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**PRODUCTIVE PERFORMANCE AND MOLECULAR GENETIC
CHARACTERIZATION OF MATERNAL SELECTED AND
RANDOMBRED LINES IN THE 6th GENERATION OF JAPANESE
QUAIL**

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ABSTRACT: The aims of the present study were to characterize the possible genetic and productive performance differences associated with the maternal selected line for six generations in Japanese quail compared to the randombred line. Body weight (BW) and shank length (SL) were recorded weekly until 5 weeks of age. BW and SL at first egg, age at first egg (AFE), first egg weight (FEW), number of days needed to produce the first 10 eggs (DN₁₀), egg weight of the tenth egg (EW₁₀) and egg mass of the first 10 eggs (EM₁₀) were individually recorded for each female. The level of polymorphism between and within maternal line and a randombred line was estimated using two PCR-based DNA marker techniques RAPD and ISSR. Each line represented by 3 females and 2 males. Six RAPD and six ISSR primers were employed to find out genetic variations and relationships among these genotypes. The results indicated that line significantly affected BW at 35 days and AFE favoring the maternal line. However, the two lines insignificantly differed for BW at 1, 7, 14, 21 and 28 days of age. Females had higher insignificant BW than males at all studied ages. Line significantly affected SL at 21, 28 and 35 days of age favoring the maternal line. However, the two lines insignificantly differed for SL at 1, 7, 14 days of age and AFE. Females had higher SL than males at all studied ages, except for 14 days of age ($P < 0.05$). Line had significant effect on AFE, maternal line attained sexual maturity at earlier age than the randombred line by 16.50 days. Maternal line had shorter DN₁₀ ($P \leq 0.05$) by 4.10 days than the randombred line. The females of the maternal line laid insignificantly higher FEW, EW₁₀ and EM₁₀ than the females of the randombred line.

Key Words: Quail; Productive performance; RAPD analysis; ISSR analysis.

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The RAPD and ISSR analyses generated a total number of 411 and 384 amplicons representing a level of polymorphism of 65.385% and 50%, and an average number of polymorphic fragments/primer of 5.667 and 4, respectively. The genetic similarity ranged from 0-1 for RAPD, ISSR, and RAPD and ISSR combination. The inter-genotyperelationships between and within the two lines based on RAPD, ISSR, and RAPD and ISSR combination revealed the highest genetic similarity between male randombred line and female maternal line, female randombred line and female maternal line, and male randombred line and female maternal line, respectively. And also revealed the lowest genetic similarity between female and female maternal line, male and female maternal line, and female and female randombred line, female and female maternal line, male and male maternal line, and female and female maternal line, respectively. The RAPD based dendrogram delimited the randombred line genotype male in the same group while, randombred line male, maternal line males, randombred line females and maternal line females were in separate clusters. The ISSR based dendrogram delimited the randombred line genotype female in separate group while, randombred line females, males, maternal line females and males were delimited in separate one cluster. The RAPD and ISSR combination based dendrogram clustered the randombred line males and female in the same group, and randombred line females, maternal line females and males genotypes in separate clusters. However, the reshuffling in the position of the maternal selected and randombred lines belonging to the individuals in the different dendrograms revealed that they share common genetic background. They might share some genes between maternal line for six generations and randombred line through selection in selected line. Moreover, each of the RAPD and ISSR was successful in identifying genotype-specific markers characterizing genotypes. The performance and molecular genetics analyses used in the present study successfully distinguished between and within the two lines and sexes.

INTRODUCTION

Japanese quail had a great impact on research field due to fast growth rate and better egg production (Minvielle, 1998), high rate of lay and much lower feed and space than domestic fowl (Adeogun and Adeoye, 2004), its unique properties of easy maintenance and low generation interval (Tarhyela et al., 2012). Thus, many countries developed quail farming into an industry accompanied by a strong interest in scientific research. Studies using Japanese quails in breeding experiments have demonstrated that this species offers scientist several advantages in exploring breeding systems and certain applied problems of poultry breeding (Okuda et al.,

2014). Selection index provides a systematic means for making selection decisions that are consistent with improved profitability. This technology permits us to exploit information on relatives and to use correlated traits to improve accuracy. Japanese quail and chicken have similar karyotypes of $2n=78$ chromosomes consisting of morphologically distinct macrochromosomes (1-8 and the ZW sex chromosomes) and cytological indistinguishable microchromosomes (Shibusawa et al., 2001). Knowledge of the relatedness between animals is very important in extended breeding programs that prevent the loss of genetic variation (Frankham et al., 2002). Conservation

genetics for preservation of species has received increasing attention (Frankham, 2003; Allendorf and Luikart, 2007). The egg layer industry is particularly challenged as most of the traits of interest are measurable only in mature females. Both males and females have the gene variants for better egg production. Molecular genetics allows direct selection to be done in males even for females limited traits. Selection based on DNA can be done at a much earlier age in the lifecycle of the chicken.

It seems that the knowledge about the genetic characterisation of Japanese quail lines and amount of genetic diversity among them is minimal. Hence, studies are needed to characterise Japanese quail lines to estimate the genetic variability for selection and breeding programs. Molecular genetics providing the tool box of the 21st century for the modern poultry breeding and enhanced selection progress. Allow for rapid and accurate identification and selection at the gene level of individuals with better performance (Fulton, 2008). Genetic markers are short DNA sequences which have slight differences between individuals. A polymorphism assay, based on the amplification of random DNA fragments using an arbitrary nucleotide sequenced primer has been developed by Williams et al. (1990). RAPD marker was found to be effective to detect polymorphism and genetic diversity in quail lines (Kumar et al., 2000; Karabağ and Balcioglu, 2010). Mansour et al. (2010) investigated the variations within four phenotypes of quail using RAPD and ISSR markers. RAPD was used to estimate genetic structures about chicken (Pipalia et al., 2006). Microsatellites have been reported in the literature for Japanese quails (Kayang et al., 2002; Guobin et al., 2006). MS map

was constructed in the Japanese quail (Kayang et al., 2004), and map was applied (Minvielle et al., 2005). MS markers have been carried out to assess genetic diversity in various populations of chickens (Tadano et al., 2007).

The aims of the current study were to identify the possible genetic variations and relationships of maternal selected and randombred lines in the 6th generation of selection, based on performance and DNA markers. The results would contribute to the appropriate managements avoiding loss of genetic variability in these lines and to future improvements.

MATERIALS AND METHODS

Flock history:

The experimental work was carried out at the Poultry Research Center, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. The birds used in this experiment were developed as follows: A maternal selected line was developed from the base population during the course of breeding experiments with Japanese quail. Selection index was applied to select females according to the aggregate breeding values of age at first egg, body weight at first egg and days needed to produce the first 10 eggs and the males kept as breeders were selected based on the performance of their females sibs for six successive generations with selection pressure of 19%. Randombred line which maintained as non-selected pedigreed population originated from the base population which the maternal selected line originated. In randombred line, all eggs laid by the two females of each family were used to obtain the parents for the next generation. Feed and water were provided ad libitum. All experimental birds were maintained as possible under the same conditions.

The following traits were estimated:

Body weight (BW) and shank length (SL) were recorded weekly until 5 weeks of age. BW and SL at first egg, age at first egg (AFE), first egg weight (FEW), number of days needed to produce the first 10 eggs (DN₁₀), egg weight of the tenth egg (EW₁₀) and egg mass of the first 10 eggs (EM₁₀) were individually recorded for each female.

Statistical analyses:

Data were subjected to analysis of variance using the General Linear Model Procedure of SPSS (SPSS, 2008). The following model was used for the growth traits to determine the effect of line and sex, $Y_{ijk} = \mu + L_i + S_j + e_{ijk}$, where Y_{ijk} =observed value in the i^{th} line in the j^{th} sex of the k^{th} individual, μ =overall mean, L_i =line effect (i =maternal selected and randombred lines), S_j =sex effect (j =males and females) 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 line effect. The statistical model used was as follows, $Y_{ij} = \mu + L_i + e_{ij}$, where Y_{ij} =observed value in the i^{th} line of the j^{th} individual, μ =overall mean, L_i =line effect (i = maternal selected and randombred lines) and e_{ij} =random error term.

Extraction of DNA

Individual blood samples were collected from 10 birds (3 females and 2 males)/line (maternal selected and randombred lines). All bird samples were phenotypically normal and healthy. Blood samples were collected from the brachial vein of each individual bird 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 blood samples from each

selected individual as described by Z10 spin column DNA Minipreps Kit (Bio basic INC.). Six and six random DNA oligonucleotide primers synthesized by Operon biotechnologies, Inc., Germany were independently used in the RAPD and ISSR-PCR reaction mixture. Table (1) lists the base sequences of these primers that produced informative polymorphic bands.

RAPD and ISSR-PCR reactions

RAPD-PCR amplification reactions were performed in a 25 μ l reaction volumes. RAPD reaction mixture containing 2.5 μ l dNTPs (2.5 mM), 1.5 μ l MgCl₂ (25 mM), 2.5 μ l 10x buffer, 2.0 μ l primer (2.5 μ M), 2.0 μ l template DNA (50 ng/ μ l), 0.3 μ l Taq DNA polymerase (5 U/ μ l) and 14.7 μ l 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 ISSR-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 polymorphism among genotypes and sexes by line tested in this study. After electrophoresis, the RAPD and ISSR patterns were visualized with UV Tec. Documentation system. Fragments sizes were estimated using ladder marker cover a wide range (100-1500 bp).

Molecular genetic analysis:

The DNA bands generated by each primer were counted and their molecular sizes were 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.

RESULTS AND DISCUSSION

Line and sex effects on BW and SL at different ages:

The results of the effects of line and sex on BW are illustrated in Fig. (1) and (2). Line significantly affected BW at 35 days and AFE favoring the maternal line. However, the two lines insignificantly differed for BW at 1, 7, 14, 21 and 28 days of age (Fig. 1). Similarly, line had significant influences on the BW at 1, 7, 21 and 28 days of age and insignificant at 14 days was reported by Baylan et al. (2009). Higher significant differences on BW in selected birds than the control at different ages were reported by Khaldari et al. (2010), Varkoohi et al. (2010) and Hussain et al. (2013). Also, in other some studies (Jatoi et al., 2013 and Akram et al., 2014) significant differences in BW among different strains of Japanese quail was observed. Females had higher insignificant

BW than males at all studied ages as shown in Fig. (2). Line had significant influence on the BW at first egg was reported by Sakunthaladevi et al. (2011). Lower values for BW at first egg were reported by Okenyi et al. (2013) and Momoh et al. (2014). Line significantly affected SL at 21, 28 and 35 days of age favoring the maternal line. However, the two lines insignificantly differed for SL at 1, 7, 14 days of age and age at first egg (Fig. 3). Females had higher SL than males at all studied ages, except for 14 days of age ($P > 0.05$) as shown in Fig. (4). 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 reported by Ojo et al. (2014). Sex differences for BW and SL were previously reported in favor of the females was cited by Nath et al. (2011), Daikwo et al. (2013) and Ojo et al. (2014).

Line effect on egg production-related traits:

Line had a significant effect on AFE, maternal line attained sexual maturity at earlier age than the randombred line by 16.50 days (Fig. 5). Similar values were reported for AFE as 55.3 days (Sakunthaladevi et al., 2011), 57.5 days (Punya et al., 2012) and 54.49 days (Momoh et al. 2014). Lower estimates were reported by Nath et al. (2011) as 51.16 days and Okuda et al. (2014) as 43.97 days. Maternal line had shorter DN_{10} ($P \leq 0.05$) by 4.10 days than the randombred line (Fig. 5). Similarly, Tawefeuk (2001) reported that there was a decrease in DN_{10} in the selected line for age at sexual maturity compare to the control line in the same generation. Mahmoud et al. (2014) reported an estimate of 15.95 days for DN_{10} in Japanese quail. The females of the maternal line laid higher FEW, EW_{10} and

EM₁₀ than the means of randombred line with insignificant differences between them (Fig. 6). These results are in accordance with previous studies reported by Nath et al. (2011) and Okenyi et al. (2013) for EW. Lower FEW of 7.83g was cited by Momoh et al. (2014). Mahmoud et al. (2014) reported an estimate of 114.23g for the first ten eggs laid in Japanese quail. From the present results, it can be concluded that maternal selected line had favored both growth traits and egg production related traits studied.

Identification of RAPD-PCR markers:

Six primers were generated reproducible and scoreable RAPD profiles (Plate 1). These primers detected scoreable polymorphisms in banding patterns among 10 individuals (Table 2). Each of the 6 primers amplified a different number of bands. In this respect, (Mansour et al., 2010) reported that RAPD exhibited polymorphism in quail. The numbers of amplified fragments are summarized in Table (3). These produced multiple band profiles with a number of amplified DNA fragments ranging from 3-10 (Table 3 and Plate 1). A total number of 411 bands were generated and polymorphism levels differed from one primer to the other. While, the number of polymorphic fragments ranged from 2-5. A maximum number of 83 amplicons was amplified with primer OPB-04, while the minimum (54) was amplified with primer OPB-01. The highest number of polymorphic bands was detected for primer OPA-01 (5 out of 8), while the lowest for primer OPB-07 (2 out of 8), however 18 bands were monomorphic. Primer OPB-11 exhibited the highest percentage (77.778%) of polymorphism, while primers OPB-07 and OPE-19 exhibited the lowest 50% (Table 4). In this respect, 85.315 polymorphism reported by Mansour et al. (2010), and

99.49% was reported by Karabağ and Balcioğlub (2010). RAPD technique has been successfully applied for estimating genetic diversity in quail lines (Kumar et al., 2000). RAPD technique using 5 primers was applied to detect genetics similarity among chickens (Ghanem et al., 2012). Table (4) also revealed that the total number of polymorphic amplicons obtained by the six studied primers was 34. This corresponds to a level of polymorphism of 65.385% and an average number of polymorphic fragments/primer of 5.667. Karabağ and Balcioğlub (2010) reported that the genetic relationships within and among selected and control lines of Japanese quail males and females lines could be estimated successfully using RAPD technique.

The specific markers for quail genotypes generated from RAPD-PCR analysis are shown in Tables (2 and 3). The highest number of RAPD specific markers was scored for female 3, male 5 maternal line, female 6, male 9 and male 10 randombred line (1 marker/primer). A number of 2 positive specific markers were scored for the presence of unique bands for a given genotype, while 8 negative specific markers was scored for the absence of a common band. The highest number of RAPD genotype-specific markers was generated by primer OPB-11 (3 markers), primers OPB-04 and OPB-07 (2 markers/primer). On the other hand, the primers OPA-01, OPB-01 and OPE-19 generated the least number (1 marker/primer). RAPD marker of individual quail showed characteristic pattern and individuals could be distinguishable from each other even within the same line.

Phylogenetic relationship among quail lines based on RAPD marker:

Based on the combined data obtained through the polymorphism of RAPD profiles, the similarity coefficient values among 10 individuals (maternal and randombred lines, females and males) were calculated according to Dice (1945) equation (Table 5). The similarity coefficient shows an average genetic distance ranging from 0-1 with a mean value of 0.5. The highest similarity index (1) was recorded between each two of male 10 randombred line and female 3 maternal line. However, the lowest (0) was observed between female 2 and female 1 maternal line, male 4 and female 2 maternal line, and female 7 and female 6 randombred line. The data obtained from the analysis of RAPD was used to draw precise relationships among individuals. The resultant dendrogram is shown in Fig. (7) using Unweighted Pair-Group Method with Arithmetical average Algorithm (UPGMA) analysis. This dendrogram clustered the genotypes into two clusters (groups). The first group involved of male 10 randombred line was delimited in separate one cluster from the rest of studied genotypes. The second group was subdivided into two subgroups. The first subgroup involved male 9 randombred line was delimited from the rest of genotypes, male 5 maternal line was delimited from the rest of genotypes, female 8 randombred line was delimited from the rest of genotypes, and the rest of genotypes in the first subgroup includes females 6 and 7 randombred line. The second subgroup was subdivided into two subgroups. The first subgroup includes female 3 maternal line was delimited in one subgroup from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup involved female 1 maternal line was delimited from the rest of genotypes, and the second subgroup includes female 2 and male 4

maternal line. Based on RAPD analysis, the randombred line male was delimited in separate group while, maternal line males, randombred line male, randombred line females and maternal line females were in separate clusters.

In conclusion, it seems that 6 primers used in the present study allowed enough distinction among the quail lines maternal selected and randombred lines, females and males. This has demonstrated the efficiency of the RAPD as a potential genetic marker.

Identification of ISSR-PCR markers:

Microsatellites are 2-6-nucleotide repeats, interspersed throughout the genome and highly polymorphic. After the generation of the first MS linkage map of quail which reported by Kayang et al. (2004), Miwa et al. (2006) mapped the panda plumage color locus on the MS linkage map of the quail. Fourteen MS markers in the inbred and randombred lines were used to differentiate the Japanese quail lines (Kim et al., 2007). In this study, 6 ISSR primers generated reproducible and scoreable ISSR profiles with the DNA of the 10 individual in the PCR reaction (Plate 2). These primers detected scoreable polymorphisms in banding patterns among the genotypes (Table 2). In this respect, Mansour et al. (2010) reported that genetic diversity of four genotypes of quail was analyzed by MS markers. Four MS markers showed that the Japanese and English white quail populations have a relatively high genetic diversity (Mamizade et al., 2013). Each of the six primers used in the present study amplified a different number of bands. The numbers of amplified fragments are summarized in Table (3). These produced multiple band profiles with a number of amplified DNA fragments ranging from 3-8. A total number of 384 bands were generated and polymorphism

levels differed from one primer to the other. While, the number of polymorphic fragments ranged from 1-4. A maximum number of 76 amplicons was amplified with primer HB-09, while the minimum (43) with primer HB-13. The highest number of polymorphic bands was detected for primer HB-13 (4 out of 7) and primer HB-14 (4 out of 11) while, the lowest for primer HB-09 (1 out of 8) and primer HB-15 (1 out of 8), however, 24 bands were monomorphic. Primer HB-13 exhibited the highest percentage (85.714%) of polymorphism, while primers HB-09 and HB-15 exhibited the lowest (25%/primer) (Table 4). In this respect, Mansour et al. (2010) found that the percentage of polymorphism in four quail phenotypes was 94.10%. Table (4) also revealed that the total number of polymorphic amplicons obtained by the 6 studied primers was 24. This corresponds to a level of polymorphism of 50% and an average number of polymorphic fragments/primer of 4. MS have much higher polymorphism and are considered to be more appropriate molecular tools for studying genetic biodiversity and relationships. Pasnin and Ranghoo-Sanmukhiya (2013) used RAPD and MS analysis to differentiate three cattle breeds, and polymorphic bands were obtained from the two techniques.

The specific markers for quail genotypes generated from ISSR-PCR analysis are shown in Tables (2 and 3). The highest number of ISSR specific markers was scored for male 4 maternal line (2 markers) and for female 8 randombred line, while the lowest (1 marker) for female 2 maternal line, female 7 and male 9 randombred line. A number of 4 positive specific markers were scored for the presence of unique bands for a given genotype, while 5 negative for the absence of a common band. The highest number of

ISSR genotype-specific markers was generated by primer HB-14 (3 markers) and primer HB-13 generated (2 markers). On the other hand, the primers 44B, HB-09, HB-10 and HB-15 generated 1 marker. ISSR marker of individual quail showed characteristic pattern and individuals could be distinguishable from each other even within the same line. This has demonstrated the efficiency of the ISSR as a potential genetic marker.

Phylogenetic relationship among quail lines based on ISSR marker:

Based on the combined data obtained through the polymorphism of ISSR profiles, the similarity coefficient values among 10 individuals (maternal and randombred lines, females and males) were calculated according to Dice (1945) equation (Table 6). The similarity coefficient shows an average genetic distance ranging from 0-1 with a mean value of 0.5. The highest similarity index (1) was recorded between each two of female 7 randombred line and female 2 maternal line. However, the lowest (0) was observed between female 2 and female 1 maternal line, and male 5 and male 4 maternal line. The data obtained from the analysis of ISSR was used to draw precise relationships among the individuals. The resultant dendrogram is shown in Fig. (8) using UPGMA analysis. This dendrogram clustered the quail genotypes into two clusters (groups). The first group consisted of female 8 randombred line was delimited in separate one cluster from the rest of genotypes. The second group was subdivided into two subgroups. The first subgroup included females 6 and 7 randombred line. The second subgroup subdivided into two subgroups, the first subgroup involved male 9 randombred line was delimited from the rest of genotypes and male 10 randombred line was delimited

from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup includes females 1 and 2 maternal line and the second subgroup involved female 3 maternal line was delimited in one subgroup from the rest of genotypes, and the other subgroup was included males 4 and 5 maternal line. Based on ISSR analysis, the randombred line female was delimited in one group while, randombred line females, randombred line males, maternal line females and males were delimited in separate one cluster.

Phylogenetic relationship among quail lines based on RAPD and ISSR markers

Both RAPD and ISSR are based on different strategies for exploring genetic diversity. While RAPD primers randomly target complementary and homologous genomic regions in the genome, ISSR primers amplify the highly repetitive inter-simple sequence repeats of the MS 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 ISSR profiles, the similarity coefficient values among 10 individuals (maternal and randombred lines, females and males) were calculated according to Dice (1945) equation (Table 7). The similarity coefficient shows an average genetic distance ranging from 0-1 with a mean value of 0.5. The highest similarity index (1) was recorded between each two of male 10 randombred line and female 3 maternal line. However, the lowest (0) was observed between females 2 and 1 maternal line. The data obtained from the analysis of RAPD and ISSR was used to draw precise relationships among the genotypes. The resultant dendrogram is shown in Fig. (9) using UPGMA analysis. This dendrogram clustered the quail

genotypes into two clusters (groups). The first group consisted of male 10 randombred line was delimited in separate one, and female 8 and male 9 randombred line were delimited in separate one cluster from the rest of genotypes. The second group was subdivided into two subgroups. The first subgroup involved females 6 and 7 randombred line were separated from the rest of genotypes, and the second subgroup includes female 3 maternal line was delimited in separate one from the rest of genotypes, male 4 maternal line was delimited in separate one and male 5 maternal line was delimited in separate one from the rest of genotypes. The other subgroup includes females 1 and 2 maternal line was separated in one cluster. Based on RAPD and ISSR combination analysis, the randombred line males and female were clustered in the same group, and randombred line females, maternal selected line females and males were in separate clusters.

The different types of markers, RAPD and ISSR revealed different levels of genetic similarity among the 10 individuals. 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 line in one group. Gathering both maternal selected and randombred

lines in one cluster. They might share some genes between maternal and randombred lines through selection in maternal line. The randombred line was the original from which the maternal line had selected. RAPD and ISSR techniques would be used for identification of males and females quail birds. Six primers with each type of marker used in the present study allowed enough distinction among the quail lines. The result of molecular genetic analysis is in agreement with the result for the performance since, there were differences between the two lines and sexes in BW and SL and between the two lines in studied egg production traits. Both was successfully distinguished among maternal and randombred lines and sexes. The study

showed that RAPD and ISSR analyses are effective methods for generating polymorphic DNA markers in the two lines. These polymorphic markers are also useful for estimating genetic distances and the genetic relationships between the lines. The level of polymorphism detected by using RAPDs and ISSRs will provide breeders with environment independent DNA markers, which should be regarded as essential tools for selection. RAPD and ISSR analyses will be practical in quail breeding and conservation of indigenous lines. It provides a mean to differentiate lines that are genetically dissimilar and members (males and females) within the lines. This will be useful in selective breeding programs in Japanese quail.

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

Marker	Primer code	Sequence (5'-3')	Marker	Primer code	Sequence (5'-3')
RAPD	OPA-01	CAG GCC CTT C	ISSR	44 B	CTC TCT CTC TCT CTC TG
	OPB-01	GTT TCG CTC C		HB-09	GTG TGT GTG TGT GG
	OPB-04	GGA CTG GAG T		HB-10	GAG AGA GAG AGA CC
	OPB-07	GGT GAC GCA G		HB-13	GAG GAG GAG GC
	OPB-11	GTA GAC CCG T		HB-14	CTC CTC CTC GC
	OPE-19	ACG GCG TAT G		HB-15	GTG GTG GTG GC

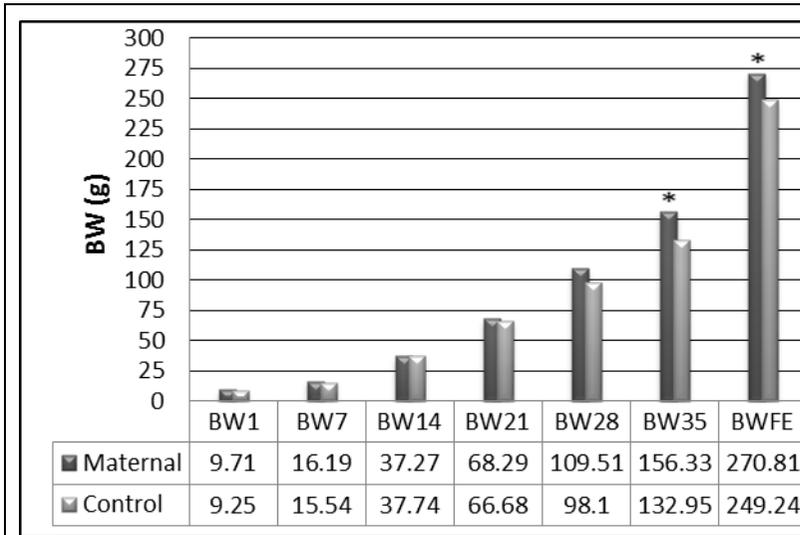


Fig. (1): Effect of line on BW at different ages (BW₁-BW₃₅=Body weight at day-old of age-35 days of age, BWFE=Body weight at first egg) of Japanese quail, *=Significant difference (P < 0.05).

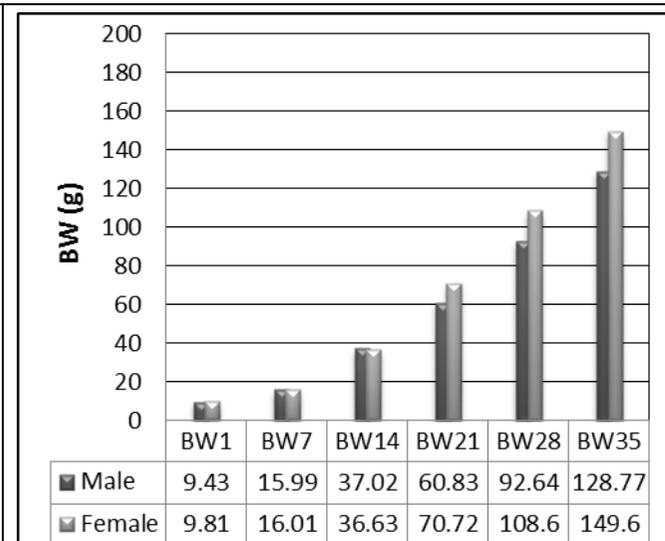


Fig. (2): Effect of sex on BW at different ages (BW₁-BW₃₅=Body weight at day-old of age-35 days of age) of Japanese quail.

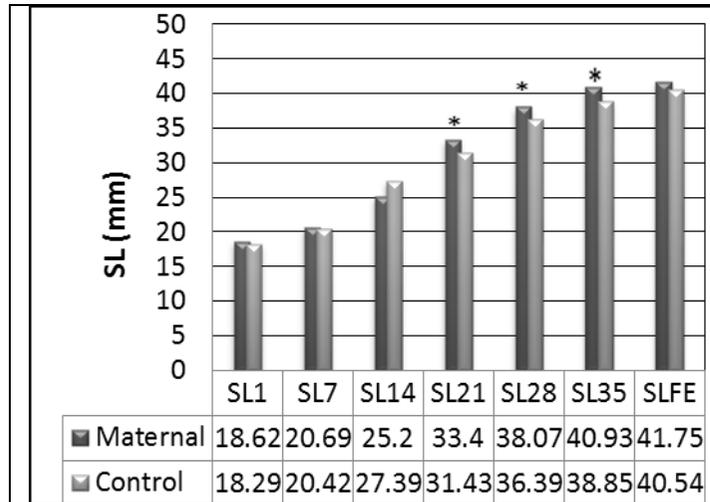


Fig. (3): Effect of line on SL at different ages (SL₁-SL₃₅=Shank length at day-old of age-35 days of age, SLFE=Shank length at first egg) of Japanese quail, *=Significant difference (P < 0.05).

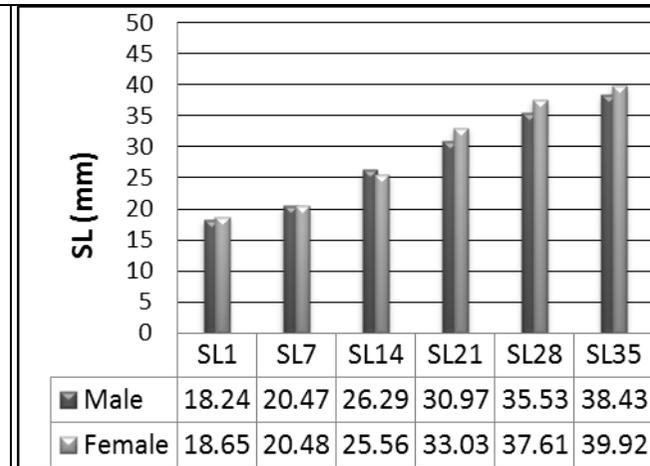


Fig. (4): Effect of sex on SL at different ages (SL₁-SL₃₅=Shank length at day-old of age-35 days of age) of Japanese quail.

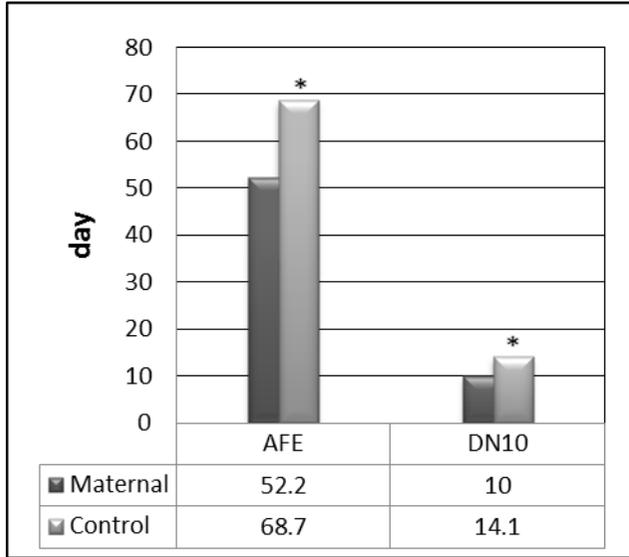


Fig. (5): Effect of line on age at first egg (AFE) and number of days needed to produce the first 10 eggs (DN₁₀) of Japanese quail, *=Significant difference (P < 0.05).

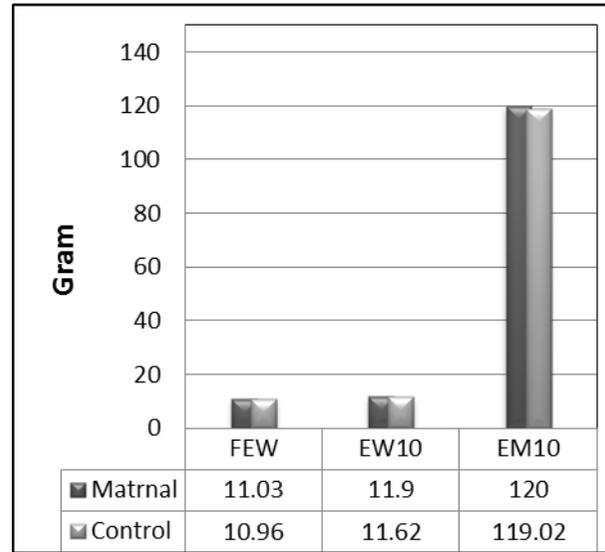


Fig. (6): Effect of line on first egg weight (FEW), egg weight of the tenth egg (EW₁₀) and egg mass of the first 10 eggs (EM₁₀) of Japanese quail.

Table (2): Banding patterns of RAPD and ISSR-PCR for two lines maternal selected and randombred (females and males) of Japanese quail.

RAPD primer	Band No.	BP	1	2	3	4	5	6	7	8	9	10
OP-A01	1	850	0	0	0	0	1	0	0	0	0	0
	2	770	1	1	0	1	1	1	1	0	1	0
	3	710	1	1	1	1	1	1	1	1	0	0
	4	685	1	1	1	1	1	1	1	1	1	1
	5	540	1	1	1	1	0	0	0	0	0	0
	6	425	1	1	1	1	1	1	1	1	1	1
	7	310	1	1	0	1	0	1	1	1	1	1
	8	220	1	1	0	1	1	1	1	1	1	0
OP-B01	1	710	1	1	1	1	1	1	1	1	1	0
	2	600	0	1	0	1	1	1	1	1	1	1
	3	475	1	1	1	1	1	1	1	1	1	1
	4	390	1	1	1	1	1	1	1	1	1	1
	5	370	0	0	0	1	1	1	1	1	1	1
	6	315	1	1	1	1	0	0	0	0	0	0
	7	240	0	0	0	0	1	1	1	1	1	1
OP-B04	1	950	1	1	1	1	1	1	1	1	0	0
	2	860	1	1	1	1	1	1	1	0	0	0
	3	840	1	1	0	1	1	1	1	0	0	0
	4	780	1	1	1	1	1	1	1	1	1	1
	5	700	1	1	1	1	1	1	1	1	1	1
	6	650	1	1	1	1	1	1	0	0	0	0
	7	630	1	1	1	1	1	1	1	1	1	1
	8	520	1	1	1	1	1	1	1	1	0	1
	9	425	1	1	0	1	1	1	1	1	1	1
	10	290	1	1	0	1	1	0	1	1	1	1

1, 2, 3=Females maternal line, 4, 5=Males maternal line, and 6, 7, 8=Females randombred line, and 9, 10=Males randombred line

Quail; Productive performance; RAPD analysis; ISSR analysis

Table (2): Cont.

RAPD primer	Band No.	BP	1	2	3	4	5	6	7	8	9	10
OP-B07	1	1420	0	0	1	0	0	0	0	0	0	0
	2	875	1	1	1	1	1	1	1	1	1	1
	3	850	1	1	1	1	1	1	1	0	0	0
	4	750	1	1	1	1	1	1	1	1	1	0
	5	620	1	1	1	1	1	1	1	1	1	1
	6	435	1	0	0	0	1	1	1	1	1	1
	7	320	1	1	1	1	1	1	1	1	1	1
	8	270	1	1	1	1	1	1	1	1	1	1
OP-B11	1	980	1	1	0	1	0	1	1	1	1	1
	2	830	0	1	1	1	1	1	1	0	1	0
	3	720	1	1	1	1	1	1	1	1	1	0
	4	600	1	1	1	1	0	1	1	1	1	1
	5	530	1	1	1	1	1	0	0	1	1	1
	6	500	1	1	1	1	1	1	1	1	1	1
	7	460	1	1	1	1	1	1	1	1	1	1
	8	280	0	0	0	1	1	1	1	1	1	1
	9	170	1	1	1	1	1	0	1	1	1	1
OP-E19	1	670	0	0	0	1	0	1	0	1	0	1
	2	460	1	0	1	1	0	1	1	1	0	1
	3	375	1	1	1	1	1	1	1	1	1	0
	4	350	1	1	1	1	1	1	1	1	1	1
	5	330	1	1	1	1	0	0	0	0	1	0
	6	315	0	0	0	0	1	0	0	0	0	1
	7	240	1	1	1	1	1	1	1	1	1	1
	8	130	1	1	1	1	1	1	1	1	1	1
	9	80	1	1	1	1	1	1	1	1	1	1
	10	60	1	1	1	1	1	1	1	1	1	1

1, 2, 3=Females maternal line, 4, 5=Males maternal line, and 6, 7, 8=Females randombred line, and 9, 10=Males randombred line

Table (2): Cont.

ISSR primer	Band No.	BP	1	2	3	4	5	6	7	8	9	10
44B	1	840	1	1	1	1	1	0	0	1	1	1
	2	725	1	1	1	1	1	1	1	1	1	1
	3	645	1	1	1	1	1	1	1	1	1	1
	4	620	1	1	1	1	1	1	1	1	1	1
	5	525	0	0	0	0	0	0	0	1	0	0
	6	365	0	0	1	1	1	1	0	1	1	0
	7	275	1	1	1	1	1	1	0	1	1	0
HB-9	1	860	1	1	1	1	1	1	1	1	1	1
	2	800	1	1	1	1	1	1	1	1	0	1
	3	700	1	1	1	1	1	1	1	1	1	1
	4	670	1	1	1	1	1	1	1	1	1	1
	5	620	1	1	1	1	1	1	0	0	0	1
	6	570	1	1	1	1	1	1	1	1	1	1
	7	535	1	1	1	1	1	1	1	1	1	1
	8	485	1	1	1	1	1	1	1	1	1	1
HB-10	1	965	1	0	0	1	1	1	1	1	0	0
	2	830	1	1	1	1	1	1	1	1	1	1
	3	600	0	0	1	1	1	1	1	1	1	1
	4	515	1	0	1	1	1	1	1	1	1	1
	5	375	1	1	1	1	1	1	1	1	1	1
	6	310	1	1	1	1	1	1	1	1	1	1
	7	280	1	1	1	1	1	1	1	1	1	1

1, 2, 3=Females maternal line, 4, 5=Males maternal line, and 6, 7, 8=Females randombred line, and 9, 10=Males randombred line

Quail; Productive performance; RAPD analysis; ISSR analysis

Table (2): Cont.

ISSR primer	Band No.	BP	1	2	3	4	5	6	7	8	9	10
HB-13	1	645	1	1	1	1	1	1	1	1	1	1
	2	510	1	1	1	1	1	0	0	1	1	1
	3	420	0	0	0	1	0	0	0	0	0	0
	4	400	0	0	0	0	0	0	1	1	0	0
	5	345	1	1	0	1	1	0	0	1	1	0
	6	220	1	1	1	0	1	1	1	1	1	1
	7	190	1	1	1	1	1	1	1	0	0	0
HB-14	1	1200	1	1	1	1	1	1	1	1	1	1
	2	770	0	0	0	0	0	1	1	0	0	0
	3	750	0	0	0	0	0	0	1	1	1	0
	4	640	1	1	1	1	1	1	1	0	1	1
	5	560	0	0	0	0	0	0	0	1	0	0
	6	500	1	1	1	1	1	1	0	1	1	1
	7	400	1	1	1	1	1	1	1	1	1	1
	8	345	1	1	1	1	1	1	1	1	1	1
	9	280	1	1	1	1	1	1	1	1	1	1
	10	260	1	1	0	1	1	0	1	1	0	1
	11	225	1	1	0	0	0	0	0	0	1	1
HB-15	1	660	1	1	1	1	1	1	1	1	1	1
	2	625	1	1	1	1	1	1	1	0	1	1
	3	540	1	1	1	1	1	1	1	1	1	1
	4	460	1	1	1	1	1	1	1	1	1	1
	5	305	1	1	1	1	1	1	1	1	1	1
	6	265	0	0	0	0	0	1	1	1	1	1
	7	240	1	1	1	1	1	1	1	1	1	1
	8	220	1	1	1	1	1	1	1	1	1	1

1, 2, 3=Females maternal line, 4, 5=Males maternal line, and 6, 7, 8=Females randombred line, and 9, 10=Males randombred line

Table (3): Number of amplified fragments markers of two quail lines maternal selected and randombred based on RAPD and ISSR-PCR analysis.

Sex/Line		RAPD primers						Total
		OPA-01	OPB-01	OPB-04	OPB-07	OPB-11	OPE-19	
1 ♀ maternal	AF	7	4	10	7	7	8	43
	SM	0	0	0	0	0	0	0
2 ♀ maternal	AF	7	5	10	6	8	7	43
	SM	0	0	0	0	0	0	0
3 ♀ maternal	AF	4	4	7	7	7	8	37
	SM	0	0	1	1	0	0	2
4 ♂ maternal	AF	7	6	10	6	9	9	47
	SM	0	0	0	0	0	0	0
5 ♂ maternal	AF	6	6	10	7	7	7	43
	SM	1	0	0	0	1	0	2
6 ♀ control	AF	6	6	9	7	7	8	43
	SM	0	0	0	0	1	0	1
7 ♀ control	AF	6	6	9	7	8	7	43
	SM	0	0	0	0	0	0	0
8 ♀ control	AF	5	6	7	6	8	8	40
	SM	0	0	0	0	0	0	0
9 ♂ control	AF	5	6	5	6	9	7	38
	SM	0	0	1	0	0	0	1
10 ♂ control	AF	3	5	6	5	7	8	34
	SM	0	1	0	1	1	1	4
PB		5	4	5	2	4	4	24
TAF		56	54	83	64	77	77	411
TSM		1	1	2	2	3	1	10
MB		2	2	3	4	2	5	18

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

Quail; Productive performance; RAPD analysis; ISSR analysis

Table (3): Cont.

Sex/Line		ISSR primers						Total
		44B	HB-09	HB-10	HB-13	HB-14	HB-15	
1 ♀ maternal	AF	5	8	6	5	8	7	39
	SM	0	0	0	0	0	0	0
2 ♀ maternal	AF	5	8	4	5	8	7	37
	SM	0	0	1	0	0	0	1
3 ♀ maternal	AF	6	8	6	4	6	7	37
	SM	0	0	0	0	0	0	0
4 ♂ maternal	AF	6	8	7	5	7	7	40
	SM	0	0	0	2	0	0	2
5 ♂ maternal	AF	6	8	7	5	7	7	40
	SM	0	0	0	0	0	0	0
6 ♀ control	AF	5	8	7	3	7	8	38
	SM	0	0	0	0	0	0	0
7 ♀ control	AF	3	7	7	4	8	8	37
	SM	0	0	0	0	1	0	1
8 ♀ control	AF	7	7	7	5	8	7	41
	SM	1	0	0	0	2	1	4
9 ♂ control	AF	6	6	6	4	8	8	38
	SM	0	1	0	0	0	0	1
10 ♂ control	AF	4	8	6	3	8	8	37
	SM	0	0	0	0	0	0	0
PB		3	1	2	4	4	1	15
TAF		53	76	63	43	75	74	384
TSM		1	1	1	2	3	1	9
MB		3	6	4	1	4	6	24

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

Table (4): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphisms as revealed by RAPD and ISSR markers among the maternal selected and randombred lines.

Marker	Primer	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	% Polymorphism
RAPD	OPA-01	8	2	6	75.000
	OPB-01	7	2	5	71.428
	OPB-04	10	3	7	70.000
	OPB-07	8	4	4	50.000
	OPB-11	9	2	7	77.778
	OPE-19	10	5	5	50.000
	Total	52	18	34	65.385
	Average	8.667	3	5.667	65.386
ISSR	44B	7	3	4	57.143
	HB-09	8	6	2	25.000
	HB-10	7	4	3	42.857
	HB-13	7	1	6	85.714
	HB-14	11	4	7	63.636
	HB-15	8	6	2	25.000
	Total	48	24	24	50.000
	Average	8	4	4	50.000

Table (5): Similarity matrix for the 10 individuals of Japanese quail on the basis of RAPD-PCR analysis.

Items	1 ♀ maternal	2 ♀ maternal	3 ♀ maternal	4 ♂ maternal	5 ♂ maternal	6 ♀ control	7 ♀ control	8 ♀ control	9 ♂ control
2 ♀ maternal	0.00								
3 ♀ maternal	0.26	0.26							
4 ♂ maternal	0.07	0.00	0.32						
5 ♂ maternal	0.38	0.30	0.58	0.28					
6 ♀ control	0.30	0.23	0.58	0.14	0.23				
7 ♀ control	0.23	0.44	0.58	0.14	0.15	0.00			
8 ♀ control	0.36	0.37	0.56	0.26	0.36	0.20	0.13		
9 ♂ control	0.45	0.82	0.76	0.35	0.37	0.37	0.37	0.18	
10 ♂ control	0.74	0.65	1.00	0.16	0.65	0.65	0.57	0.20	0.39

Quail; Productive performance; RAPD analysis; ISSR analysis

Table (6): Similarity matrix for the 10 individuals of Japanese quail on the basis of ISSR-PCR analysis.

Items	1 ♀ maternal	2 ♀ maternal	3 ♀ maternal	4 ♂ maternal	5 ♂ maternal	6 ♀ control	7 ♀ control	8 ♀ control	9 ♂ control
2 ♀ maternal	0.00								
3 ♀ maternal	0.32	0.34							
4 ♂ maternal	0.23	0.40	0.24						
5 ♂ maternal	0.07	0.24	0.08	0.00					
6 ♀ control	0.56	0.74	0.25	0.47	0.31				
7 ♀ control	0.80	1.00	0.83	0.87	0.71	0.41			
♀ control	0.76	0.94	0.18	0.67	0.52	0.85	0.78		
9 ♂ control	0.56	0.57	0.41	0.62	0.47	0.64	0.90	0.54	
10 ♂ control	0.32	0.34	0.34	0.56	0.40	0.57	0.67	0.78	0.41

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

Items	1 ♀ maternal	2 ♀ maternal	3 ♀ maternal	4 ♂ maternal	5 ♂ maternal	6 ♀ control	7 ♀ control	8 ♀ control	9 ♂ control
2 ♀ maternal	0.00								
3 ♀ maternal	0.37	0.37							
4 ♂ maternal	0.15	0.16	0.38						
5 ♂ maternal	0.37	0.38	0.54	0.25					
6 ♀ control	0.51	0.59	0.63	0.32	0.34				
7 ♀ control	0.55	0.63	0.89	0.50	0.45	0.17			
8 ♀ control	0.65	0.81	0.92	0.53	0.54	0.55	0.45		
9 ♂ control	0.65	0.58	0.84	0.58	0.53	0.61	0.58	0.40	
10 ♂ control	0.78	0.87	1.00	0.79	0.74	0.75	0.72	0.53	0.52

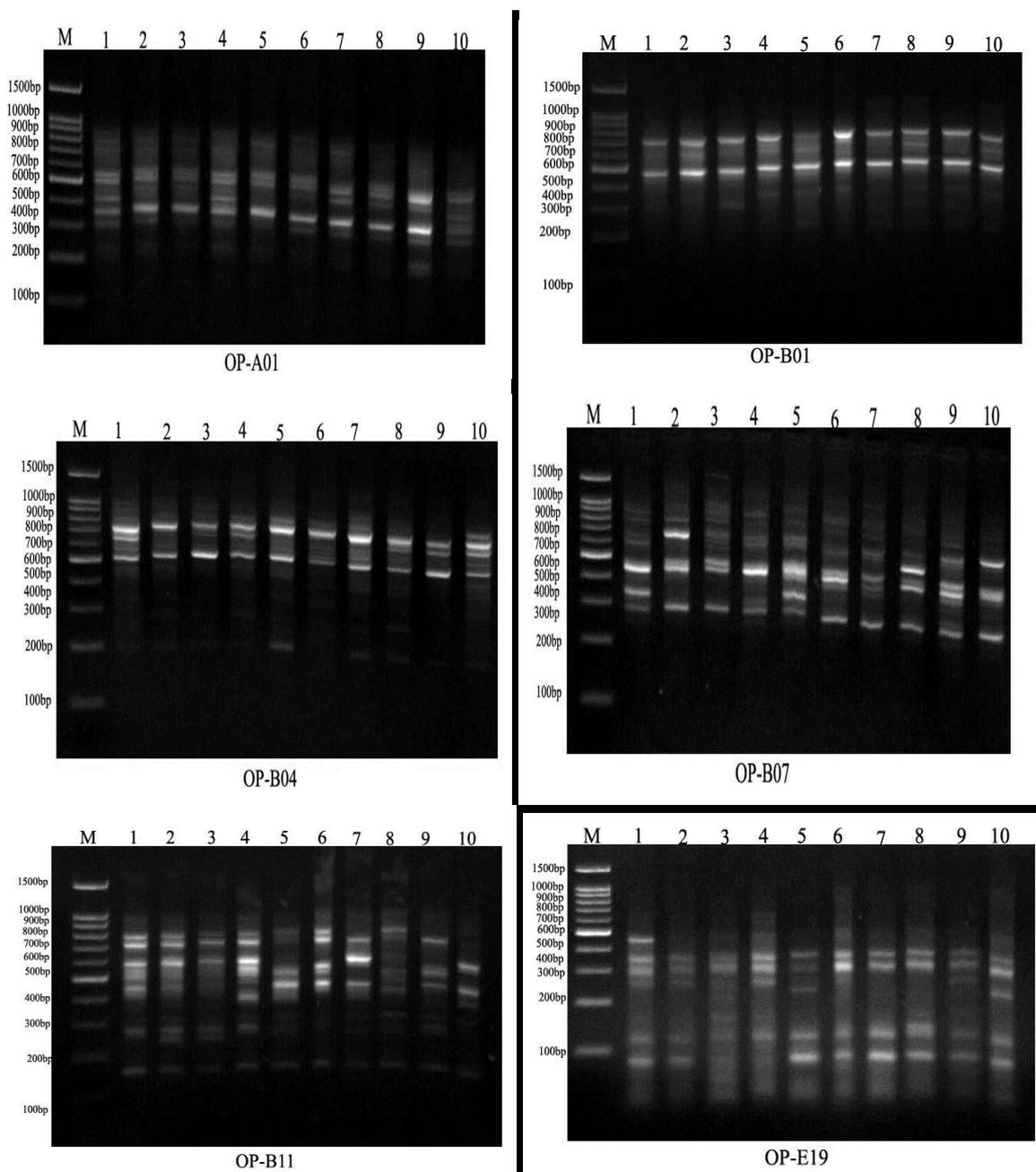


Plate (1): RAPD profile of the maternal selected (S) and randombred control (C) Japanese quail lines amplified with 6 different RAPD primers. M=Ladder marker, 1, 2 and 3=Females S, 4 and 5=Males S, 6, 7 and 8=Females C and 9 and 10=Males C.

Quail; Productive performance; RAPD analysis; ISSR analysis

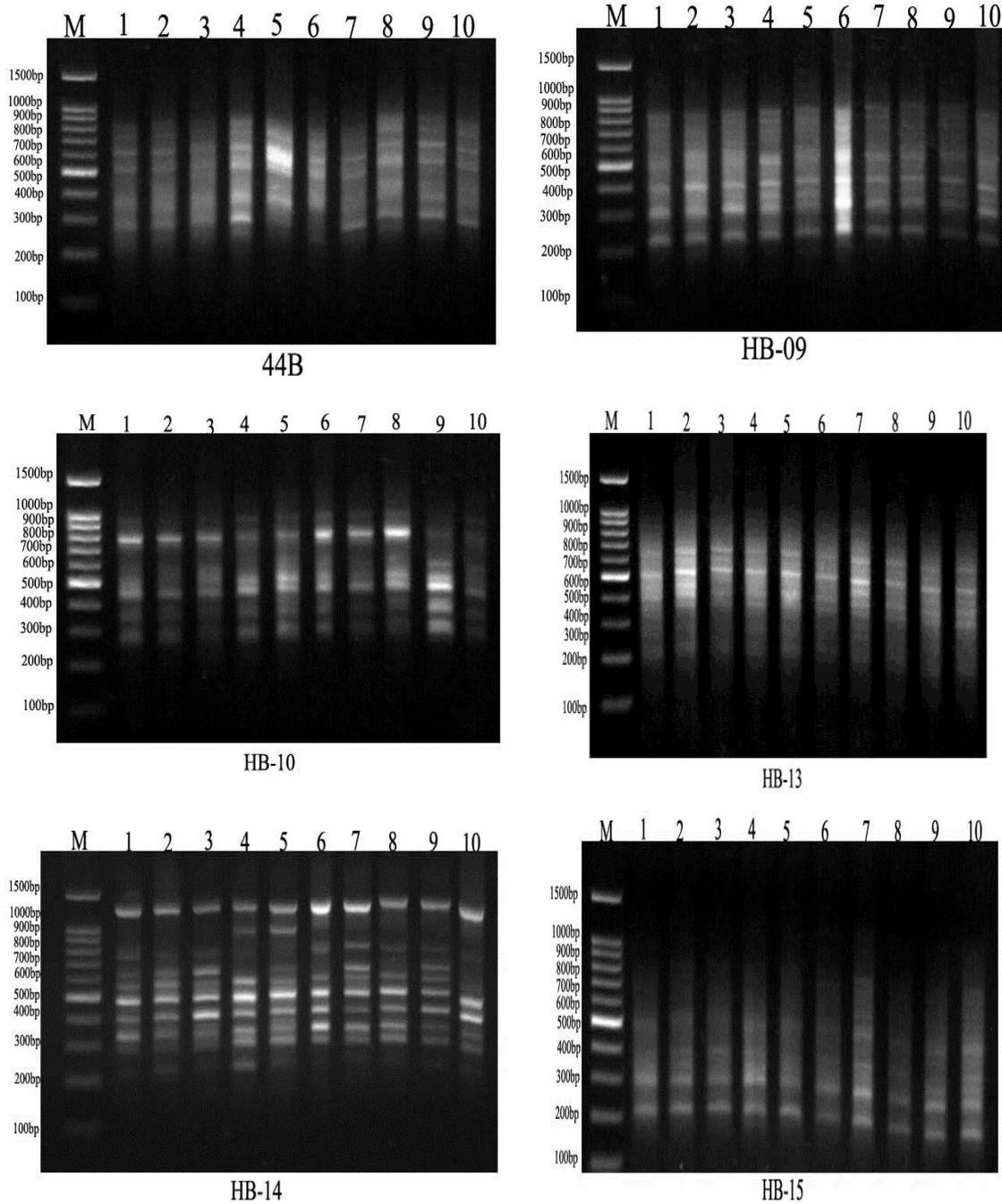


Plate (2): ISSR profile of the maternal selected (S) and randombred control (C) Japanese quail lines amplified with 6 different ISSR primers. M=Ladder marker, 1, 2 and 3=Females S, 4 and 5=Males S, 6, 7 and 8=Females C and 9 and 10=Males C.

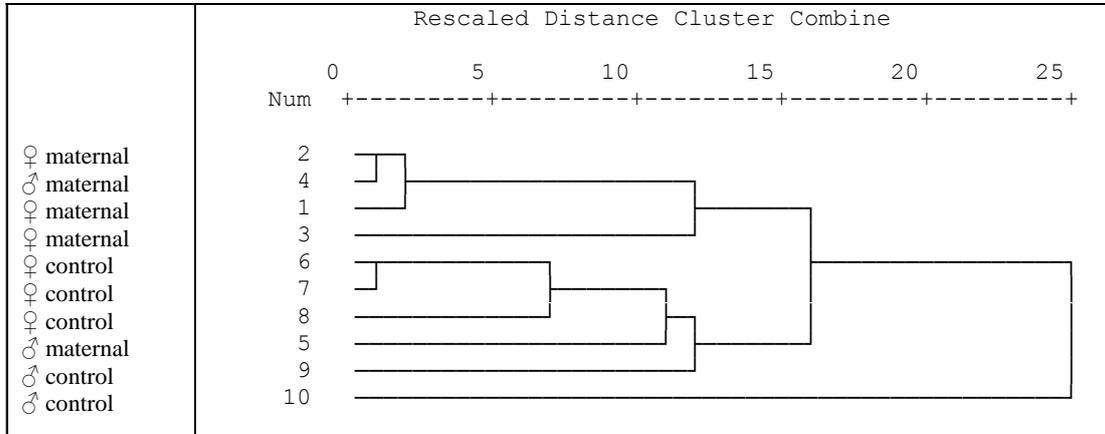


Fig. (7): Dendrogram for the 10 individual quails constructed from the RAPDs data using UPGMA and similarity matrix computed according to Dice coefficient.

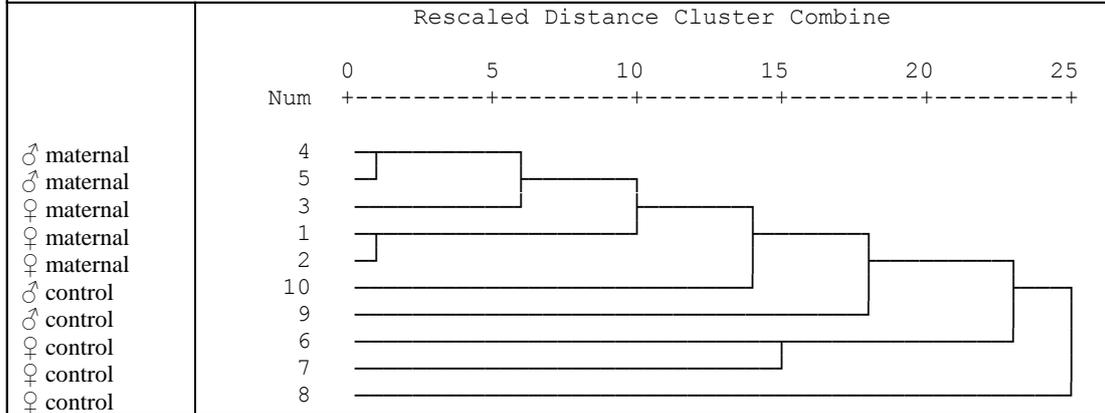


Fig. (8): Dendrogram for the 10 individual quails constructed from the ISSRs data using UPGMA and similarity matrix computed according to Dice coefficient.

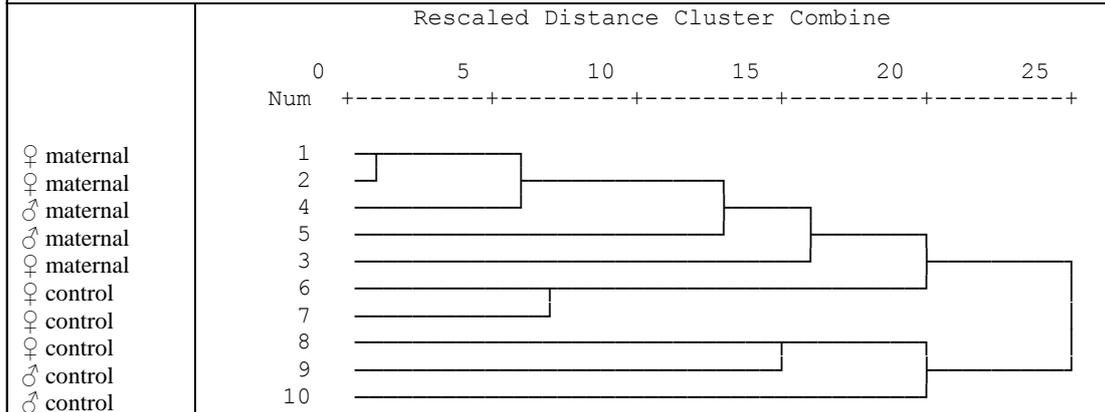


Fig. (9): Dendrogram for the 10 individual quails constructed from the RAPDs and ISSRs data using UPGMA and similarity matrix computed according to Dice coefficient.

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Quail; Productive performance; RAPD analysis; ISSR analysis

أن ذكور وأنثى ناتجة بالتزاوج العشوائي قد تجمعت في عنقود واحد و اناث ناتجة بالتزاوج العشوائي و اناث و ذكور منتخبة قد انفصلت في عنقود واحد. وعلى الرغم من أنه قد حدث تغيير في مواقع بعض الأفراد التابعة للخط المنتخب أميا والخط الناتج بالتزاوج العشوائي في أشجار القرابة المختلفة مما يظهر تقاربهما في الخلفية الوراثية. وأن الخطين ربما يكونوا مشتركين في بعض الجينات بين الخط المنتخب لسنة أجيال والخط المتزاوج عشوائيا من خلال الانتخاب بداية من العشيرة القاعدية التي نشأ منها الخطان. بالإضافة الى ذلك فقد نجحت كل من تقنيتي ال RAPD وال ISSR في تحديد دلائل جزيئية مميزة لعشرة تراكيب وراثية من السمان الياباني. مما يدل على كفاءة هذه التقنيات في التمييز بين التراكيب الوراثية المتقاربة. وهذه النتائج إتفقت مع نتائج الأداء الإنتاجي المتمثلة في وجود إختلافات بين الخطين وبين الجنسين في صفات النمو وبين الخطين في صفات إنتاج البيض المدروسة. كما توضح الدراسة أن إستخدام طرق الوراثة الجزيئية تعتبر وسائل ذات كفاءة عالية للتمييز بين خطوط السمان المختلفة في برامج التربية تحت ظروف الانتخاب أو التزاوج العشوائي ويمكن أن تكون ذات فائدة كبيرة في تحديد الطرز الوراثية وحفظ الاصول الوراثية.