

PRODUCTIVE PERFORMANCE AND MOLECULAR GENETIC CHARACTERIZATION OF BROWN AND WHITE JAPANESE QUAIL GENOTYPES USING RAPD AND ISSRs-PCR MARKERS

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The Japanese quail bred for meat and egg production, reaching table weight at six weeks and coming into lay at the same age. Thus, quail has the potential to serve as an excellent and cheap source of animal protein (Vali, 2008). Associated effects of quail plumage colour genetic variants on performance traits have only been studied for a few mutations, such as the roux mutation, which can be used for auto-sexing at one day of age. Roux mutation is associated with lower body weight and abdominal fat (Minvielle *et al.*, 1999). Decreased hatchability with recessive white gene (Petek *et al.*, 2004), or the curly feather mutation, with increased body weight (Minvielle *et al.*, 2005). Lavender plumage color in Japanese quail is associated with birds lighter body weight, lower body temperature, increased feed consumption (Bed'hom *et al.*, 2012). Differences in egg production traits between Pharaoh and Manchurian golden breeds (Genchev, 2012), emphasize the need for further studies of plumage colour effect on production performance.

Random amplified polymorphic DNA (RAPD) and inter simple sequence

repeats (ISSRs) are the molecular techniques available for characterization of the variation at the DNA level. RAPD technique (Williams *et al.*, 1990 and 1993) was the first PCR-based marker system used in genetic analysis showed high level of polymorphism. DNA fingerprinting of each individual quail could be distinguishable even within a line (Mannen *et al.*, 1993; Ye *et al.*, 1998). RAPD markers were effective to detect polymorphism and genetic diversity in quail lines (Kumar *et al.*, 2000; Sharma *et al.*, 2000; Karabağ and Balcioglu, 2010). Also, Mansour *et al.* (2010) investigated the variations within four phenotypes of quail using RAPD and ISSRs markers.

In an independent studies in different locations and different breeds using RAPD phylogenetic relationship and diversity were established by some authors in chickens (Monira *et al.*, 2011; Yap and Kumaran, 2011; Tamara *et al.*, 2012; Alatafi *et al.*, 2013), in ducks (Alyethodi *et al.*, 2010) and in rabbit breeds (El-Bayomi *et al.*, 2013). Random and microsatellite markers were used to detect the genetic lineage and to determinate

genetic variation in chicken (Olowofeso *et al.*, 2005; Tadano *et al.*, 2013). ISSRs technique is a way to assess genetic diversity, to identify closely related genotypes in many species and to permit the detection of polymorphisms in microsatellites loci. The efficacies of microsatellite markers with populations have been reported by Romanov and Weigend (2001). Also, microsatellites have been reported in the literature for quails (Pang *et al.*, 1999; Kayang *et al.*, 2002 and 2004). The first genetic map for quail was produced with AFLP markers (Roussot *et al.*, 2003). Genetic coadaptability of quail populations was studied using microsatellite markers (Guobin *et al.*, 2006). Phylogenetic relationships between various species were examined using random and microsatellite markers (Emara and Kim, 2003).

The use of these markers with chicken breeds had been reported by Zhang *et al.* (2002). Genetic diversity of guineafowl based on microsatellite analysis was reported by Kayang *et al.* (2010). Microsatellite markers were used also to assess the DNA patterns of native Egyptian chickens (El-Komy, 2011). Also, RAPD and microsatellite markers were used as the basis for improving broiler performance (Nassar, 2013). Limited reports have addressed the genetic diversity of the indigenous quail using productive performance and DNA fingerprinting technique.

The aims of the present study were to characterize the possible genetic and productive traits differences associated

with the white plumage in Japanese quail compared to the brown colour quail. Productive performance and DNA markers were used to identify these genotypes. In addition, the genetic variations and relationships among them were determined using RAPD and ISSRs-PCR analyses to test the use of results for exploring genetic diversity.

MATERIALS AND METHODS

Birds and husbandry

The experimental work was carried out at the Poultry Research Center, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. A white color genotype produced from some genetic segregation from the brown genotype during the course of breeding experiments with Japanese quail. The white colour genotype was deliberately identified and propagated for the study of possible productive performance and genetic differences between brown and white genotypes. Two hundred and forty quail (120 brown and 120 white) were obtained from 20 sires and 40 dams for each genotype (two females were randomly assigned to each male), in one hatch following a two-week egg collection period. Feed and water were provided *ad libitum*. All experimental birds were maintained as possible under similar conditions.

The following productive performance were estimated

Body weight (BW) and shank length (SL) at 1, 7, 14, 21, 28 and 35 days

of age and at first egg, age at first egg (AFE), first egg weight (FEW), first egg shape index (FESI), number of days needed to produce the first 10 eggs (DN₁₀), number of days needed to produce the first 30 eggs (DN₃₀), egg mass of the first 10 eggs (EM₁₀), egg mass of the first 30 eggs (EM₃₀), number of egg produced in the first month (EN_{FM}), number of egg produced in the second month (EN_{SM}), number of egg produced in the first two months (EN_{F_{TM}}), egg mass of the first month (EM_{FM}), egg mass of the second month (EM_{SM}) and egg mass of the first two months (EM_{F_{TM}}). The data were recorded for each female.

Statistical analysis

Data were subjected to analysis of variance using the General Linear Model Procedure of SPSS (SPSS, 2008) and significant differences among the means were tested by Duncan's multiple range test (Duncan, 1955). The following model was used for the growth traits to determine the effect of genotype, sex and the interaction between genotype and sex, $Y_{ijk} = \mu + G_i + S_j + GS_{ij} + e_{ijk}$ where Y_{ijk} =observed value in the i^{th} genotype in the j^{th} sex of the k^{th} individual, μ =overall mean, G_i =genotype effect (i =brown and white), S_j =sex effect (j =male and female), GS_{ij} =the interaction between genotype and sex and e_{ijk} is the error term associated with the Y_{ijk} . While data of egg production-related traits were subjected to a one-way analysis of variance with genotype effect. The statistical model used was as follows, $Y_{ij} = \mu + G_i + e_{ij}$, where Y_{ij} =observed value in the i^{th} genotype of the j^{th} individ-

ual, μ =overall mean, G_i =genotype effect (i =brown and white) and e_{ij} =random error term.

Extraction of DNA

Blood samples were collected individually from each of the 10 birds (three females and two males) for each genotype (brown and white). All birds were phenotypically normal and healthy. Blood sample was collected from the brachial vein of each individual in a tube containing EDTA solution (pH 8.0) as anticoagulant reagent and stored at -20°C until DNA extraction. DNA extraction was performed from the blood samples of each individual as described by Z10 spin column DNA Minipreps Kit (Bio basic INC.). In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, MgCl₂ and Taq DNA polymerase. A total of 21 and 15 random DNA oligonucleotide primers synthesized by Operon biotechnologies, Inc. Germany were independently used in the RAPD and ISSRs-PCR reactions mixture, respectively. Table (1) lists the base sequences of these primers that produced informative polymorphic bands.

RAPD and ISSRs-PCR reactions

The RAPD-PCR amplification reactions were performed in a 25 μ l reaction volumes. Briefly, the RAPD reaction mixture containing 2.5 μ l dNTPs (2.5 mM),

1.5 µl MgCl₂ (25 mM), 2.5 µl 10x buffer, 2.0 µl primer (2.5 µM), 2.0 µl template DNA (50 ng/µl), 0.3 µl Taq DNA polymerase (5 U/µl) and 14.7 µl of sterile ddH₂O. Amplification was performed in a DNA thermal cycler (Techni TC-512 PCR). The RAPD-PCR reaction was subjected to one cycle at 95°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 37°C for 30 sec, and 72°C for 30 sec, then a final cycle of 72°C for 12 min.

The ISSRs-PCR amplification reactions were performed in the same reaction volumes as used with RAPD-PCR with little modifications in the reaction mixture. Also, amplification was performed in a DNA thermal cycler (Techni TC-512 PCR) as programmed in RAPD reaction with some modifications. PCR products were separated by agarose (1.5%) gel electrophoresis, stained with ethidium bromide at 100 V to detect polymorphisms among genotypes and sexes. After electrophoresis, the RAPD and ISSRs patterns were visualized with UV Tec. Documentation system. Fragments sizes were estimated with the 1500-100 bp DNA ladder markers.

Molecular genetic analysis

The DNA bands generated by each primer were detected with their molecular sizes and compared with those of the ladder marker. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded

1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the studied genotypes.

Calculation was achieved using Dice similarity coefficients (Dice, 1945) as implemented in the computer program software SPSS-17 (SPSS, 2008). Mathematically, similarity coefficients or band sharing (BS) could be expressed as $BS = 2C_{ab} / (N_a + N_b)$ where BS is band sharing, C_{ab} is the number of common bands shared by individuals a and b, N_a and N_b are the total number of bands for individuals a and b (Dunnington *et al.*, 1990).

RESULTS AND DISCUSSION

Genotype, sex and their interaction effects on body weight and shank length

The results of the effects of genotype, sex and the genotype by sex interaction on both BW and SL are presented in Table (2). Genotype affected significantly BW at 1, 14 and 21 days of age and SL at all studied ages favoring the brown genotype with heavier BW and longer SL at these ages at 21, 28 and 35 days of age. However, the two genotypes insignificantly differed for BW at 1, 28 and 35 days of age. Sex effect on BW and SL was significant at all studied ages, except for one day old, females had significantly higher BW and SL than males. The interaction effect between genotype and sex on BW was significant at all studied ages except for one day old. Brown females had the highest BW at 7, 14, 21 and 35 days of

age and brown males had the lowest BW at 7 and 14 days of age. While white males had the lowest BW at 21, 28 and 35 day old. The interaction effect between genotype and sex on SL was significant at all studied ages, except for 1 and 14 days, brown females had the highest SL, while white males had the lowest SL.

Similar trend to our results that plumage color is associated with lower growth has been previously reported in albino by Minvielle *et al.* (1999). Also, the range of BW was significantly smaller for the white quail with lower growth until maturity was reported by Minvielle *et al.* (2010). On the opposite, white plumage quails significantly exceeded brown quails in their live BW at 1, 14 and 28 days of age (Jassim *et al.*, 2006). Values of SL obtained in the present study were in agreement with Ojedapo (2013) and Akram *et al.* (2013). Lower values for BW and SL at 14 and 28 days of age were stated by Ojo *et al.* (2014) in the brown genotype.

The differences between the estimates in the literature for BW and SL of quail and the present recorded results at a particular age may be due to the differences in the environmental conditions and to the possible differences in genetic make-up of the different flocks. Sexual dimorphism has previously been reported in favour of the female in white and brown genotypes (Jassim *et al.*, 2006) and in brown genotype (Daikwo *et al.*, 2013).

Genotype effect on egg production related traits

Means of BW and SL at first egg and egg production-related traits for brown and white genotypes are present in Table (3). The results showed that brown genotype had significantly heavier BW (240.81 g) than the white genotype (214.67 g). However, the two genotypes insignificantly differed for SL at first egg. The genotype had significant influence on the BW at first egg reported by Sakunthaladevi *et al.* (2011). Lower value for BW at first egg in brown genotype (154.64 g) reported by Okenyi *et al.* (2013). The white genotype had significantly higher shape index of the first egg (80.92) than the brown genotype (77.42), this result was almost similar to the values obtained on brown quail by Alkan *et al.* (2010). The brown genotype matured at earlier 6.06 days ($P \leq 0.05$) than the white genotype and consequently had shorter by 8.47 days ($P \leq 0.05$) to produce the first 30 eggs, and white genotype had shorter by 1.20 days ($P > 0.05$) to produce the first 10 eggs than the brown genotype. The mean of the white genotype laid higher first egg weight (11.44 g), EM_{10} (114.46) and EM_{30} (350.18) than the egg weight means of brown genotype (11.08 g), EM_{10} (113.00) and EM_{30} (350.11) with insignificant differences between them.

Table (3) showed that brown genotype laid significantly more number of eggs during the first, the second and the first two months than the white genotype.

Difference means between the two genotypes were 3.31, 4.39 and 8.24 eggs, respectively. Brown genotype had significantly higher egg mass than the white genotype during the first month, the second month and the first two months.

These results are in accordance with previous studies reported by El-Fiky *et al.* (2000) and Sakunthaladevi *et al.* (2011) for AFE. While, lower estimates for AFE with no statistical differences between Pharaoh and Manchurian golden breeds were obtained by Genchev (2012). Similar trends were reported for EN₃₀ and egg weight by Okenyi *et al.* (2013), while higher values were obtained by El-Fiky *et al.* (2000) and Aboul-Hassan (2001). However, EN was not affected by the genotype of quail (Minvielle *et al.*, 2010). Similar results were reported for EM_{FTM} (El-Fiky *et al.*, 2000; Aboul-Hassan, 2001) in brown and white genotypes and Badawy (2008) in brown genotype.

From the present results, it can be concluded that brown genotype had favored growth and egg production related-traits. The results of productive performance could be used in breeding programs to improve the quails and produce new genotypes.

Identification of RAPD-PCR markers

Six out of 21 random primers generated reproducible and scorable RAPD profiles (Plate 1). These primers detected scorable polymorphisms in banding pat-

terns among the 10 quail individuals under investigation (Table 4). Each of the 6 primers used for analysis of individual quail genotypes amplified a different number of bands. In this respect, Sharma *et al.* (2000) reported that 6 out of 20 pre-screened decamer primers revealed polymorphic and reproducible results, while 20 initially screened primers exhibited polymorphism in quail (Mansour *et al.*, 2010). The numbers of amplified fragments from all primers for all genotypes are summarized in Table (5). These produced multiple band profiles with a number of amplified DNA fragments ranging from 4-11 (Table 5 and Plate 1). A total number of 442 DNA bands was generated by the 6 random primers for the 10 quail individuals, females and males used in the present study. Polymorphism levels differed from one primer to the other. While, the number of polymorphic fragments ranged from 2-8. A maximum number of 91 amplicons was amplified with primer OPE-19, while the minimum number of fragments (61) was amplified with primer OPB-10. The highest number of polymorphic bands was detected for primer OPA-18 (8 out of 12 amplified bands) while the lowest number of polymorphic bands was detected for primer OPB-10 (2 out of 6 amplified bands). However, 17 bands were common (monomorphic) for all primers and individuals. Primer OPA-18 exhibited the highest percentage (91.67%) of polymorphism, while primer OPB-10 exhibited the lowest percentage (62.50%) of polymorphism (Table 6).

In this respect, Sharma *et al.* (2000) found that the percentage of polymorphic was 31.7%, while 85.315 polymorphism reported by Mansour *et al.* (2010), and Karabağ and Balcioglu (2010) reported that polymorphism rate was calculated as 99.49%. RAPD-PCR technique has been successfully applied for estimating genetic diversity in quail lines (Kumar *et al.*, 2000). Table (6) also revealed that the total number of polymorphic amplicons obtained by the 6 studied primers was 49. This corresponds to a level of polymorphism of 74.24% and an average number of polymorphic fragments/primer of 8.17 (Table 6). In this respect, RAPD marker technique has been used to detect genetic diversity and polymorphism in various quail lines (Sharma *et al.*, 2000; Karabağ and Balcioglu, 2010). Mannen *et al.* (1993) reported that DNA fingerprinting of Japanese quail showed characteristic pattern for each individual and these characteristic could be distinguishable from each other even within a line. No commonly shared DNA fingerprints band was observed among 18 quails examined. Ye *et al.* (1998) found that in Japanese quail, the DNA fingerprinting technique produced distinct banding patterns.

The specific markers for quail genotypes generated from RAPD-PCR analysis are shown in Tables (4 and 5). As shown in Table (5), the highest number of RAPD-PCR specific markers were scored for male (5) brown (2 markers) and male (10) white (2 markers), while the lowest (one marker) were scored for male brown

(5) and female white (7). A number of 4 positive specific markers were scored for the presence of unique bands for a given genotype, while 12 negative specific markers was scored for the absence of a common band. In the meantime, the highest number of RAPD-PCR genotype-specific markers was generated by primer OPB-10 (4 markers), primers OPA-18, OPE-06 and OPE-19 generated (3 markers/primer). On the other hand, the primer OPB-02 generated 2 markers and the primer OPA-10 generated the least number of RAPD-PCR specific markers (one marker). RAPD marker of individual quail showed characteristic pattern and individuals could be distinguishable from each other even within the same color.

Phylogenetic relationship among quail genotypes based on RAPD marker

Based on the combined data obtained through the polymorphism of RAPD profiles, the similarity coefficient values among the studied 10 individuals of quail (brown and white, females and males) were calculated according to Dice (1945) equation (Table 8). The similarity coefficient between the females and males of the 10 individual quails (brown and white) shows an average genetic distance ranging from 0.00-1.00 with a mean value of 0.50. The highest similarity index (1.00) was recorded between brown male (5) and brown female (2). However, the lowest similarity index (0.00) was observed between brown female (2) and brown female (1). The data obtained from the analysis of RAPD was used to draw

precise relationships among the 10 tested individuals. The resultant dendrogram is shown in Fig. (1) using Unweighed Pair-Group Method with Arithmetical average algorithm (UPGMA) analysis. This dendrogram clustered the quail genotypes into two clusters (groups). The first group consisted of brown female (1) and brown female (2) were delimited in separate one cluster from the rest of studied quail genotypes. The second group was subdivided into two subgroups. The first subgroup involved brown male (5) was separated from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup includes brown female (3) and brown male (4) were separated in one sub-group from the rest of genotypes, and the second subgroup was subdivided into two sub-groups. The first subgroup involved white female (6) and white female (7), and the other subgroup includes white female (8) and white male (9). The second subgroup includes white male (10) was delimited in separate group.

Based on RAPD analysis, the brown genotype female and male, and white genotype females and males were clustered in the same group while, brown female and brown male were in separate clusters.

In conclusion, 6 primers used in the present study allowed enough distinction among the quail genotypes brown and white, females and males. This has demonstrated the efficiency of the RAPD in discrimination among genotypes.

Identification of ISSRs-PCR markers

Microsatellites are two-six-nucleotide repeats, interspersed throughout the genome and highly polymorphic. After the generation of the first microsatellite linkage map of quail which reported (Kayang *et al.*, 2004), microsatellite-based markers were used in quail genetic analysis such as estimating inbreeding by pedigree (Kim *et al.*, 2007). Six out of 15 primers generated reproducible and scorable ISSRs profiles (Plate 2). These primers detected scorable polymorphisms in banding patterns among the 10 individual quail under investigation (Table 4). In this respect, Mansour *et al.* (2010) reported that genetic diversity of four genotypes of quail was analyzed by 15 microsatellite markers with high polymorphism including the same 6 ISSRs primers which used in the present study. Each of the 6 primers used for the analysis of individual quail genotypes amplified a different number of bands. The numbers of amplified fragments from all primers for all genotypes are summarized in Table (5). These produced multiple band profiles with a number of amplified DNA fragments ranging from 3-11 (Table 5 and Plate 2). A total number of 467 DNA bands were generated by the 6 random primers for the 10 individuals, females and males used in the present study. Polymorphism levels differed from one primer to the other. While, the number of polymorphic fragments ranged from 3-7. A maximum number of 95 amplicons was amplified with primer HB-10, while the minimum number of fragments (56) was amplified with primer

HB-14. The highest number of polymorphic bands was detected for primer HB-13 (7 out of 14 amplified bands) and primer HB-14 (7 out of 10 amplified bands) while, the lowest number of polymorphic bands was detected for primer HB-15 (3 out of 11 amplified bands). However, 19 bands were common (monomorphic) for all primers and genotypes. Primer HB-14 exhibited the highest percentage (90%) of polymorphism, while primer HB-15 exhibited the lowest percentage (45.45%) of polymorphism (Table 6). In this respect, Mansour *et al.* (2010) found that the percentage of polymorphism in four quail phenotypes was 94.10%. Table (6) also revealed that the total number of polymorphic amplicons obtained by the 6 studied primers was 51. This corresponds to a level of polymorphism of 72.86% and an average number of polymorphic fragments/ primer of 8.5 (Table 6). Microsatellites have much higher polymorphism and are considered to be more appropriate molecular tools for studying genetic biodiversity and relationships.

The specific markers for quail genotypes generated from ISSRs-PCR analysis are shown in Tables (4 and 5). As shown in Table (5), the highest number of ISSRs-PCR specific markers was scored for female (1) brown (8 markers), while the lowest (one marker) was scored for females, brown (2 and 3), female white (8), male white (9), female white (6) and male brown (5). A number of 8 positive specific markers were scored for the presence of unique bands for a given genotype, while 12 negative specific markers

was scored for the absence of a common band. In the meantime, the highest number of ISSRs-PCR genotype-specific markers was generated by primer HB-10 (8 markers) and primer HB-13 generated (4 markers). On the other hand, the primers 44B, HB-09, HB-14 and HB-15 generated 2 markers. ISSRs marker of individual quail showed characteristic pattern and individuals could be distinguishable from each other even within the same color. This has demonstrated the efficiency of the ISSRs as a potential genetic marker.

Phylogenetic relationship among quail genotypes based on ISSRs marker

Based on the combined data obtained through the polymorphism of ISSRs profiles, the similarity coefficient values among the studied 10 individuals of quail (brown and white, males and females) were calculated according to Dice (1945) equation (Table 8). The similarity coefficient between the females and males of the 10 quail individuals (brown and white) shows an average genetic distance ranging from 0.00-1.00 with a mean value of 0.50. The highest similarity index (1.00) was recorded between white female (7) and brown female (1). However, the lowest similarity index (0.00) was observed between white female (7) and white female (6). The data obtained from the analysis of ISSRs was used to draw precise relationships among the 10 tested individuals. The resultant dendrogram is shown in Fig. (2) using UPGMA analysis. This dendrogram clustered the quail genotypes into two clusters (groups). The first

group consisted of brown female (1) was delimited in separate one cluster from the rest of studied quail genotypes. The second group was subdivided into two subgroups. The first subgroup subdivided into two subgroups, the first subgroup involved white male (10) was separated from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup includes white female (6) and white female (7) were separated in one sub-group from the rest of genotypes, and the second subgroup was included white female (8) and white male (9) were separated in one subgroup. The second subgroup was subdivided into two subgroups, the first subgroup involved brown female (2) was separated from the rest of genotypes, and the other subgroup includes brown female (3) and brown male (4). The second subgroup includes brown male (5) was delimited in separate group.

Based on ISSRs analysis, the white color female and male, and brown color females and males were clustered in the same group while, brown female was delimited in separate one cluster.

Phylogenetic relationship among quail genotypes based on RAPD and ISSRs markers

Both RAPD and ISSRs are based on different strategies for exploring genetic diversity. While RAPD primers randomly target complementary and homologous genomic regions in the genome, ISSRs primers amplify the highly repetitive inter-simple sequence repeats of the

microsatellite regions. The combination of both techniques will enhance the screening of diversity between and within genotypes. Based on the combined data obtained through the polymorphism of RAPD and ISSRs profiles, the similarity coefficient values among the studied 10 individuals genotypes (brown and white, males and females) were calculated according to Dice (1945) equation (Table 9). The similarity coefficient shows an average genetic distance ranging from 0.00-1.00 with a mean value of 0.50. The highest similarity index (1.00) was recorded between white female (6) and brown female (1). However, the lowest similarity index (0.00) was observed between white female (7) and white female (6). The data obtained from the analysis of RAPD and ISSRs was used to draw precise relationships among the 10 tested quail genotypes. The resultant dendrogram is shown in Fig. (3) using UPGMA analysis. This dendrogram clustered the quail genotypes into two clusters (groups). The first group consisted of brown female (1) and brown female (2) were delimited in separate one cluster from the rest of studied quail genotypes. The second group was subdivided into two subgroups. The first subgroup involved brown female (3) and brown male (4) were separated from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup includes brown male (5) was delimited in separate one cluster from the rest of studied quail genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup involved white male (10) was delimited in separate

one cluster from the rest of studied quail genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup includes white female (6) and white female (7). The second subgroup includes white female (8) and white male (9) was delimited in separate group.

Based on RAPD and ISSRs combination analysis, the brown color females were clustered in the same group, and female and male white, and brown male and brown color female were in separate clusters.

The different types of markers, RAPD and ISSRs revealed different levels of genetic similarity among the 10 individuals quail. This could be due to the difference in the polymorphism detection mechanisms by the different types of markers. DNA sequence variation at primer binding sites and DNA length differences between primer binding sites produce the RAPD polymorphisms. ISSR polymorphism is the result of differences in the number of repetitive di-tri- or tetra-nucleotide units. Therefore, combining the data obtained from the different types of markers may reveal more informative genetic relationships.

In conclusion, this result seems to be reliable since it goes with the expectation of clustering males and females in the same genotype in one group. Gathering both brown and white color genotypes in one cluster even though, brown females delimited in separate group. They might share some genes between brown and white genotypes through mutation in

brown color genotype. The brown color genotype was the original color from which the white color genotype had segregated. RAPD and ISSRs techniques would be used for identification of male and female quail birds. Six primers with each type of marker used in the present study allowed enough distinction among the quail genotypes.

The result of molecular genetic analysis used in the present study is in agreement with the result of productive performance. The productive performance and molecular genetic analysis used in the present study successfully distinguished between the two genotypes of quail, brown and white colures, males and females.

SUMMARY

The aims of the present study were to characterize the possible genetic and productive traits differences associated with the plumage colour in two genotypes of Japanese quail. Productive performance and DNA markers were used to identify these genotypes. Genotype effect showed that the brown genotype had significantly heavier body weight (BW) at 7, 14 and 21 days of age and longer shank length (SL) at all studied ages, except for 1 and 14 days than the white genotype. Sex effect on BW and SL was significant at all studied ages, except for one day old, females had significantly higher BW and SL than males. The interaction effect between genotype and sex on BW was significant at all studied ages except for one day old. The interaction effect between

genotype and sex on SL was significant at all studied ages, except for 1 and 14 days. Brown genotype had significantly heavier BW at first egg than the white genotype. The white genotype had significantly higher shape index than brown genotype. The brown genotype matured at earlier age ($P \leq 0.05$) than the white genotype and had shorter days ($P \leq 0.05$) to produce the first 30 eggs, and had shorter days ($P > 0.05$) to produce the first 10 eggs than the brown genotype. Brown genotype laid significantly more number of eggs during the first, the second and the first two months than the white genotype and had significant higher egg mass during the different study periods. From the present results, it can be concluded that brown genotype had favored growth traits and most of egg production related traits during annual egg production.

The level of polymorphism among two Japanese quail genotypes brown and white, was estimated using two PCR-based marker techniques RAPD and ISSRs. Six RAPD and six ISSRs primers were employed to find out genetic variations and relationships among these genotypes of quail. RAPD and ISSRs analysis generated a total number of 442 and 467 amplicons representing a level of polymorphism of 74.24% and 72.86%, and an average number of polymorphic fragments/ primer of 8.17 and 8.5, respectively. The genetic relationships among the 10 individuals of quail were estimated in terms of similarity using Dice coefficients. The genetic similarity ranged from 0.00-1.00 for RAPD, ISSRs, and RAPD and

ISSRs combination. The inter-genotype relationships among the two quail genotypes based on RAPD, ISSRs, and RAPD and ISSRs combination revealed the highest genetic similarity between the genotype brown male (5) and brown female (2), white female (7) and brown female (1), and white female (6) and brown female (1), respectively. The inter-genotype relationships among the two quail genotypes based on RAPD, ISSRs, and RAPD and ISSRs combination revealed the lowest genetic similarity between the genotype brown female (2) and brown female (1), white female (7) and white female (6), and white female (7) and white female (6), respectively. The RAPD based dendrogram clustered the brown genotype female and male, and white genotype females and males in the same group while, brown female and brown male genotypes were in separate clusters. The ISSRs based dendrogram clustered the white genotypes female and male, and brown genotypes females and males in the same group while, brown female was delimited in separate one cluster. The RAPD and ISSRs combination based dendrogram clustered the brown genotype females in the same group, and female and male white genotypes, and brown male and brown female genotypes in separate clusters. However, the reshuffling in the position of the brown and white genotypes belonging to the individuals in the different dendrograms revealed that they share common genetic background. They might share some genes between brown and white genotypes through mutation in brown color genotype. Moreover, each of

the RAPD and ISSRs was successful in identifying genotype-specific markers characterizing 10 individuals of quail. The productive performance and molecular genetic analysis used in the present study successfully distinguished between the two genotypes of quail, brown and white colures, males and females.

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Table (1): The nucleotide sequences of 6 primers used for RAPD-PCR and 6 primers used for ISSRs-PCR analyses.

RAPD Marker		ISSRs Marker	
Primer code	Sequence (5'-3')	Primer code	Sequence (5'-3')
OPA-10	GTG ATC GCA G	44 B	CTC TCT CTC TCT CTC TG
OPA-18	AGG TGA CCG T	HB-09	GTG TGT GTG TGT GG
OPB-02	TGA TCC CTG G	HB-10	GAG AGA GAG AGA CC
OPB-10	CTG CTG GGA C	HB-13	GAG GAG GAG GC
OPE-06	AGA TGC AGC C	HB-14	CTC CTC CTC GC
OPE-19	ACG GCG TAT G	HB-15	GTG GTG GTG GC

Table (2): Means and standard errors for genotype, sex and genotype by sex interaction effects on body weight and shank length at different ages of Japanese quail

Item	Genotype effect		Sex effect		Genotype by sex interaction effect			
	Brown	White	Males	Females	Brown		White	
					Males	Females	Males	Females
BW ₁	7.95±0.20	8.36±0.19	8.11±0.19	8.20±0.20	8.09±0.28	7.81±0.30	8.13±0.27	8.95±0.28
BW ₇	26.92 ^a ±1.25	23.05 ^b ±1.19	21.98 ^b ±1.19	27.99 ^a ±1.25	21.66 ^b ±1.72	32.18 ^a ±1.82	22.30 ^b ±1.74	23.80 ^b ±1.62
BW ₁₄	48.26 ^a ±2.05	42.77 ^b ±2.00	41.46 ^b ±2.00	49.57 ^a ±2.05	41.25 ^b ±2.82	55.26 ^a ±2.97	41.68 ^b ±2.82	43.87 ^b ±2.82
BW ₂₁	79.34 ^a ±2.50	66.57 ^b ±2.38	66.70 ^b ±2.38	79.21 ^a ±2.50	73.96 ^b ±3.45	84.73 ^a ±3.63	59.45 ^c ±3.29	73.69 ^b ±3.45
BW ₂₈	100.40±3.03	99.67±2.88	90.23 ^b ±2.88	109.84 ^a ±3.03	95.4 ^{bc} ±4.17	105.39 ^{ab} ±4.40	85.04 ^c ±4.17	114.30 ^a ±4.17
BW ₃₅	131.94±3.75	122.32±3.56	112.84 ^b ±3.56	141.42 ^a ±3.75	122.47 ^b ±5.16	141.44 ^a ±5.44	103.20 ^c ±4.92	141.43 ^a ±5.16
SL ₁	17.72±0.20	17.53±0.19	17.56±0.19	17.57±0.20	17.71±0.28	17.73±0.29	17.64±0.26	17.42±0.28
SL ₇	25.15 ^a ±0.40	23.82 ^b ±0.38	23.47 ^b ±0.38	25.50 ^a ±0.40	23.52 ^b ±0.55	26.88 ^a ±0.58	23.42 ^b ±0.52	24.11 ^b ±0.55
SL ₁₄	29.49±0.43	28.85±0.42	28.26 ^b ±0.42	30.07 ^a ±0.43	28.27±0.59	30.70±0.63	28.25±0.59	29.44±0.59
SL ₂₁	35.10 ^a ±0.51	33.25 ^b ±0.48	33.25 ^b ±0.48	35.38 ^a ±0.51	33.97 ^b ±0.70	36.22 ^a ±0.74	32.54 ^b ±0.67	34.54 ^{ab} ±0.70
SL ₂₈	38.42 ^a ±0.41	37.17 ^b ±0.43	36.89 ^b ±0.40	38.70 ^a ±0.40	38.04 ^a ±0.57	38.80 ^a ±0.60	35.74 ^b ±0.57	38.60 ^a ±0.19
SL ₃₅	40.03 ^a ±0.46	38.51 ^b ±0.44	38.47 ^b ±0.44	40.07 ^a ±0.46	39.76 ^a ±0.64	40.30 ^a ±0.67	37.18 ^b ±0.61	39.85 ^a ±0.64

BW₁-BW₃₅=Body weight at 1-35 days of age, SL₁-SL₃₅=Shank length at 1-35 days of age and ^{a, b and c}= Means within the same effect with different letters are significantly differed.

Table (3): Comparisons between brown and white genotypes of Japanese quail for egg production-related traits (mean \pm SE).

Item	Genotypes	
	Brown	White
Body weight at first egg (gram)	240.81 ^a \pm 7.95	214.67 ^b \pm 7.54
Shank length at first egg (mm)	40.48 \pm 0.50	40.87 \pm 0.48
Age at first egg (day)	56.24 ^b \pm 2.28	62.30 ^a \pm 2.16
First egg shape index %	77.42 ^b \pm 1.04	80.92 ^a \pm 0.99
First egg weight (gram)	11.08 \pm 0.31	11.44 \pm 0.29
Number of days needed to produce the first 10 eggs (day)	15.00 \pm 0.78	13.80 \pm 0.74
Number of days needed to produce the first 30 eggs (day)	40.33 ^b \pm 2.41	48.80 ^a \pm 2.29
Number of eggs produced in the first month (egg)	24.11 ^a \pm 1.14	20.80 ^b \pm 1.08
Number of egg produced in the second month (egg)	23.33 ^a \pm 1.70	18.40 ^b \pm 1.61
Number of egg produced in the first two months (egg)	47.44 ^a \pm 2.49	39.20 ^b \pm 2.36
Egg mass of the first 10 eggs (gram)	113.00 \pm 1.74	114.46 \pm 1.65
Egg mass of the first 30 eggs (gram)	350.11 \pm 5.76	350.18 \pm 5.46
Egg mass of the first month (gram)	295.11 ^a \pm 15.06	243.63 ^b \pm 14.29
Egg mass of the second month (gram)	285.33 ^a \pm 18.99	218.67 ^b \pm 18.01
Egg mass of the first two months (gram)	580.44 ^a \pm 28.54	462.30 ^b \pm 27.08

Table (4): Cont.'

Primer	Band No.	BP	1	2	3	4	5	6	7	8	9	10	Primer	Band No.	BP	1	2	3	4	5	6	7	8	9	10	
OP-E06	1	1030	0	0	1	1	1	1	1	1	1	1	HB-14	1	1240	1	1	1	1	1	1	1	1	1	1	
	2	920	0	1	1	1	1	1	1	1	1	1		2	890	0	0	0	1	1	0	0	0	1	1	
	3	785	1	1	1	1	1	1	1	1	1	1		3	830	1	1	1	0	0	0	0	0	0	0	0
	4	635	0	0	0	0	0	0	1	1	0	0		4	760	0	0	0	0	1	1	1	1	1	1	1
	5	620	1	1	1	1	1	1	1	1	1	1		5	620	0	0	0	0	1	1	1	1	1	1	0
	6	580	1	1	1	1	1	1	1	1	1	0		6	540	1	1	1	1	1	1	1	0	1	1	1
	7	490	1	1	1	1	1	1	1	1	1	1		7	485	0	0	0	0	0	1	1	1	1	1	0
	8	435	0	0	0	0	1	1	0	0	0	0		8	410	0	0	0	0	0	1	1	1	1	1	0
	9	350	1	1	1	1	0	0	0	0	0	0		9	320	1	0	1	1	1	1	1	1	1	1	1
	10	290	1	1	1	1	1	1	1	1	1	1		10	250	0	0	0	0	0	0	1	0	1	0	0
	11	250	1	0	1	1	1	1	1	1	1	1														
	12	220	1	1	0	0	0	1	1	0	0	1														
OP-E19	1	1280	0	0	0	0	0	1	1	0	0	0	HB-15	1	1680	0	0	0	0	0	0	0	0	1	0	
	2	1160	0	0	1	1	0	0	0	0	0	0		2	1290	1	0	1	1	1	1	1	1	1	0	
	3	950	0	0	0	0	0	1	1	1	1	1		3	1000	1	1	1	1	1	1	1	1	1	1	
	4	880	1	1	1	1	1	1	1	1	1	1		4	980	1	1	1	1	1	1	1	1	1	1	
	5	650	0	0	0	0	0	1	1	1	1	1		5	830	1	1	1	1	1	1	1	1	1	1	
	6	600	1	1	1	1	0	1	1	1	1	1		6	800	1	1	1	1	1	1	1	1	1	0	
	7	580	1	1	1	1	1	1	1	1	1	1		7	760	1	1	1	1	1	1	1	1	1	1	
	8	540	1	1	1	1	0	0	0	0	0	0		8	540	1	1	1	1	1	1	1	1	1	1	
	9	420	1	1	1	1	1	1	1	1	1	1		9	410	0	1	1	1	0	0	0	0	1	0	
	10	400	1	1	1	1	1	1	1	1	1	1		10	345	1	1	1	1	1	1	1	1	1	1	
	11	350	1	1	1	1	1	1	1	1	1	1		11	275	1	1	1	1	0	0	0	1	1	1	
	12	305	1	1	1	1	1	0	1	1	1	1														
	13	260	0	0	0	0	1	0	0	0	0	0														
	14	200	0	0	0	0	1	0	1	1	0	1														

1, 2, 3=Females brown, 4, 5=Males brown, and 6, 7, 8=Females white, and 9, 10=Males white

Table (5): Number of amplified fragments markers of two quail genotypes brown and white based on RAPD and ISSRs-PCR analyses.

Genotypes		RAPD primers							ISSRs primers						
		OPA-10	OPA-18	OPB-02	OPB-10	OPE-06	OPE-19	Total	44B	HB-09	HB-10	HB-13	HB-14	HB-15	Total
1 Female brown	AF	5	8	5	6	8	8	40	4	6	10	9	4	9	42
	SM	0	1	0	0	1	0	2	0	1	8	0	0	0	9
2 Female brown	AF	6	7	4	6	8	8	39	4	7	8	9	3	9	40
	SM	0	1	0	0	1	0	2	0	0	0	0	1	0	1
3 Female brown	AF	8	9	4	6	9	9	45	7	8	8	7	4	10	44
	SM	0	0	0	0	0	0	0	1	0	0	0	0	0	1
4 Male brown	AF	7	8	6	6	9	9	45	7	9	7	8	4	10	45
	SM	1	0	0	0	0	0	1	0	0	0	2	0	0	2
5 Male brown	AF	5	4	7	7	9	8	40	8	8	9	8	6	8	47
	SM	0	1	1	1	0	2	5	0	0	0	0	0	0	0
6 Female white	AF	7	7	8	6	10	9	47	8	7	10	8	7	8	48
	SM	0	0	0	0	0	1	1	0	0	0	0	0	0	0
7 Female white	AF	5	7	8	5	10	11	46	9	6	10	9	7	8	49
	SM	0	0	0	1	0	0	1	1	1	0	0	1	0	3
8 Female white	AF	5	9	7	7	9	10	47	8	8	11	9	7	9	52
	SM	0	0	1	0	0	0	1	0	0	0	1	0	0	1
9 Male white	AF	8	9	7	7	7	9	47	8	7	11	8	9	11	54
	SM	0	0	0	0	1	0	1	0	0	0	0	0	1	1
10 Male white	AF	6	9	7	5	9	10	46	7	7	11	90	5	7	46
	SM	0	0	0	2	0	0	2	0	0	0	1	0	1	2
PB		6	8	7	2	3	6	32	5	4	4	7	7	3	30
TAF		62	77	63	61	88	91	442	70	73	95	84	56	89	467
TSM		1	3	2	4	3	3	16	2	2	8	4	2	2	20
MB		3	1	1	3	4	5	17	2	4	3	3	1	6	19

AF=Amplified fragments, SM=Marker including either the presence or absence of a band in quail genotypes brown and white, PB=Polymorphic bands, TAF=Total number of amplified fragments, TSM=Total number of specific markers across brown and white genotypes and MB=Monomorphic bands.

Table (6): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphisms as revealed by RAPD and ISSRs markers among the brown and white genotypes.

Marker	Primer	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	Polymorphism %
RAPD	OPA-10	10	3	7	70.00
	OPA-18	12	1	11	91.67
	OPB-02	10	1	9	90.00
	OPB-10	8	3	5	62.50
	OPE-06	12	4	8	66.67
	OPE-19	14	5	9	64.28
	Total	66	17	49	74.24
	Average	11	2.8	8.17	74.27
ISSRs	44B	9	2	7	77.78
	HB-09	11	4	7	63.64
	HB-10	15	3	12	80.00
	HB-13	14	3	11	78.57
	HB-14	10	1	9	90.00
	HB-15	11	6	5	45.45
	Total	70	19	51	72.86
	Average	11.67	3.17	8.5	72.84

Table (7): Similarity matrix for the 10 individuals of Japanese quail on the basis of RAPD-PCR analysis.

Genotypes	1 Female brown	2 Female brown	3 Female brown	4 Male brown	5 Male brown	6 Female white	7 Female white	8 Female white	9 Male white
2 Female brown	0.00								
3 Female brown	0.38	0.44							
4 Male brown	0.48	0.65	0.001						
5 Male brown	0.92	1.00	0.79	0.69					
6 Female white	0.67	0.73	0.56	0.47	0.46				
7 Female white	0.73	0.90	0.62	0.43	0.42	0.03			
8 Female white	0.77	0.83	0.56	0.56	0.67	0.26	0.13		
9 Male white	0.67	0.63	0.37	0.37	0.57	0.17	0.31	0.17	
10 Male white	0.73	0.90	0.52	0.43	0.63	0.43	0.27	0.27	0.22

Table (8): Similarity matrix for the 10 individuals of Japanese quail on the basis of ISSRs-PCR analysis.

Genotypes	1 Female brown	2 Female brown	3 Female brown	4 Male brown	5 Male brown	6 Female white	7 Female white	8 Female white	9 Male white
2 Female brown	0.31								
3 Female brown	0.65	0.20							
4 Male brown	0.78	0.33	0.03						
5 Male brown	0.57	0.32	0.11	0.06					
6 Female white	0.79	0.54	0.32	0.53	0.09				
7 Female white	1.00	0.85	0.62	0.74	0.38	0.00			
8 Female white	0.74	0.59	0.46	0.58	0.23	0.02	0.14		
9 Male white	0.80	0.57	0.36	0.40	0.14	0.02	0.21	0.06	
10 Male white	0.90	0.65	0.43	0.56	0.27	0.22	0.51	0.02	0.11

Table (9): Similarity matrix for the 10 individuals of Japanese quail on the basis of RAPD and ISSRs-PCR analyses.

Genotypes	1 Female brown	2 Female brown	3 Female brown	4 Male brown	5 Male brown	6 Female white	7 Female white	8 Female white	9 Male white
2 Female brown	0.17								
3 Female brown	0.59	0.35							
4 Male brown	0.83	0.54	0.01						
5 Male brown	0.83	0.71	0.48	0.39					
6 Female white	1.00	0.71	0.49	0.56	0.28				
7 Female white	0.86	0.99	0.70	0.67	0.45	0.00			
8 Female white	0.84	0.80	0.57	0.64	0.48	0.14	0.14		
9 Male white	0.94	0.87	0.41	0.43	0.36	0.08	0.28	0.28	
10 Male white	0.75	0.88	0.53	0.50	0.49	0.34	0.34	0.45	0.27

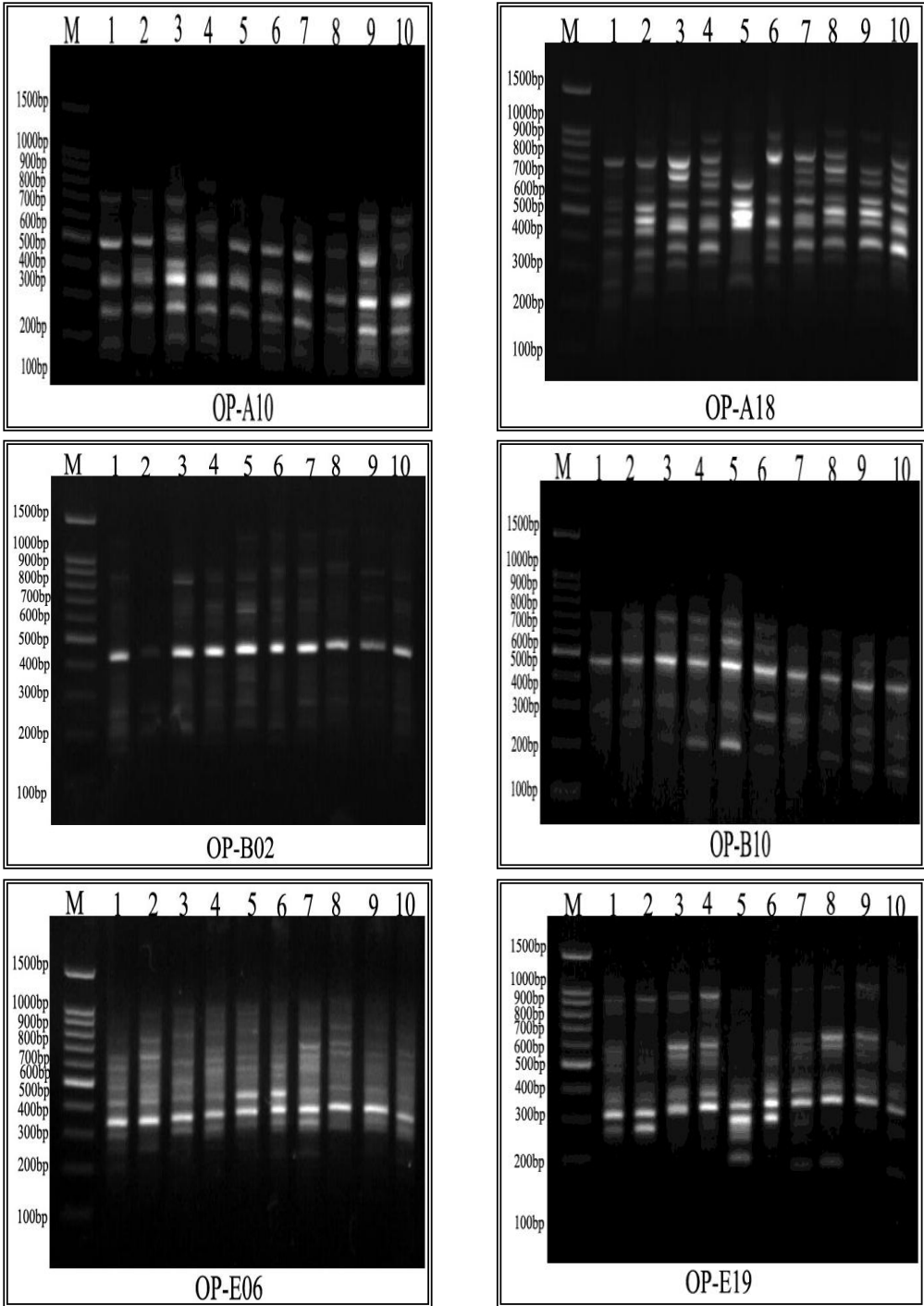


Plate (1): RAPD profile of the brown (B) and white (W) Japanese quail genotypes amplified with 6 different RAPD primers. M = Ladder marker, 1, 2 and 3 = Females B, 4 and 5 = Males B, 6, 7 and 8 = Females W and 9 and 10 = males W.

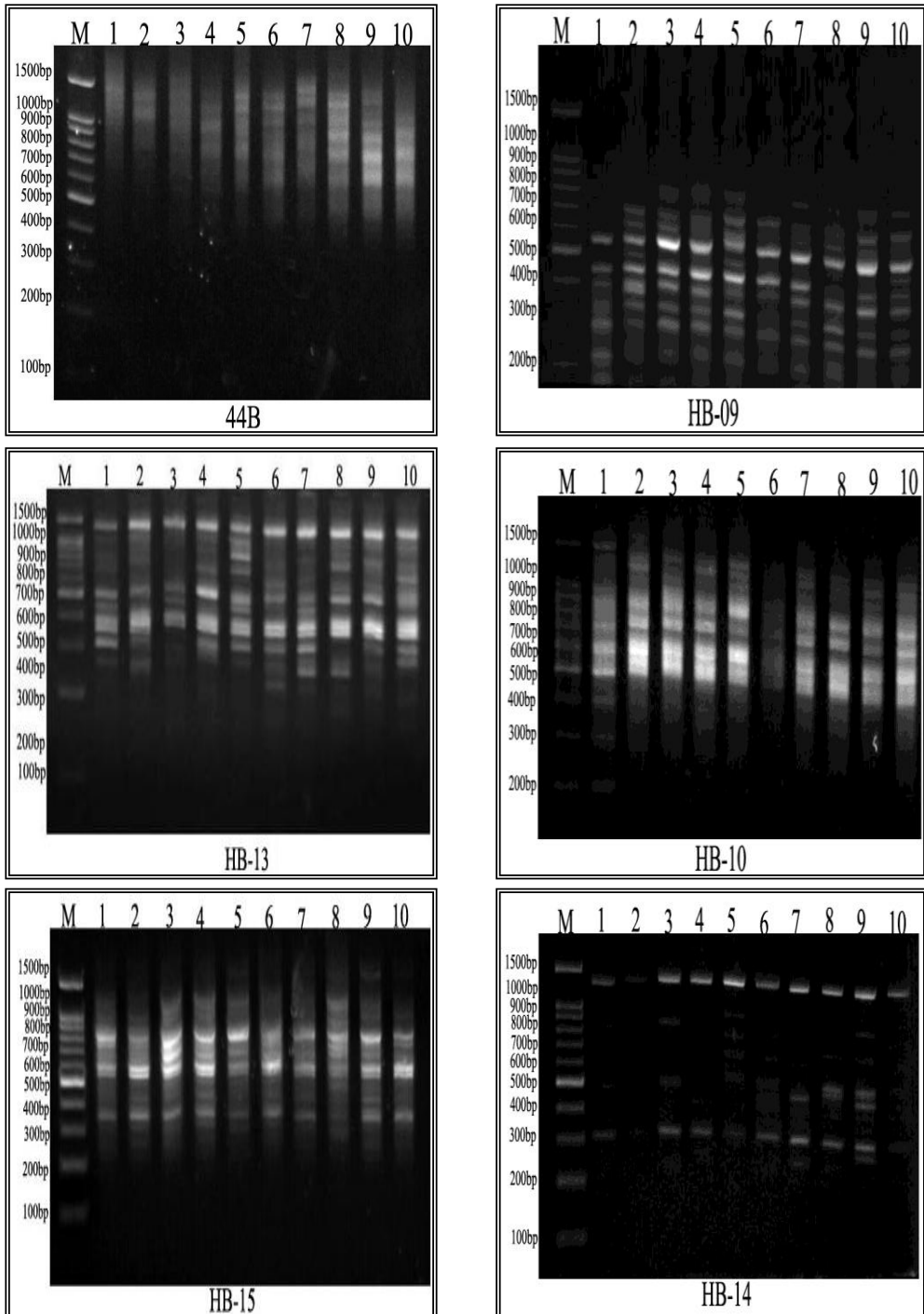


Plate (2): ISSRs profile of the brown (B) and white (W) Japanese quail genotypes amplified with 6 different ISSRs primers. M = Ladder marker, 1, 2 and 3 = Females B, 4 and 5 = Males B, 6, 7 and 8 = Females W and 9 and 10 = Males W.

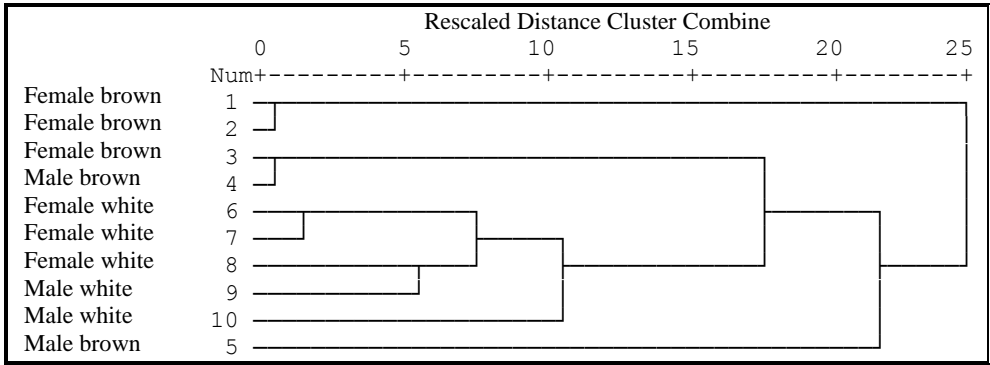


Fig. (1): Dendrogram for the 10 individual quails constructed from the RAPDs data using UPGMA and similarity matrix computed according to Dice coefficient.

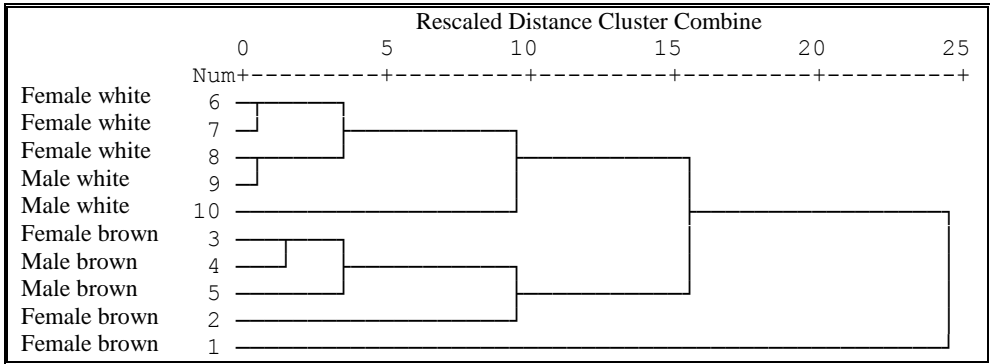


Fig. (2): Dendrogram for the 10 individual quails constructed from the ISSRs data using UPGMA and similarity matrix computed according to Dice coefficient.

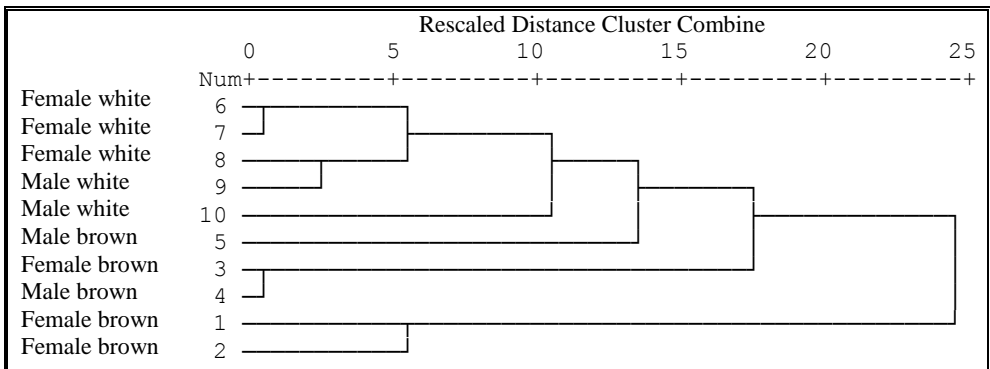


Fig. (3): Dendrogram for the 10 individual quails constructed from the RAPDs and ISSRs data using UPGMA and similarity matrix computed according to Dice coefficient.