EVALUATION OF GENETIC DIVERSITY IN PEAFOWL COLOR MUTANTS

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ABSTRACT: *Peafowl (Genus: Pavo) belongs to the peasant family, Phasinaidea which is of enormous aesthetic value. In Khyber Pakhtunkhwa, five colored mutants belonging to Pavo muticus and Pavo cristatus are found. Nine (9) genotypes of the five colour mutants were analyzed by RAPD PCR for the evaluation of genetic diversity. Our results revealed that out of the total 41 amplified bands, 40 were found polymorphic. Shannon's index was found to be 0.5046 and Nei' diversity was 0.7543 while mean Nei' identity ranged from 0.36-0.73.*

INTRODUCTION

The word Peafowl refers to any species of birds in genus Pavo and may refers to the AfroPavo of the pleasant family, Phasianidae. The three species in Phasianidae are *Pavo cristatus* (Blue Peafowl), *Pavo muticus* (Green Peafowl) and *Afropavo congensis* (Congo Peafowl). Blue Peafowl is a native breed of the India, Pakistan, Bangladesh and Sri Lanka. There are certain colour mutants of this species e.g., white Peafowl, black shoulder, Pied etc. Blue Peafowl is the Provincial bird of Punjab (Pakistan) and is a national bird of India.

Pavo muticus (Green Peafowl) is native to Burma in the east of Java that is why they are also called as the Burmese or Java green. Green Peafowl have larger size than blue Peafowl. *Pavo muticus* (Green Peafowl) is listed as Vulnerable, by IUCN [1]. Congo Peafowl differs markedly from the previously mentioned species mainly because of its featherless neck. It was discovered in 1936. It is a very rare, little-known species and is found in the tropical forests of Zaire (Africa).

Peafowl are important birds as they posses aesthetic, medicinal and economic value. There are various colored mutants, which are reared for their beautiful colors. Peafowls are found in a wide range of habitats including tropical and subtropical as well as evergreen and deciduous forests, grassland and farmland edge. It forages and nests on the ground but roosts on the top of trees.

Recently there is a trend in research and improvement of Peafowl production, and hence in Peafowl breeding. There are a few reports about the genetic information of Peafowl populations. Advances in molecular tools have enabled us to disclose the genetic differentiation and the genetic diversity between and among various breeds. There are many methods, which are used for documenting genetic information. These methods are consisting of DNA barcoding, DArT, AFLP, isozyme analysis, RFLP, and RAPD [2]. Due to its universal set of primers, no requirement for preliminary work such as filter preparation, probe isolation and nucleotide sequencing, RAPD (Randomly amplified polymorphic DNA) analysis is economical and useful as compared to other techniques of genetic documentation such as RFLP etc [3]. The ease and simplicity of the RAPD technique make it ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics. In many instance, only a small number of primers are necessary to identify polymorphism within species [4]. Moreover, a single primer may be sufficient to differentiate all of the varieties [5]. In the Khyber Pakhtunkhwa province of Pakistan, five color mutants of peafowl are found. Genetic diversity and population size determines the survival of organisms in diverse environments. The population of peafowl in KPK is scarce and genetic diversity among the species has never studied. This study aimed at determining genetic diversity in the peafowl color mutants found in KPK.

MATERIALS AND METHODS

Blood samples of male and female Peafowl color mutants (Blue Peafowl male, Black Shoulder Peafowl, Pied Peafowl and White Peafowl) were selected from *Pavo cristatus* species. The two (02) genotypes (Green Java male and Green Java female) were selected from *Pavo muticus* specie a harem of nine (09) members of each of the colored mutants of peafowl were identified and selected for blood samples and were shifted to the laboratory for DNA extraction.

Genomic DNA Extraction: DNA from whole blood was extracted at IBGE. 0.8 ml of 1X SSC solution and 0.5 ml of whole blood were mixed up and centrifuged for 2 min in an eppendorf tube at 10,000 rpm. The supernatant was discarded and left 0.1 ml in the tube to which 0.5 ml of buffered phenol 0.5 ml of lysis buffer and were added and centrifuged at 14,000 rpm for 5 min. The 0.5 ml of Phenol/chloroform/Isoamyl alcohol (25:24:1) was then added to the aqueous part collected from the previous step and centrifuged at 14,000 rpm for 5 min. The aqueous fraction was transferred to a new tube where it was added 0.05 ml of 3M sodium acetate, 1 ml chilled ethanol (95%) and centrifuged at 14,000 rpm for 10 min. Then pellets were obtained which were washed with 70% ethanol. Pellets were

dried and re-suspended in 50 µl of TE buffer. For complete dissolution, DNA samples were kept at 70 ^{o}C for 10 minutes and then stored at -20 ^{o} C. Eight (08) different RAPD primers were employed for the molecular study of Peafowl.

Polymerase chain reaction (PCR): Polymerase chain reaction (PCR) was carried out according to the protocol of Devous and Gale [6] with some modifications. Thermal profile of PCR is given in Table 1.

Table.1. Thermal profile of the PCR reaction for Peafowl

Step	Duration	Temperature
Hot start	4min	95^{o}C
Denaturation	1min	94^{o}C
Primer annealing	1min	33^{o}C
Extension	2min	72^{o}C
Final extension	10min	72^{o}C

For RAPD-PCR, Randomly Amplified Polymorphic DNA markers were obtained from the gene link technology (GLT), USA and were used for the analysis of genetic diversity. Information about primer sequences are given in Table 2.

Table.2. List of primers used for the determination of genetic diversity in different color mutants of Peafowl

S. No.	Primer	Sequence	Size
1	A-04	AATCGGGCTG	10bp
2	A-14	TCTGTGCTGG	10bp
3	A-16	AGCCAGCGAA	10bp
4	B-05	TGCGCCCTTC	10bp
5	B-14	TCCGCTCTGG	10bp
6	F-19	CCTCTAGACC	10bp
7	G-13	CTCTCCGCCA	10bp
8	H-19	CTGACCAGCC	10bp

Data analysis: Data scoring was carried out, based on the presence or absence of the PCR products for the computer analysis. Presences of amplified PCR products were scored as 1 while their absence as 0. Then computer program "Pop gene 32" version 1.31 was used to construct Dendrogram by UPGMA using dissimilarity coefficients.

RESULTS

Table 3 shows genetic relatedness among different Peafowl colored mutants. The highest genetic Identity (0.7317) was observed between genotypes of BPF (Blue peafowl female) and PPF (Pied peafowl female) and between genotypes of PPM (Pied peafowl male) and PPF (Pied peafowl female) while the lowest genetic identity (0.3659) between genotypes of GJF (Green java female) and WPM (White peafowl male).

Where GJM (Green Java Male), GJF (Green Java Female), BPM (Blue Peafowl Male), BPF (Blue Peafowl Female), BSM (Black Shoulder Male), BSF (Black Shoulder Female), PPM (Pied Peafowl Male), PPF (Pied Peafowl Female), WPM (White Peafowl Male) are nine (09) Peafowl colored mutants Dendrogram produced by the same software gave the two clusters of genotypes as given in the figure 1.

Cluster analysis: The genetic dissimilarity coefficient matrices of nine (09) Peafowl genotypes based on eight RAPD primers using UPGMA method Nie and Lie [7] to construct Dendrogram using computer programme "pop gene" (Figure 1). The genotypes were classified into four groups:

Group A comprises of GJM (Green java male), GJF (Green java female),

Group B comprises of BPM (Blue Peafowl male), BSM (Black shoulder male) and BSF (Black shoulder female)

Group C contains BPF (Blue Peafowl female), PPF (Pied Peafowl female) and PPM (Pied Peafowl male) and

Group D contains WPM (white Peafowl male).

Table.3. Nei's genetic identity among all Peafowl genotypes

	GJM	GJF	BPM	BPF	BSM	BSF	PPM	PPF	WPM
GJM	-								
GJF	0.6341	-							
BPM	0.4878	0.6585	-						
BPF	0.5366	0.6585	0.5610	-					
BSM	0.5122	0.5854	0.6829	0.6829	-				
BSF	0.4878	0.6098	0.6585	0.6585	0.6829	-			
PPM	0.4390	0.6098	0.6585	0.6585	0.5610	0.6585	-		
PPF	0.5610	0.6341	0.6341	0.7317	0.5610	0.6829	0.7317	-	
WPM	0.5366	0.3659	0.5610	0.5610	0.6829	0.5610	0.6585	0.5854	-

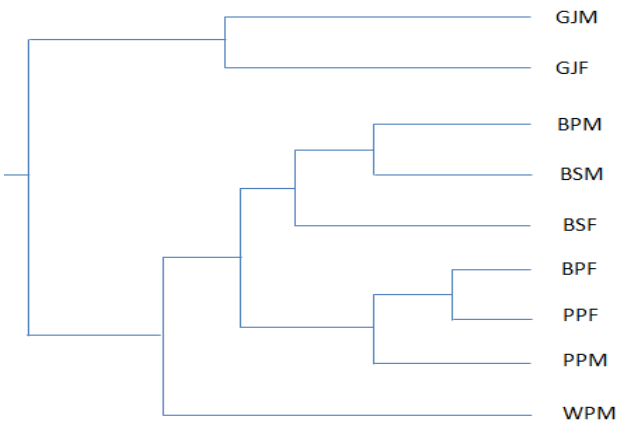

Figure.1. Dendrogram analysis of nine (09) peafowl genotypes using RAPD primers using computer program "Pop gene 32"

Populations of group A were found to be the most dissimilar groups from the rest of the population. Therefore, maximum pair wise genetic distances were observed between GJF (a member of Group A) and WPM (member of Group D). Minimum distance was observed between BPF (Blue peafowl female) and PPF (both are members of group C). Likewise, distance was observed between PPM and PPF (both are members of Group C). Members of one group were found to be at least genetic distance with the members of the same group and distantly related with the members of the other group. Results presented by Dendrogram are in close agreement with the average dissimilarity matrix presented in Table 4, and also with the Nei,s Identity as shown in Table 3. Shannon's diversity index was also calculated and was 0.5.

Table 4: Shannon's Index and Nie genetic diversity for each primer

Primer	Shannon Index (Mean)	Nie Genetic diversity (Mean)
A-04	0.6204	0.8522
A-14	0.5915	0.7588
A-16	0.5879	0.8621
B-05	0.3089	0.8580
B-14	0.3309	0.6943
F-19	0.5179	0.6870
G-14	0.6744	0.6744
H-19	0.5406	0.4412
Overall Mean	0.5046	0.7543
Standard deviation	0.2492	0.1847

DISCUSSION

RAPD is a very efficient molecular technique for the recognition of species and for establishing phylogenetic correlation among and between species, and is based on Polymerase chain reaction [8]. To establish relationship among closely associated breeding lines RAPD markers are more efficient because they are faster and cheaper to handle [9]. The RAPD markers are similar in function to RFLPs but are advantageous to RFLPs because they are cheaper, less time consuming and needs no managing skills [10, 11, 12].

Forty primers were primarily screened for their ability to generate polymorphic bands among these primers eight primers were chosen which gave reproducible and distinctive polymorphic product. Total 41, RAPD bands were obtained out of which 40 or 97.56% were considered as polymorphic. All of the genotypes could be separated from each other by the deviation in their banding profile. Occurrences of bands in each one of genotypes were recognized as monomorphic bands/alleles while their nonexistence from any of the genotypes is called polymorphic bands. The absence of the bands in the genotypes is due to the failure of the RAPD primers to anneal with the DNA of the genotypes because of the absence of the complementary sites for the respective primers [13]. The probable cause of higher average number of the score able and polymorphic bands/loci may be due to the higher concentration of the GC content in the primers.

The data suggested that members of group A in general and GJF (Green java female) in specific are found to be the most unrelated group from the rest of the Peafowl population under study. Therefore highest pair wise genetic distances were found between GJF (Green java female) and WPM (White peafowl male) followed by the genetic distance between GJM (Green java male) and PPM (Pied Peafowl male). Similarly results of Table 4 of Nei's genetic Identities showed that maximum genetic Identities are found between genotypes GJM (Green java male) and GJF (Green java female) and also among genotypes from BPM (Blue peafowl male) to WPM (White peafowl male). Table 3 also showed that minimum genetic Identities were observed when genotypes of GJM (Green java male) and GJF (Green java female) were compared with genotypes from BPM (Blue peafowl male) to WPM (White peafowl male). The Dendrogram showed that genotype of GJM (green java male) is closely related to genotype of GJF (green java female) and they are distant from other genotypes. If the member of group A (green java male and green java female) were crossed with member of group C (blue Peafowl female, pied Peafowl female, pied Peafowl male and white Peafowl male) a very diverse group may be developed and so chances of the conservation of Peafowl be increased.

CONCLUSION

From the present research study, it can be concluded that species of genus Pavo, belongs to a very diverse background having; genetic diversity which is of utmost importance for the various breeding programs in Peafowl conservation and is also helpful for the development of other molecular techniques for species identification.

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