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ASSESSMENT OF RAPD MARKERS VARIABILITY OF LOCAL TURKEY (MELEAGRIS GALLOPAVO) POPULATION IN EGYPT

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ABSTRACT: This study was performed to assess the diversity level within the local turkey population in Egypt in comparison with the commercial turkeys. Ten mature males and ten mature females of each population were used. RAPD-PCR technique was applied using ten 10-mer random primers. Altogether 2790 bands were detected, with an average of 6.975 bands overall individuals and primers. In general, the amplified bands were higher in commercial individuals (1695 bands) than the local ones (1690 bands). The total fragment number (TFN) was 115 with an average of 11.5 fragment/primer. The average of PIC percentage of 59% overall individuals. Nevertheless, no specific or unique band was detected. Shannon information index was higher in Baladi (0.615) than in commercial turkeys (0.488). Shannon diversity index also was higher in Baladi (0.433) compared to commercial turkeys (0.328). Analysis of molecular variance (AMOVA) showed that 79% of the total variation was attributed to the within-population variance. The principal component analysis (PCA) results showed similar trend, 83.3% of the total variance was explained by the first 3 axes partitioned to 75.96%, 4.46% and 2.88% for the first, second and third axis, respectively. The study highlighted the high level of within-population genetic variability in the local Egyptian turkeys.

keywords: Diversity-local turkeys-Meleagris gallopavo-PIC-RAPD-PCR

INTRODUCTION

Overall the world, poultry share about 36% of meat production, turkeys (Meleagris gallopavo) contribute about 5% of poultry meat production followed to chickens (89% of poultry meat production). According to FAO statistics, turkey production in Egypt has increased and reached 1950K heads in 2017 compared to 1633K heads in 2010 (FAO, 2017), this increase is mainly attributed to the use of commercial lines rather than local breeds. Despite of local breeds are considered a key factor in sustainable development plans, Egyptian turkey local breed (Baladi) is neglected, and there is no any breeding or management program designed for it. Moreover, maintaining the genetic variation within local breeds allow them for genetic response for selection as well as adaptability for environmental conditions (El-Gendy and Helal, 2014).

Genetic variability is a crucial platform in animal genetic improvement. Estimation of within-population genetic variability reflects the true situation of the population, and provides powerful genetic information for designing breeding programs and for conservation as well. There are different approaches for studying within-population variability. However, since the discovery of DNA-based techniques, the molecular approaches dominated the study of variability and diversity within- and between-populations (Lamare and Rao, 2015). Molecular approaches including different techniques that rely on the use of nuclear DNA markers to amplify specific regions of DNA using polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR or microsatellite). Although the effectiveness of RAPD technique in breed characterization has many limitations, it is results can give insights about the level of diversity either between or within breeds and species (El-Gendy et al., 2005). RAPD has been widely used to study diversity and variability within and between different local poultry populations including chickens, ducks, geese and quail as well as ostrich (Abu Shnaf and Anwar, 2018; Basha et al., 2016; Eissa et al., 2014; El-Gendy et al., 2005; El-Sabrout et al., 2015; Helal and Ahmed, 2018; Hinckley et al., 2005; Ibrahim et al., 2015; Kameshpandian et al., 2018; Singh and Sharma, 2002). Nevertheless, very few reports were investigated the diversity level within turkey populations (Al-Barzinji et al., 2015; Al-Barzinji and Fatah, 2016; Ameen, 2013; Amin, 2017; Smith et al., 1996; Vergara et al., 2018), while no reports were found on the study of the genetic diversity on Egyptian local turkeys. Accordingly, this study was performed to evaluate the level of diversity within the local turkey population in Egypt in comparison with commercial line.

MATERIALS AND METHODS

Populations

The study was performed using commercial and local birds obtained from the turkey farm of poultry services center at Faculty of Agriculture, Cairo University. The commercial line was a light body weight strain with an average body weight of 4.3 and 7.2 kg for hens and toms, respectively. The Baladi turkeys were lighter than the commercial and averaged 2.1 and 3.7 for hens and toms, respectively.

Blood sampling, DNA extraction and amplification

Blood samples were randomly collected from the jugular vein of 40 mature individuals of Baladi and commercial line turkeys (10 samples/sex/population).

Blood samples were collected in tubes containing EDTA solution (anticoagulant, pH=0.8) and stored at -20 C until the extraction of genomic DNA. Bioflux® extraction kit was used for genomic DNA extraction according to the manufacturer's instructions. The quality and quantity of the extracted DNA were determined after the extraction. DNA was thereafter amplified using 10-mer random primers as shown in Table (1). The PCR machine was programmed as described by (Helal and Ahmed, 2018), the program was consisted of initial denaturation (94℃ for 10 min), then 40 cycles of denaturation at 92℃ for 2 min, annealing at 35℃ for 1 min, and elongation at 72℃ for 2 min, the final elongation step was set at 72℃ for 10 min. The total volume of PCR mixture was 15 µl (2 µl of DNA template, 2 µl of primer, 1 µl of $MgCl_2$, 7 µl of PCR master-mix and 3 µl of nuclease-free water). PCR products were then separated using 2% agarose gel, stained using ethidium bromide, photographed and ultraviolet light.

Band scoring and statistical Analysis

The resulted banding patterns were scored (1 for presence and 0 for absence) and then analyzed using GENALEX version 6.5 (Peakall and Smouse, 2012). The different genetic parameters were calculated as follows:

Polymorphic information content = 1 - (total number of detected bands/ genotypes)^2, number of effective alleles (Ne)v= $1/\sum p_i^2$, Shannon's information index (I) = $-1^* \sum p_i \ln p_i$, Shannon's diversity (h) = $1-\sum p_i^2$, unbiased diversity (uh) = (N/(N-1))h, and PHI_{PT} = variance among populations/ total variance.

RESULTS AND DISCUSSION

Banding patterns and polymorphism

The total number of amplified bands was 2790, with an average of 6.975 bands overall individuals and primers as shown in table (1). In general, the amplified bands were higher in commercial individuals (1695 bands) than the local ones (1690 bands). The total fragment number (TFN) was 115 with an average of 11.5 fragment/primer. Similar results were found by (Al-Barzinji et al., 2015), they reported an average TFN of 14.8 in local turkeys in Erbil. The highest TFN detected by RAPD in local turkeys was reported for local turkeys populations in Kurdistan and reached 79 as an average of all primers (Ameen, 2013). TFN reached 18 in the local Mexican turkey populations (Chassin-Noria et al., 2005) and 24 in USA (Smith et al., 1996).

Frequency of alleles, number of different alleles (Na) and number of effective alleles (Ne) are presented in figure (1) and table (2). The number of different alleles was higher in Baladi (1.957±0.019) than commercial turkeys (1.878±0.031), same trend was found for the number of effective alleles. As a percentage, Ne was found to be 92.6% and 83.0% of the total number of the observed alleles in local and commercial turkeys, respectively. The number of observed alleles was 7.82 per locus for four Mexican turkey populations (Vergara et al., 2018).

The percentage of polymorphic loci was 95.65% in Baladi population and 87.83% in commercial turkeys. Lower percentages were obtained for two native Mexican turkeys (58.33 and 53.33% compared to 41.17% for commercial turkeys (Cano Camacho et al., 2003). Fortunately, it is obvious from the polymorphism results that the local population did not suffer from genetic bottlenecks. Heterozygosity is very important parameter in animal populations, but the accurate estimation of heterozygosity using RAPD is very difficult due to the dominant nature of RAPD markers, it however can be

determined using polymorphic information content (PIC) which is considered a measure of gene diversity as a higher PIC value indicates more allelic diversity and therefore greater polymorphism for a particular locus (Ali et al., 2008; Muhammad et al., 2017). Table (3) shows PIC values, all primers revealed a high level of polymorphism, the average of PIC percentage was 59% overall individuals. Nevertheless, no specific or unique band was detected. PIC percentages were predominantly higher in Baladi turkeys than in the commercial line, this may be a clue on the high percentage of heterozygosity in local turkeys, Naylor (1962) stated that population under random mating system are sufferable form the increase of heterozygosity. PIC percentages were as low as zero in the males of commercial line by primer OPB-03, and reached to the maximum (94%) in the males of Baladi turkeys by primer OPA-01. In general, the percentages of PIC were higher in males than females either in commercial or local turkeys. Similar PIC (59.9%) was obtained using microsatellites in four Mexican turkey populations (Vergara et al., 2018). Gorji et al. (2011) reported that the PIC results of RAPD should be similar to ISSR markers as a single primer is used as forward and reverse primers. The average percentage of PIC in the current study (59%) is an average value between the percentages of 31.49% which was found in local turkeys in Sulamania in Iraq (Ameen, 2013) and 100% which was found in the local Mexican turkeys (Chassin-Noria et al., 2005).

Genetic diversity and Analysis of Molecular Variance (AMOVA)

The value of Shannon information analysis was higher in Baladi (0.615) than that of commercial turkeys (0.488). Also, the values for females were slightly higher than males for both populations. Shannon diversity index (table 2) also was higher in Baladi (0.433) compared to commercial turkeys (0.328). Similar results were obtained in Mexico by Chassin-Noria et al. (2005), they reported higher Shannon information index in a domestic Mexican population (0.332) than the commercial turkeys (0.164). It is very clear from the diversity results that the local Baladi is more diverse population than the commercial turkeys, probably due to the lack of selection program for the local population.

AMOVA of the two populations revealed that most of the variation was attributed to the within-population variance (79 % of the total variance) and only 21% is due to the differences between the two populations (table 4). López-Zavala et al. (2013) reported that 86% of the total variation of Mexican domesticated turkeys was due to the within population variance. Forasmuch PHI_{PT} is an analogue of $F_{st,}$, it suppresses the intra-individual variance and therefore permits the calculation of genetic differences of populations (Yamasaki and Ideta, 2013), PHI_{PT} was statistically significant ($p<0.00$) with a value of 0.212.

For better understanding of the distribution of the variation, principal component analysis (PCA) was conducted. The PCA results showed a similar trend, 83.3% of the total variance was explained by the first 3 axes partitioned to 75.96%, 4.46% and 2.88% for the first, second and third axis, respectively (figure 2). The first principal coordinate (75.96%) separated the local Baladi population from the commercial turkeys. These three principal components (83.3%) were enough to figure out the variance of the populations as the

eigenvalue of the fourth component was very low.

CONCLUSION

RAPD-PCR has effectively revealed the genetic variation within local turkey population in Egypt. Concludingly, the study proved the high level of diversity within the local Egyptian turkey population. The high variability within the local turkeys may be attributed to the adaptive performance of the local turkeys to enable them to cope with the subtropical environmental conditions in Egypt. Accordingly, local turkey population contains unexploited genetic reserve, including genes with prominent traits and designing breeding program for exploiting that genes is vital for sustainable development

Table (1): Primer Sequence, total number of detected bands and average number of bands by primer

Primer	Sequence	Detected bands			Average /individual	
		All individuals	Baladi turkeys	commercial line	Baladi turkeys	commercial line
OPA-01	CAGGCCCTTC	230	95	135	4.75	6.75
OPA-03	AGTCAGCCAC	320	117	203	5.85	10.15
OPA-05	TGCGCCCTTC	290	97	193	4.85	9.65
OPA-06	GGTCCCTGAC	143	32	111	1.60	5.55
OPA-08	GTGACGTAGG	224	69	155	3.45	7.75
OPA-18	AGGTGACCGT	255	84	171	4.20	8.55
OPB-03	CATCCCCCTG	274	107	167	5.35	8.35
OPB-07	GGTGACGCAG	371	152	219	7.60	10.95
OPB-14	TCCGCTCTGG	293	162	131	8.10	6.55
OPC-06	GAACGGACTC	390	180	210	9.00	10.50
Total		2790	1095	1695		
Average		6.975			5.475	8.475

Table (2): Number of alleles (Na), effective number of alleles (Ne), Shannon information index (I), Shannon diversity (h) and unbiased diversity (Uh)

Population	Sex		Na	Ne	I	h	Uh
Baladi	unisex	Mean	1.957	1.812	0.615	0.433	0.455
		SE	0.019	0.024	0.014	0.011	0.011
Commercial	unisex	Mean	1.878	1.559	0.488	0.328	0.345
		SE	0.031	0.030	0.020	0.014	0.015
Baladi	Males	Mean	1.957	1.724	0.581	0.402	0.447
		SE	0.019	0.025	0.015	0.011	0.012
	Females	Mean	1.948	1.742	0.582	0.404	0.449
		SE	0.021	0.027	0.016	0.012	0.014
Commercial	Males	Mean	1.748	1.534	0.444	0.304	0.338
		SE	0.041	0.034	0.025	0.018	0.02
	Females	Mean	1.870	1.541	0.477	0.320	0.356
		SE	0.032	0.029	0.02	0.015	0.016

Table (3): Polymorphic information content, by primer, for the detected allelic bands

Primer	All individuals	Baladi	Commercial	Baladi		Commercial	
				Male	Female	Male	Female
OPA-01	0.72	0.90	0.54	0.94	0.84	0.48	0.56
OPA-03	0.65	0.82	0.47	0.91	0.70	0.50	0.43
OPA-05	0.58	0.81	0.35	0.83	0.77	0.38	0.31
OPA-06	0.74	0.96	0.52	0.92	0.97	0.65	0.36
OPA-08	0.64	0.87	0.40	0.93	0.77	0.48	0.31
OPA-18	0.43	0.77	0.09	0.83	0.68	0.12	0.06
OPB-03	0.38	0.62	0.13	0.61	0.63	0.00	0.25
OPB-07	0.58	0.72	0.44	0.63	0.77	0.38	0.47
OPB-14	0.66	0.60	0.72	0.49	0.69	0.78	0.65
OPC-06	0.54	0.61	0.46	0.54	0.64	0.44	0.52
Average ±SE	0.59±0.04	0.77±0.04	0.41±0.06	0.76±0.06	0.75±0.03	0.42±0.07	0.39±0.05

Table (4): Analysis of molecular variance

Source	df	SS	MS	Est. Var.	PVC %	Φ_{PT}	*P* value
Among Pops	1	146.600	146.600	6.179	21%	0.212	0.001
Within Pops	38	875.000	23.026	23.026	79%		
Total	39	1021.600		29.205	100%		

Figure (1): Allele frequency generated by RAPD markers for Baladi and commercial turkeys

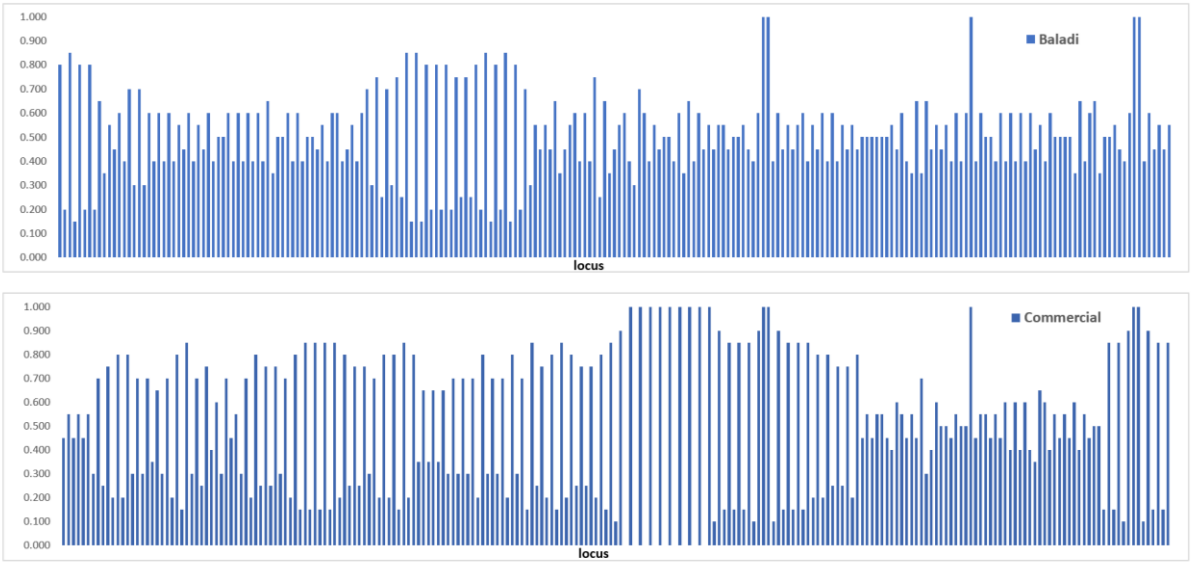
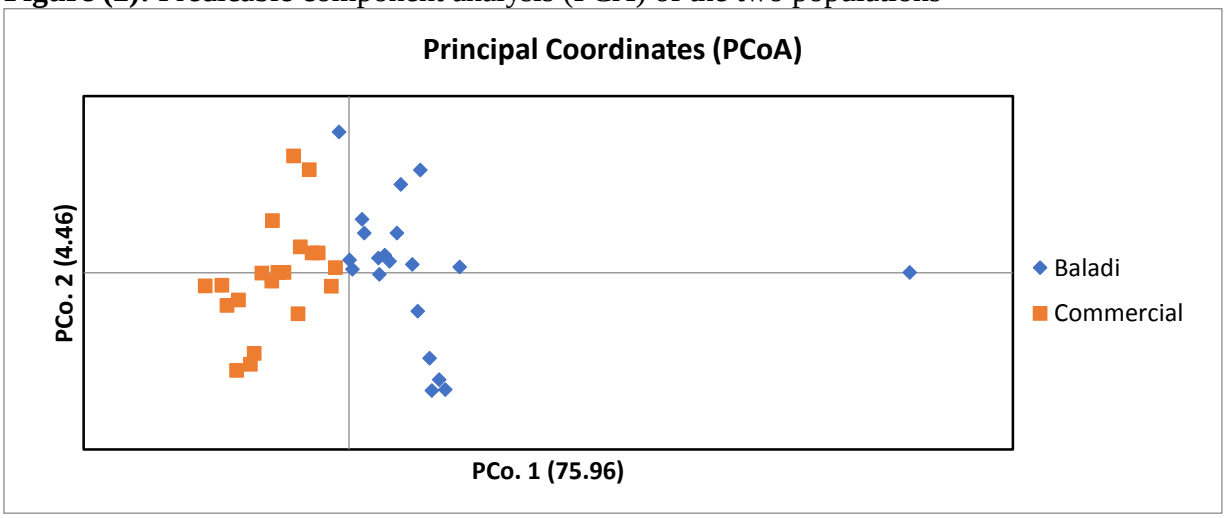


Figure (2): Predicable component analysis (PCA) of the two populations



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الملخص العربي

دراسة التباين الوراثي في عشيرة الدجاج الرومي المحلي في مصر باستخدام التضخيم العشوائي للمادة الوراثية

مصطفي هلال

قسم الإنتاج الحيواني -كلية الزراعة – جامعة القاهرة

أجريت هذه الدراسة بهدف تقييم مستوى التنوع الوراثي داخل عشيرة الدجاج الرومي المحلية في مصر بالمقارنة مع سلالة تجارية خفيفة الوزن، حيث تم استخدام 40 طائراً بالغاً (10 ذكور 10 إناث من كل عشيرة). وتمت دراسة التباين على مستوي المادة الوراثية باستخدام تقنية تفاعل البلمرة المتسلسل وتم تضخيم المادة الوراثية باستخدام عشرة واسمات عشوائية كل منها يتكون من 10 قواعد نيتروجينية. ظهر جلياً من بداية رصد حزم المادة الوراثية وجود اختلاف في طرز الحزم بين السلالة التجارية والمحلية حيث كان عدد حزم المادة الوراثية التي تم تضخيمها 2790 حزمة، بمتوسط 6.975 حزمة/فرد/واسم. كانت حزم المادة الوراثية المضخمة بشكل عام أعلى في افراد السلالة التجارية (1695 حزمة) من افراد السلالة المحلية (1690 حزمة). وكان إجمالي عدد المواقع التي تم فيها تضخيم للمادة الوراثية 115 موقع وراثي بمتوسط 11.5 موقع / واسم وكان متوسط نسبة محتوي المعلومات المتباينة (PIC) 59 ٪. ومع ذلك، لم يتم الكشف عن حزمة محددة أو فريدة من نوعها في إحدى السلالتين. كان مؤشر شانون للمعلومات أعلى في السلالة المحلية (0.615) منه في التجارية (0.488). وكان مؤشر شانون للتنوع أعلى أيضًا في السلالة المحلية (0.433) مقارنة بالتجارية (0.328). وأظهر تحليل التباين على المستوي الجزيئي (AMOVA) أن 79 ٪ من التباين الكلي يعزى إلى التباين داخل العشائر و21%. أظهرت نتائج تحليل المكون الرئيسي (PCA)اتجاهًا مشابهًا، حيث تم تفسير 83.3٪ من التباين الكلي من خلال أول 3 محاور مقسمة إلى 75.96٪، 4.46٪ و2.88٪ للمحور الأول والثاني والثالث، على التوالي. سلطت الدراسة الضوء على ارتفاع معدل التباين الوراثي داخل السلالة المحلية للدجاج الرومي المصري وهي النتيجة التي توضح اهمية حفظ الأصول الوراثية للدجاج الرومي المحلي مما يسمح بالبدء في برامج التحسين الوراثي لها.