PRODUCTIVE TRAITS AND MOLECULAR GENETICS CHARACTERIZATION (RAPD AND ISSR) OF SELECTED LONG SHANK LENGTH AND CONTROL LINES IN THE 6th GENERATION OF JAPANESE QUAIL

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Japanese quail (*Coturnix japonica*) are an economically important species valued for its egg, meat, and as a biological animal model in Asia, Europe, the Middle East and America (Minvielle, 2004). Research on Japanese quail has led to the development of commercial strains for meat, egg production and knowledge of its genetics (Tsudzuki, 2008). Many of body measurements such as length of shank and keel may be used as good indicators of skeletal size. The length of shank is a better measure for the genetics of size than body weight (Nordskog, 1976). Shank length was used to predict live body weight (Amao *et al*., 2010; Ojo *et al*., 2014). The knowledge about the genetic characterization of various well-developed quail lines and amount of genetic diversity among them is minimal. Hence studies are needed to characterize quail lines genetically and to estimate the genetic variability in order to enhance selection and breeding programs. Genetic improvement of livestock is dependent on the fact that genetic variation exists within breeds allowing them to respond to selection (Sruoga *et al*., 2007). Among the most popular molecular markers were the RAPD, described first by Welsh and McClelland (1990). RAPD were commonly used for the genetic mapping and selection criteria for characters in poultry. DNA fingerprint patterns (Piao *et al*., 2003) were characterized in two lines of Japanese quail that are differentiated by large and small body sizes and developed by selection. RAPD methods have been used to detect specific markers, genetic similarity in Japanese quail lines (Sharma *et al*., 2000; Karabağ and Balcioğlub, 2010). Mansour *et al*. (2010) investigated the variations within four phenotypes of Japanese quail using RAPD and ISSR markers. The second widely used DNA marker is based on MS, SSR which are efficient for estimating genetic variation between populations of the same species. The modern genetic tools developed specifically for quail analyses were MS

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markers (Kayang *et al*., 2002 and 2004; Mannen *et al*., 2005). Moe *et al*. (2007) reported that a nonsynonymous SNP in the gene for insulin-like growth factor1 receptor from selected Japanese quail was associated with body weight in the large and small body sizes lines. Molecular markers have opened up new horizons for estimating genetic relationships among animal populations and serve as an important initial guide to develop conservation strategies (Davila *et al*., 2009). Genetic diversity measures using MS yield reliable estimations of variability within and genetic relationships among chicken populations (Kayang *et al*., 2010). The use of molecular information in selection and breeding programs has the power to increase productivity of Japanese quail and to maintain genetic diversity. Characterization of the genetic diversity of indigenous quail lines is a prerequisite for providing needed information for the conservation of useful genotypes.

Japanese quail DNA markers effort was initiated in our laboratory based on the characterization of RAPD and ISSR markers. The objective of the present work was to characterize the possible genetic variations and relationships based on productive traits and DNA markers associated with the selected line for long shank length in the six generation of selection compared to the control line. The development of molecular techniques has been created new possibilities for the selection and genetic improvement of Japanese quail.

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 long shank length selected line was produced from the base population during the course of breeding experiments with Japanese quail. Individual phenotypic selection was carried out at four weeks of age separately for each sex for six generations. Higher shank length (one male and two females) were selected according to their deviation from the mean of their corresponding sexes to produce the parents for the next generation. Randombred control which was maintained as non-selected pedigreed population originated from the unselected base population from which the selected line originated. In control, all eggs laid by the two females of each family were used to produce the parents for the next generation. All birds were housed in the same room in order to keep temperature, humidity, light intensity and other variables uniform as possible. The same diets were provided to birds on the selection process across various generations. Feed and water were provided *ad libitum*. All experimental birds were maintained as possible under the same conditions.

I- Productive traits studies

Traits recorded individually in the present work were 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), number of days needed to produce the first 10 eggs (DN_{10}), egg weight of the tenth egg (EW_{10}) and egg mass of the first 10 eggs (EM_{10}).

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=selected long shank length and control), S_j=sex effect (j=male and female) 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=selected long shank length and control) and e_{ij}=random error term.

II- Molecular studies

Extraction of DNA

Individual blood samples were collected from 10 birds (three females and two males)/line (selected long shank length and control). 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 6 random DNA oligonucleotide primers for RAPD and ISSR synthesized by Operon Biotechnologies, Inc., Germany were independently used in the RAPD and ISSR-PCR reaction mixture, respectively. The primers succeeded to generate reproducible polymorphic DNA products. Table (1) lists the base sequences of these primers that produced informative polymorphic bands.

RAPD and ISSR-PCR reactions

The RAPD-PCR amplification reactions were performed in a 25 µl reaction volumes. RAPD reaction mixture containing 2.5 µl dNTPs (2.5 mM), 1.5 µl $MgCl_2$ (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 ddH_2O. 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 lines and sexes by genotype tested in this study. After electrophoresis, the RAPD and ISSR patterns were visualized with UV Tec. Documentation system. Fragments sizes were estimated with the 1500-100 bp ladder marker.

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 lines. 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=2Cab/(Na+Nb) where BS is band sharing, Cab is the number of common bands shared by individuals a and b, Na and Nb are the total number of bands for individuals a and b.

RESULTS AND DISCUSSION

I- Productive traits studies

Line and sex effects on body weight and shank length at different ages

The results of the effects of line and sex on BW are given in Figs. (1 and 2). Line significantly affected BW at 14, 21, 28, 35 days and age at first egg favoring the long shank length line. However, the two lines insignificantly differed for BW at 1 and 7 days of age (Fig. 1). Sex effect on BW was insignificant at all studied ages, females had higher BW than males at all studied ages except for one day old of age as shown in Fig. (2). The genotype had significant influence on the BW at first egg as reported by Sakunthaladevi *et al.* (2011). Lower value for BW at first egg in brown genotype (154.64 g) was reported by Okenyi *et al.* (2013). Line significantly affected SL at 14, 21, 28 and 35 days and age at first egg favoring the long shank length line. However, the two lines insignificantly differed for SL at 1 and 7 days of age (Fig. 3). Females had higher SL than males at all studied ages except for one day old 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). Sexual dimorphism for BW and SL has been previously reported in favor of the females as cited by Abo Samaha *et al.* (2010), Nath *et al.* (2011), Daikwo *et al*. (2013) and Ojo *et al*. (2014).

Line effect on egg production related traits

Line had significant effect on age at first egg, long shank length line matured at earlier age than the control line by 5.90 days (Fig. 5). Lower values were reported for age at first egg of 51.16 days

(Nath *et al.*, 2011), 55.3 days (Sakunthaladevi *et al*., 2011) and 57.5 days (Punya *et al*., 2012). Long shank length line needed fewer days to produce the first 10 eggs ($P \leq 0.05$) by (two days than the control line, Fig. 5). Bahie El-Deen and El-Sayed (1999) reported that the period needed to produce the first 10 eggs for the control and selected line were 13.32 and 13.10 days after 3 generations of selection for BW at 6 weeks. Similarly, Tawefeuk (2001) reported that there were a decrease in days needed to produce the first 10 eggs in the selected line for age at sexual maturity and days needed to produce the first 10 eggs from 100% to 54% (relative to control line in the same generation) in the base population to the 4^{th} generations. Mahmoud *et al.* (2014) reported an estimate of 15.95 days for days needed to produce the first 10 eggs in Japanese quail. The females of the selected line laid higher first egg weight (11.88 g), EW_{10} (11.60 g) and EM_{10} (116.00 g) than the first egg weight means of control line (10.87 g), EW_{10} (11.59 g) and EM_{10} (112.8 g) with insignificant differences between them (Fig. 6). These results are in accordance with previous studies reported by Nath *et al.* (2011) 7.60-12.81 g and Okenyi *et al.* (2013) 11.41 g for egg weight. Mahmoud *et al.* (2014) reported an estimate of 114.23 g for the first ten eggs laid in Japanese quail. From the present results, it can be concluded that selected long shank length line had favored growth traits and studied egg production related traits.

II- Molecular studies

Identification of RAPD-PCR markers

Six random primers with the DNA of the 10 individuals quail in the PCR reaction generated reproducible and scoreable RAPD profiles (Plate 1). These primers detected scoreable polymorphisms in banding patterns among the individuals (Table 2). The results are in accordance with the earlier observations. RAPD markers were tested to detect polymorphism in quail lines, and were found to be effective using 6 primers (Sharma *et al*., 2000) and 31.7% bands were found to be polymorphic. Two selected quail and control lines were analyzed using RAPD-PCR with 6 primers (Ali *et al*., 2002), and the data suggested that RAPD markers are useful for studying the genetic variability among lines. The numbers of amplified fragments are summarized in Table (3). These produced multiple band profiles with a number of amplified DNA fragments ranging from 4-11. A total number of 456 bands were generated and polymorphism levels differed from one primer to the other. While, the number of polymorphic fragments ranged from 2-6. A maximum number of 95 amplicons was amplified with primer OPQ-15, while the minimum (58) was amplified with primer OPA-10. The highest number of polymorphic bands was detected for primer OPA-18 (6 out of 11) while the lowest was detected for primer OPC-03 and OPQ-15 (2 out of 8 and 2 out of 11, respectively). However, 31 bands were monomorphic for all primers and genotypes. Primer OPA-10 exhibited the highest percentage

(72.727%) of polymorphism, while primer OPC-03 exhibited the lowest (25%) (Table 4). The average percentage of polymorphism was 48.333%, while 85.315% polymorphism was reported by Mansour *et al*. (2010) and Karabağ and Balcioğlub (2010) reported that polymorphism was 99.49%. The total number of polymorphic amplicons obtained by the 6 studied primers was 29. This corresponds to a level of polymorphism of 48.333% and an average number of polymorphic fragments/primer of 4.833. The specific markers for quail genotypes generated from RAPD analysis are shown in Tables (2 and 3). The number of RAPD specific markers were scored for female 3 and male 4 of the selected line (1 marker), one marker was scored for females 6, 7 and males 9, 10 of the control line. A number of 4 positive specific markers were scored for the presence of unique bands for a given genotype, while 4 negative specific markers were scored for the absence of a common band. The highest number of RAPD genotype-specific markers was generated by primer OPA-10 and OPQ-15 (3 markers/primer), primers OPA-18 and OPB-10 generated the least number (1 markers/primer). On the other hand, the primer OPC-03 and OPZ-09 generated zero markers. RAPD marker of individual quail showed characteristic pattern and individuals could be distinguishable from each other even within the same line. RAPD technique based on 6 primers was applied to detect genetic similarity between selected chicken strains (Ali *et al*., 2003), while 12 primers were used by Okumus and Kaya (2005). RAPD markers can be generated and giving a genotype-specific pattern in quails (Salem *et al*., 2005). Two chicken breed individuals males and females were analyzed using RAPD and SSR markers to characterize the genotypes and to estimate their genetic diversity (Al-Jallad *et al*., 2012).

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 (selected long shank length and control, 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 female 7 of the control and female 3 of the selected line. However, the lowest similarity index (0) was observed between male 10 and female 8 of the control and male 10 and male 9 of the control. 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 quail genotypes into two clusters (groups). The first group consisted of female 1 of the selected line which was delimited from the rest genotypes, female 3 and male 4 of the selected line were separated in one cluster from the rest of studied quail genotypes. The se-

cond group was subdivided into two subgroups. The first subgroup involved female 6 control was delimited from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup includes female 2 selected line was delimited from the rest of genotypes, female 7 control was delimited from the rest of genotypes, male 5 selected line was delimited from the rest of genotypes and female 8 control was delimited from the rest of genotypes, and the second subgroup was involved males 9 and 10 control. Based on RAPD analysis, the selected long shank length genotype females and male were clustered in the same group while, selected female and male, and control females and control males were in separate clusters.

In conclusion, 6 primers used in the present study allowed enough distinction among the quail genotypes selected long shank length and control, females and males. A RAPD marker is effective to detect similarity between Japanese quail lines and is potential tool for studying genetic relationships.

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 was reported by Kayang *et al.* (2004), MS-based markers were used in quail genetic analysis (Kim *et al*., 2007). Six random ISSR primers generated reproducible and scoreable ISSR profiles with the DNA of the 10 individual quails in the PCR reaction (Plate 2). These primers detected scoreable polymorphisms in banding patterns (Table 2). In this respect, Mansour *et al*. (2010) reported that genetic diversity of four genotypes of quail was analyzed by 15 MS markers. Each of the 6 primers used for analysis of individual quail lines 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 5-12. A total number of 470 bands were generated, and polymorphism levels differed from one primer to the other. While, the number of polymorphic fragments ranged from 2-3. A maximum number of 96 amplicons was amplified with primer HB-14, while the minimum (63) with primer HB-09. The highest number of polymorphic bands was detected for primer 44B (3 out of 9) and primer HB-09 (3 out of 8) and primer HB-10 (3 out of 11) while, the lowest was for primer HB-13 (2 out of 8) and primer HB-15 (2 out of 10). However, 31 bands were common for all primers and genotypes. Primer 44B exhibited the highest percentage (66.667%) of polymorphism, while primer HB-13 exhibited the lowest 25% (Table 4). In this respect, Mansour *et al*. (2010) found that the percentage of polymorphism in four quail phenotypes was 94.10%. The total number of polymorphic amplicons was 27. This corresponds to a level of polymorphism of 46.552% and an average number of polymorphic fragments/primer of 4.5. ISSR are considered

to be appropriate molecular tools for studying genetic biodiversity and relationships.

The specific markers for quail lines generated from ISSR-PCR analysis are shown in Tables (2 and 3). The highest number of ISSR specific markers was scored for female 7 control (two markers), while the lowest (one marker) was scored for females, 1 and 2 and male 5 selected line. A number of 4 positive specific markers were scored for the presence of unique bands for a given lines, while 7 negative specific markers was scored for the absence of a common band. The highest number of ISSR genotype-specific markers was generated by primer 44B, HB-10 and HB-14 (3 markers) and primer HB-15 generated (two markers). On the other hand, the primers HB-09 and HB-13 generated zero markers. 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. Kayang *et al*. (2002) reported that 98 MS markers were polymorphic in the Japanese quail genome. Farrag *et al*. (2011) examined three Japanese quail lines genetically to detect genetic diversity using 13 MS markers. Genomic SSRs, MS have been used for estimation of genetic diversity, phylogenetic and conservation genetic purposes in farm animal breeding (Teneva *et al*., 2013).

Phylogenetic relationship among quail genotypes based on ISSR marker

Based on the combined data obtained through the polymorphism of ISSR profiles, the similarity coefficient values among the studied 10 individuals of quail (selected long shank length and control, males and females) were calculated according to Dice (1945) equation (Table 6). The similarity coefficient showed an average genetic distance ranging from 0-1 with a mean value of 0.5. The highest similarity index (1) was recorded between male 10 of the control and male 5 of the selected line. However, the lowest similarity index (0) was observed between female 8 of the control and male 4 of the selected line. The data obtained from the analysis of ISSR was used to draw precise relationships. 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 males 9 and 10 control which were delimited in separate one cluster from the rest of studied quail genotypes. The second group was subdivided into two subgroups. The first subgroup was subdivided into two subgroups, the first subgroup involved female 7 control and was delimited from the rest of genotypes, and the second subgroup was subdivided into two subgroups. The first subgroup included females 1 and 2 selected line and were separated in one subgroup from the rest of genotypes, and the second subgroup included male 5 selected line and female 6 control and were separated in one subgroup. The second subgroup

was subdivided into two subgroups, the first subgroup involved female 3 selected line and was delimited from the rest of genotypes, and the other subgroup included male 4 selected line and female 8 control. Based on ISSR analysis, the control males genotypes were delimited in the same group while, selected long shank length females, males and control females were clustered in separate one cluster.

In conclusion, six primers used in the present study allowed enough distinction among the quail genotypes selected long shank length and control, females and males. An ISSR marker is effective to detect similarity between Japanese quail lines and is potential tool for studying genetic relationships.

Phylogenetic relationship among quail genotypes 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 intersimple 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 the studied 10 genotypes were calculated according to Dice (1945) equation (Table 7). The similarity coefficients show an average genetic distance ranging from 0-1 with a mean value of 0.5. The highest similarity index (1) was recorded between male 9 of the control and female 1 of the selected line. However, the lowest similarity index (0) was observed between male 9 and female 8 of the control. The data obtained from the analysis of RAPD and ISSR was used to draw precise relationships among 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 two subgroups, the first subgroup included females 1 and 2 selected line and the second subgroups involved female 3 and male 4 selected line. The second group was subdivided into two subgroups. The first subgroup involved female 7 control which was delimited from the rest of genotypes, and the second subgroup involved male 5 selected line and female 6 control line. The second subgroup was subdivided into two subgroups. The first subgroup includes male 10 control delimited in separate one cluster from the rest of quail genotypes, and the second subgroup included female 8 and male 9 control line. Based on RAPD and ISSR combination analysis, the selected genotype females and male were clustered in the same group, and selected genotype male and control females and males genotypes were in separate clusters.

The different types of DNA 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 tetranucleotide units. Therefore, combining the data obtained from the different types of markers may reveal more informative genetic relationships. Dendrogram based on the RAPD and ISSR data showed a clear separation between individuals belonging to each line.

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 selected and control genotypes in one cluster even though, selected genotype females and males delimited in separate group. They might share some genes between selected long shank length and control lines through six generations of selections in selected line. The control line was the original line from which the selected long shank length line had selected. RAPD and ISSR 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 lines. We have described informative Japanese quail RAPD and ISSR markers that would form a useful base of DNA markers as part of our initiative to develop a genetic map for Japanese quail. The result of molecular genetic analysis is in agreement with the result of productive traits. The productive traits and molecular genetic analysis successfully distinguished between the two lines of quail, selected line for long shank length and control line, females and males. The results obtained with the different markers proved the presence of genetic variability between the individuals analyzed within and between the two lines.

SUMMARY

The main objectives of this investigation were to characterize the possible genetic and productive traits differences associated with the selected line of long shank length after six generations of selections in Japanese quail compared to the control line. Productive traits and DNA markers were used to identify these lines. Line significantly affected BW and SL at 14, 21, 28 and 35 days and age at first egg favoring the selected line. Females had higher insignificant ($P > 0.05$) BW and SL than males at all studied ages except for one day old of age. Selected line matured at earlier ($P \leq 0.05$) age and had shorter ($P \leq 0.05$) days needed to produce the first 10 eggs than the control line. The selected line laid higher first egg weight, EW_{10} and EM_{10} than the control line with insignificant differences between them. From the present results, it can be concluded that selected long shank length line had favored growth traits and studied egg production related traits. The level of polymorphism among two Japanese quail lines was estimated using two PCR-based DNA marker techniques RAPD and ISSR. Each line represented by three females and two

males. Six RAPD and 6 ISSR primers were employed to find out genetic variations and relationships among these genotypes. RAPD and ISSR analysis generated a total number of 456 and 470 amplicons representing a level of polymorphism of 48.333% and 46.552%, and an average number of polymorphic fragments/primer of 4.833 and 4.5, respectively. The genetic relationships among the 10 individuals of quail were estimated in terms of similarity using Dice coefficients. The genetic similarity ranged from 0-1 for RAPD, ISSR, and RAPD and ISSR combination. The inter-line relationships among the two quail lines based on RAPD, ISSR, and RAPD and ISSR combination revealed the highest genetic similarity between female of the control line and female of the selected line, male control and male selected line, and male control and female selected line, respectively. The inter-line relationships among the two quail lines based on RAPD, ISSR and RAPD and ISSR combination revealed the lowest genetic similarity between male and female control line and male and male control line, female control and male selected line, and male and female control line, respectively. The RAPD based dendrogram clustered the selected long shank length females and male genotypes in the same group while, selected female, selected male and control females and males were in separate clusters. The ISSR based dendrogram clustered the control males in the same group while, control females and selected females and males were delimited in separate one cluster. The RAPD and ISSR combination based dendrogram clustered the selected females and males in the same group, and selected male and control females and males in separate clusters. However, the reshuffling in the position of the selected long shank length and control genotypes belonging to the individuals in the different dendrograms revealed that they share common genetic background. They might share some genes between selected and control lines through selection in selected line. Moreover, each of the RAPD and ISSR was successful in identifying genotype-specific markers characterizing 10 individuals of Japanese quail. The productive traits and molecular genetic analysis used in the present study successfully distinguished between the two lines, selected line of long shank length and control line, females and males to estimate the genetic variability between them in order to enhance selection and breeding programs.

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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-10	GTG ATC GCA G	ISSR	44B	CTC TCT CTC TCT CTC TG
	OPA-18	AGG TGA CCG T		HB-09	GTG TGT GTG TGT GG
	OPB-10	CTG CTG GGA C		HB-10	GAG AGA GAG AGA CC
	OPC-03	GGG GGT CTT T		HB-13	GAG GAG GAG GC
	OPQ-15	GAT GAC CGC C		HB-14	CTC CTC CTC GC
	OPZ-09	CAC CCC AGT C		HB-15	GTG GTG GTG GC

Table (2): Banding patterns of RAPD and ISSR-PCR for two lines (selected for long shank length and control, females and males) of Japanese quail.

RAPD Primers	Band No.	BP	1	2	3	4	5	6	7	8	9	10	ISSR Primers	Band No.	BP	1	2	3	4	5	6	7	8	9	10
OP-A10	1	700	1	1	1	0	0	0	0	0	0	0	44B	1	600	1	1	1	1	1	1	1	1	1	0
	2	600	0	1	0	0	0	0	1	0	0	0		2	550	0	0	0	0	0	0	1	0	0	0
	3	540	0	1	1	1	1	1	0	1	1	1		3	490	1	1	1	1	1	1	0	1	1	1
	4	520	0	0	0	0	0	0	0	0	1	0		4	415	0	0	0	0	0	0	1	0	1	1
	5	415	0	1	0	0	1	1	0	0	1	1		5	390	0	1	1	1	1	1	0	1	1	1
	6	375	0	0	0	0	0	1	0	0	0	0		6	340	1	1	1	1	1	1	1	1	1	1
	7	350	1	1	1	1	1	1	1	1	1	1		7	300	1	1	1	1	1	1	1	1	1	1
	8	265	1	1	1	1	1	1	1	1	1	1		8	260	1	1	1	1	1	1	1	1	1	1
	9	210	1	1	0	0	1	0	1	1	1	1		9	200	1	1	0	1	1	1	0	1	1	1
	10	190	0	0	0	0	0	0	1	0	0	0													
	11	160	1	1	1	1	1	1	1	1	1	1													
OP-A18	1	950	1	1	1	1	1	1	1	1	1	1	HB-09	1	840	1	1	1	1	1	1	1	1	1	1
	2	845	1	1	1	1	1	1	1	1	1	1		2	810	1	1	1	1	1	1	1	1	1	1
	3	730	0	0	0	0	1	1	1	1	1	1		3	770	0	0	0	0	1	1	1	0	0	0
	4	700	0	0	1	1	1	1	1	1	1	0		4	650	0	0	0	0	1	1	1	0	0	0
	5	580	1	1	1	1	1	1	1	1	1	1		5	540	0	1	1	1	1	1	1	1	0	0
	6	520	1	1	1	1	1	1	1	1	1	1		6	410	1	1	1	1	1	1	1	1	1	1
	7	445	1	0	0	0	1	1	0	0	0	0		7	315	1	1	1	1	1	1	1	1	1	1
	8	310	1	1	1	0	1	1	1	1	1	1		8	225	1	1	1	1	1	1	1	1	1	1
	9	290	0	1	0	0	1	1	1	1	1	1													
	10	220	0	1	0	0	0	0	1	1	1	1													
	11	205	0	1	0	0	0	1	0	1	0	1													

Table (2): Cont'

RAPD Primers	Band No.	BP	1	2	3	4	5	6	7	8	9	10	ISSR Primers	Band No.	BP	1	2	3	4	5	6	7	8	9	10
OP-B10	1	795	0	0	0	0	1	0	1	0	0	0	HB-10	1	1790	0	0	0	0	0	0	0	0	1	0
	2	710	1	1	1	1	1	1	1	1	1	0		2	1580	0	0	1	1	0	0	1	1	1	1
	3	680	1	1	1	1	1	1	1	1	1	1		3	1400	1	0	1	1	0	1	1	1	1	1
	4	615	1	1	0	0	0	0	0	1	0	0		4	940	1	0	1	1	1	1	1	1	1	1
	5	510	1	1	1	1	1	1	1	1	1	1		5	800	1	1	1	1	1	1	1	1	1	0
	6	460	1	1	1	1	1	1	1	1	1	1		6	715	1	1	1	1	1	1	1	1	1	1
	7	250	0	0	0	1	0	1	1	0	0	0		7	625	1	1	1	1	1	1	1	1	1	1
	8	150	1	1	1	1	1	1	1	1	1	1		8	550	1	1	1	1	1	1	1	1	1	1
	9	80	1	1	1	1	1	1	1	1	1	1		9	480	1	1	1	1	1	1	1	1	1	1
														10	400	1	1	1	1	1	1	1	1	1	1
														11	340	1	1	1	1	0	1	1	1	0	0
OP-C03	1	790	1	1	0	1	1	0	1	1	1	1	HB-13	1	860	0	0	0	0	1	1	1	1	1	0
	2	675	1	1	1	1	1	1	1	1	1	1		2	790	0	0	0	1	1	0	0	1	1	0
	3	570	1	1	1	1	1	1	1	1	1	1		3	700	1	1	1	1	1	1	1	1	1	1
	4	450	1	1	1	1	1	1	1	1	0	0		4	650	1	1	1	1	1	1	1	1	1	1
	5	340	1	1	1	1	1	1	1	1	1	1		5	515	1	1	1	1	1	1	1	1	1	1
	6	280	1	1	1	1	1	1	1	1	1	1		6	490	1	1	1	1	1	1	1	1	1	1
	7	245	1	1	1	1	1	1	1	1	1	1		7	440	1	1	1	1	1	1	1	1	1	1
	8	220	1	1	1	1	1	1	1	1	1	1		8	380	1	1	1	1	1	1	1	1	1	1
OP-Q15	1	765	1	1	1	1	1	0	1	1	1	1	HB-14	1	1285	1	1	1	1	1	1	1	1	1	1
	2	470	0	1	1	1	1	0	1	1	1	1		2	960	1	1	0	1	1	1	1	0	0	1
	3	450	1	1	1	1	1	1	1	1	1	1		3	790	1	0	1	1	0	1	1	0	0	1
	4	400	1	1	1	1	1	1	1	1	1	1		4	680	1	1	1	1	1	1	1	1	1	1
	5	360	1	1	1	1	1	1	1	1	1	1		5	590	1	1	1	1	1	1	1	1	0	1
	6	340	1	1	1	1	1	1	1	1	1	1		6	490	0	0	0	0	0	0	0	0	0	1
	7	315	1	0	0	1	1	1	1	1	1	1		7	400	1	1	1	1	1	1	1	1	1	1
	8	240	0	0	0	0	0	0	1	0	0	0		8	330	1	1	1	1	1	1	1	1	1	1
	9	190	1	1	1	1	1	1	1	1	1	1		9	300	1	1	1	1	1	1	1	1	1	1
	10	165	1	1	1	1	1	1	1	1	1	1		10	280	1	1	1	1	1	1	1	1	1	1
	11	115	1	1	0	1	1	1	1	1	1	1		11	260	1	1	1	1	0	1	1	1	1	1
														12	170	0	0	0	0	0	0	1	1	1	1
OP-Z09	1	1250	1	1	0	0	0	0	0	0	0	0	HB-15	1	1410	0	0	1	1	1	1	1	1	1	0
	2	800	0	0	1	1	0	0	0	0	0	0		2	1360	0	1	1	1	1	1	1	1	1	1
	3	730	1	1	1	1	1	1	1	1	1	1		3	1160	1	1	1	1	1	1	1	1	1	1
	4	640	1	1	1	1	1	1	1	1	1	1		4	1120	1	1	1	1	1	1	1	1	1	1
	5	550	1	1	1	1	1	1	1	1	1	1		5	875	1	1	1	1	1	1	1	1	1	1
	6	490	1	1	1	1	1	1	1	1	1	1		6	745	1	1	1	1	1	1	1	1	1	1
	7	435	1	1	1	1	1	1	1	1	1	1		7	690	0	0	0	1	1	1	1	1	1	1
	8	400	1	1	1	1	1	1	1	1	1	1		8	525	1	1	1	1	1	1	1	1	1	1
	9	350	0	1	1	0	0	0	0	1	1	1		9	410	0	0	0	0	0	0	0	0	0	1
	10	280	1	1	1	1	1	1	1	1	1	1		10	300	1	1	1	1	1	1	1	1	1	1

1, 2, 3 = Females selected long shank length, 4, 5 = Males selected long shank length, and 6, 7, 8 = Females control and 9, 10 = Males control.

Table (3): Number of amplified fragments markers of two quail lines (selected long shank length and control) based on RAPD and ISSR-PCR analysis.

lines		RAPD primers							ISSR primers						
		OPA-10	OPA-18	OPB-10	OPC-03	OPQ-15	OPZ-09	Total	44B	HB-09	HB-10	HB-13	HB-14	HB-15	Total
1 Female long	AF	5	6	7	8	9	8	43	6	5	9	6	10	6	42
	SM	0	0	0	0	0	0	0	0	0	0	0	0	1	1
2 Female long	AF	8	8	7	8	9	9	49	7	6	7	6	9	7	42
	SM	0	0	0	0	0	0	0	0	0	1	0	0	0	1
3 Female long	AF	5	6	6	7	8	9	41	6	6	10	6	9	8	45
	SM	0	0	0	0	1	0	1	0	0	0	0	0	0	0
4 Male long	AF	4	5	7	8	10	8	42	7	6	10	7	10	9	49
	SM	0	1	0	0	0	0	1	0	0	0	0	0	0	0
5 Male long	AF	6	9	7	8	10	7	47	7	8	7	8	8	9	47
	SM	0	0	0	0	0	0	0	0	0	0	0	1	0	1
6 Female control	AF	6	10	7	7	8	7	45	7	8	9	7	10	9	50
	SM	1	0	0	0	1	0	2	0	0	0	0	0	0	0
7 Female control	AF	6	9	8	8	11	7	49	6	8	10	7	11	9	51
	SM	1	0	0	0	1	0	2	2	0	0	0	0	0	2
8 Female control	AF	5	10	7	8	10	8	48	7	6	10	8	9	9	49
	SM	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9 Male control	AF	7	9	6	7	10	8	47	8	5	10	8	8	9	48
	SM	1	0	0	0	0	0	1	0	0	1	0	1	0	2
10 Male control	AF	6	9	5	7	10	8	45	7	5	8	6	12	9	47
	SM	0	0	1	0	0	0	1	1	0	1	0	1	1	4
PB		5	6	3	2	2	3	21	3	3	3	2	3	2	16
TAF		58	81	67	76	95	79	456	68	63	90	69	96	84	470
TSM		3	1	1	0	3	0	8	3	0	3	0	3	2	11
MB		3	4	5	6	6	7	31	3	5	5	6	6	6	31

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

Marker	Primer	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	Polymorphism%
RAPD	OPA-10	11	3	8	72.727
	OPA-18	11	4	7	63.636
	OPB-10	9	5	4	44.444
	OPC-03	8	6	2	25.000
	OPQ-15	11	6	5	45.454
	OPZ-09	10	7	3	30.000
	Total	60	31	29	48.333
	Average	10	5.167	4.833	48.333
ISSR	44B	9	3	6	66.667
	HB-09	8	5	3	37.500
	HB-10	11	5	6	54.545
	HB-13	8	6	2	25.000
	HB-14	12	6	6	50.000
	HB-15	10	6	4	40.000
	Total	58	31	27	46.552
	Average	9.667	5.167	4.500	46.550

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

Genotypes	1 Female long	2 Female long	3 Female long	4 Male long	5 Male long	6 Female control	7 Female control	8 Female control	9 Male control
2 Female long	0.61								
3 Female long	0.80	0.71							
4 Male long	0.61	0.87	0.33						
5 Male long	0.46	0.57	0.74	0.38					
6 Female control	0.85	0.93	0.95	0.71	0.27				
7 Female control	0.71	0.64	1.00	0.64	0.35	0.86			
8 Female control	0.71	0.32	0.64	0.46	0.16	0.52	0.24		
9 Male control	0.89	0.48	0.82	0.64	0.16	0.69	0.41	0.08	
10 Male control	0.82	0.24	0.92	0.74	0.25	0.61	0.50	0.00	0.00

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

Genotypes	1 Female long	2 Female long	3 Female long	4 Male long	5 Male long	6 Female control	7 Female control	8 Female control	9 Male control
2 Female long	0.23								
3 Female long	0.30	0.30							
4 Male long	0.27	0.27	0.01						
5 Male long	0.81	0.46	0.69	0.32					
6 Female control	0.35	0.33	0.25	0.07	0.08				
7 Female control	0.76	0.93	0.49	0.45	0.63	0.22			
8 Female control	0.61	0.44	0.17	0.00	0.32	0.23	0.45		
9 Male control	0.88	0.88	0.51	0.40	0.57	0.63	0.69	0.08	
10 Male control	0.64	0.81	0.89	0.41	1.00	0.72	0.78	0.65	0.57

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

Genotypes	1 Female long	2 Female long	3 Female long	4 Male long	5 Male long	6 Female control	7 Female control	8 Female control	9 Male control
2 Female long	0.37								
3 Female long	0.57	0.53							
4 Male long	0.48	0.66	0.09						
5 Male long	0.73	0.59	0.78	0.38					
6 Female control	0.67	0.74	0.61	0.32	0.16				
7 Female control	0.90	0.69	0.84	0.55	0.49	0.53			
8 Female control	0.66	0.31	0.39	0.21	0.25	0.40	0.42		
9 Male control	1.00	0.74	0.72	0.53	0.36	0.71	0.63	0.00	
10 Male control	0.86	0.60	0.90	0.71	0.74	0.78	0.80	0.36	0.27

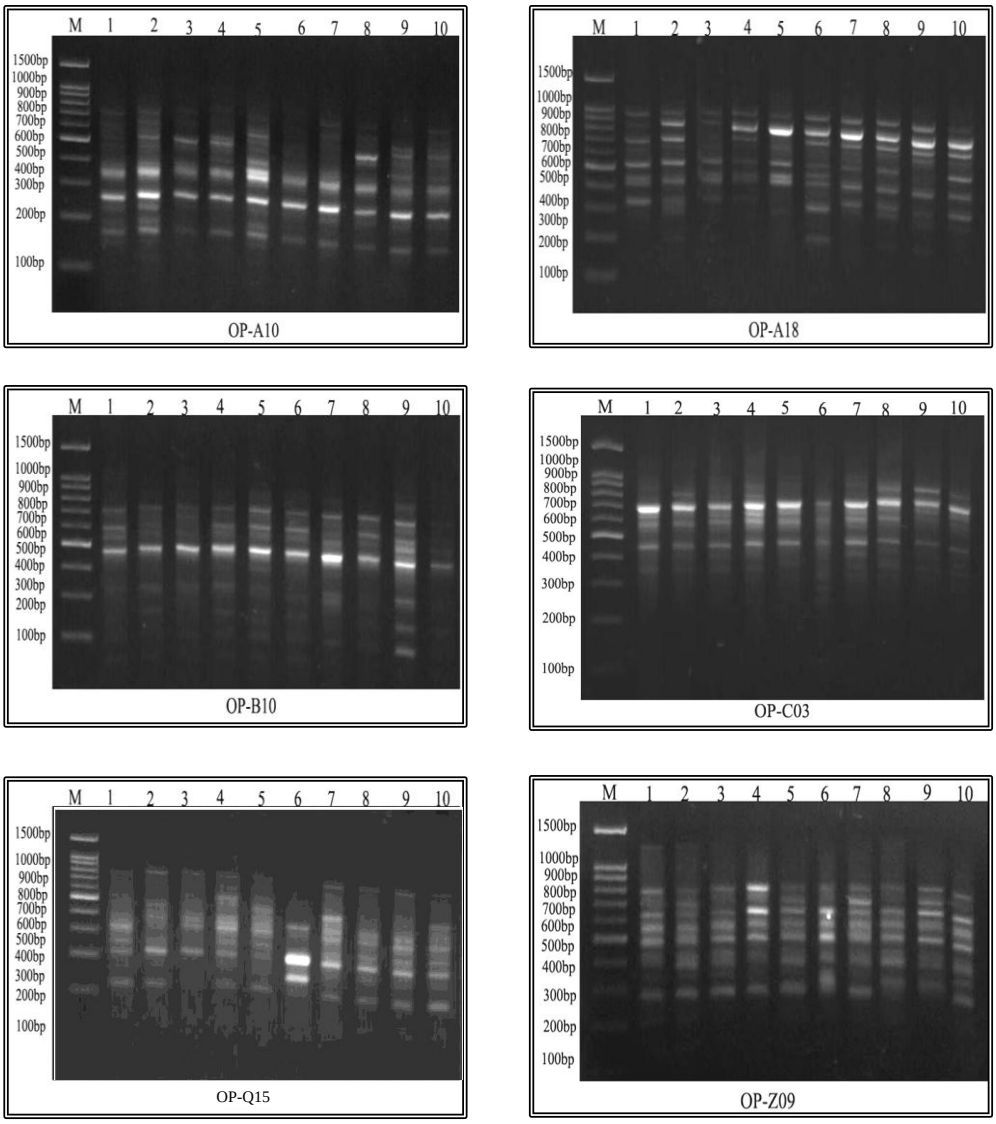


Plate (1): RAPD profile of the selected long shank length (SL) and control (C) Japanese quail lines amplified with 6 different RAPD primers. M=Ladder marker, 1, 2 and 3=Females SL, 4 and 5=Males SL, 6, 7 and 8=Females C and 9 and 10=males C.

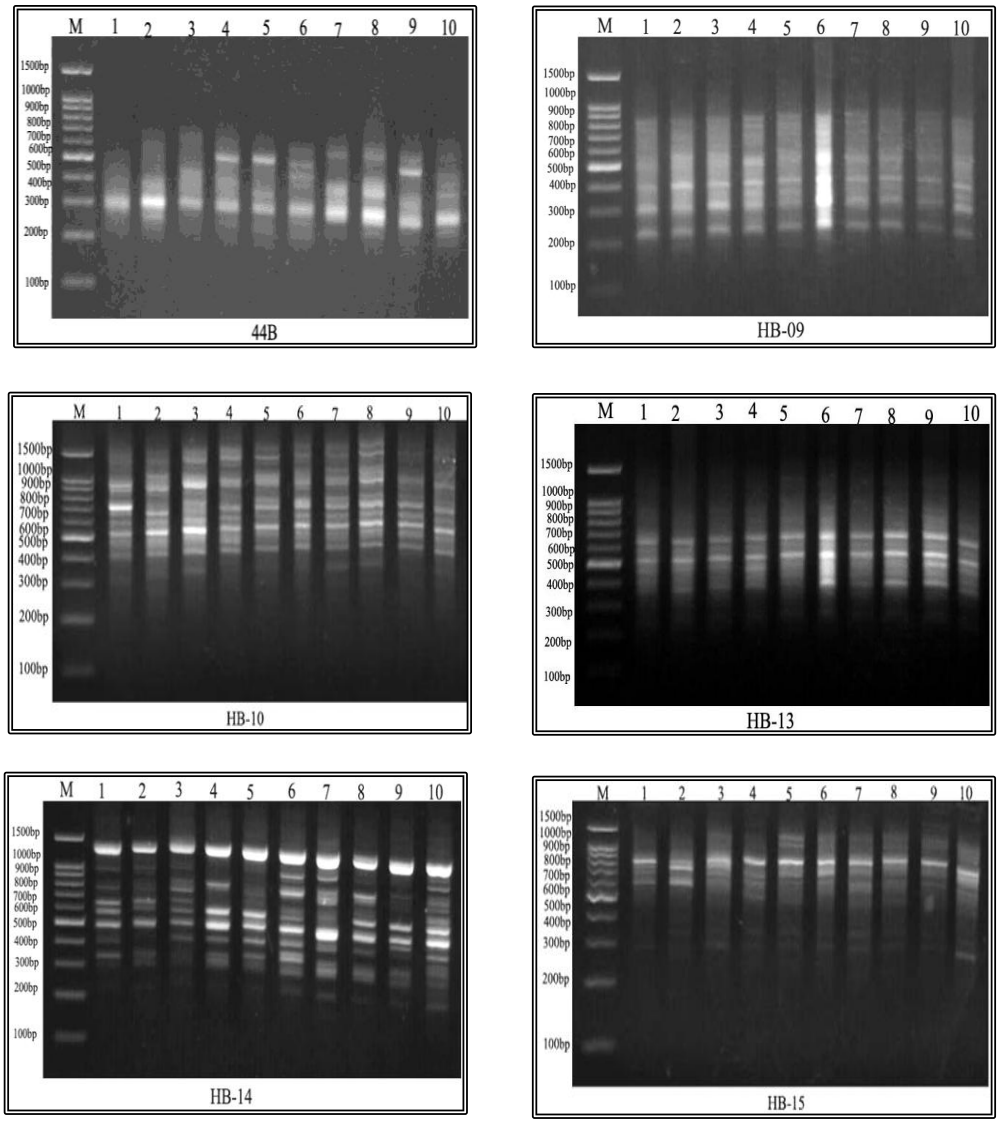


Plate (2): ISSR profile of the selected long shank length (SL) and control (C) Japanese quail lines amplified with 6 different ISSR primers. M=Ladder marker, 1, 2 and 3=Females SL, 4 and 5=Males SL, 6, 7 and 8=Females C and 9 and 10=Males C.

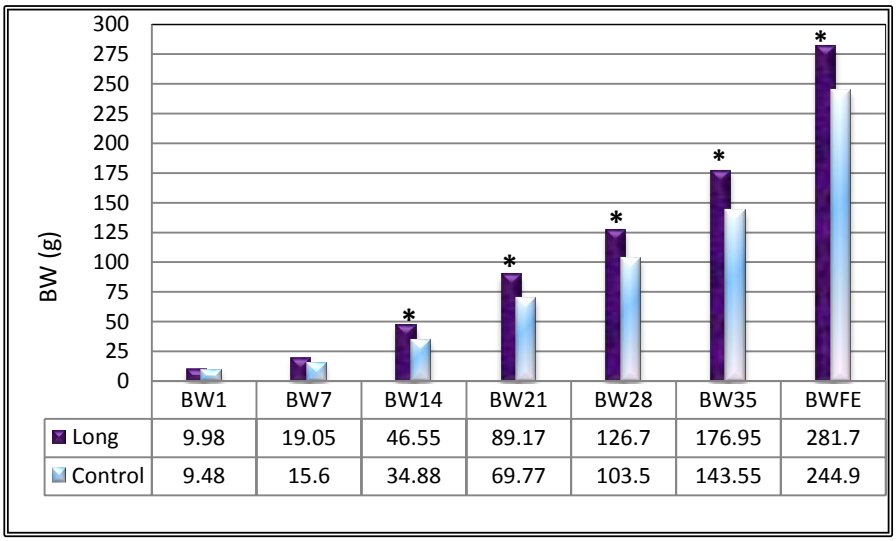


Fig. (1): Effect of line on BW at different ages (BW_1-BW_{35}=Body weight at one day-old to 35 days old, BWFE=Body weight at first egg) of Japanese quail, *= Significant difference ($P < 0.05$).

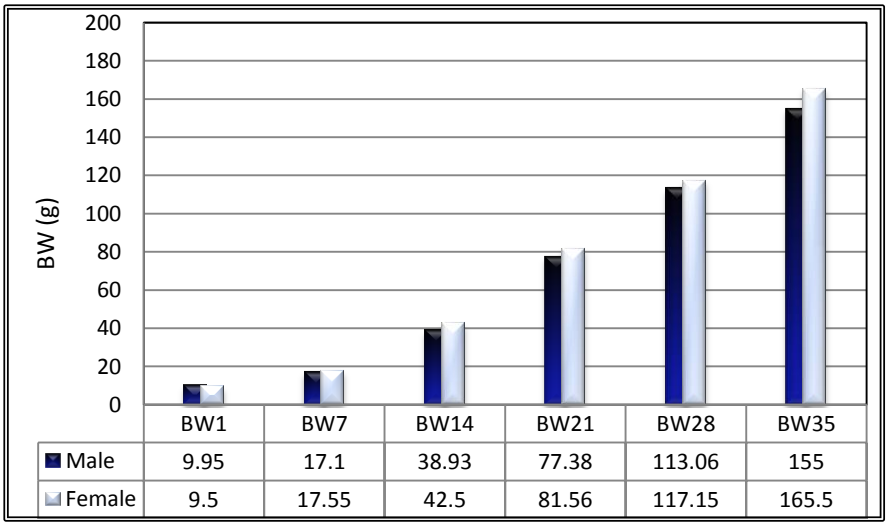
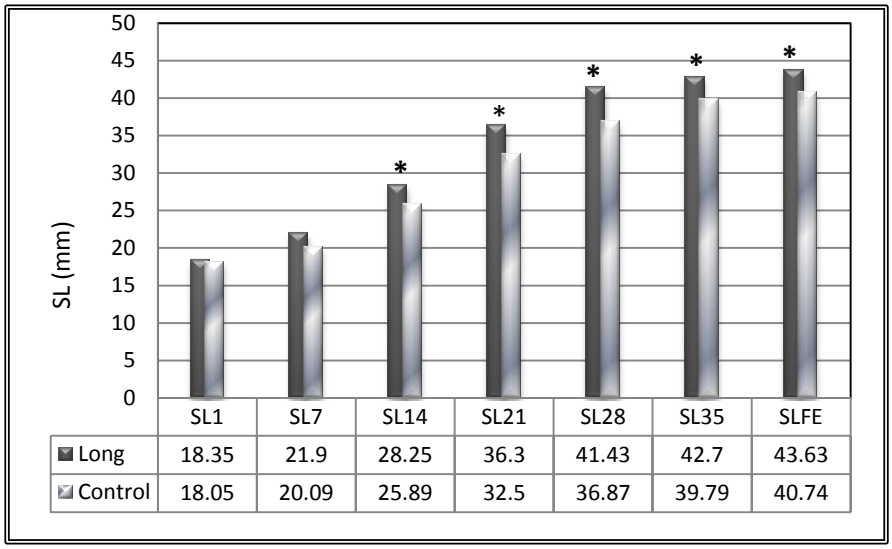
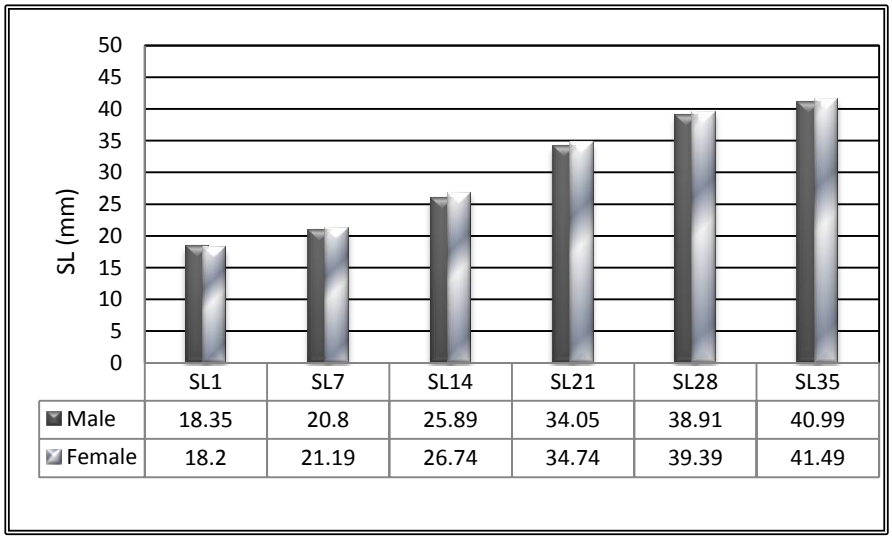


Fig. (2): Effect of sex on BW at different ages (BW_1-BW_{35}=Body weight at one day-old to 35 days old) of Japanese quail.



	SL1	SL7	SL14	SL21	SL28	SL35	SLFE
Long	18.35	21.9	28.25	36.3	41.43	42.7	43.63
Control	18.05	20.09	25.89	32.5	36.87	39.79	40.74

Fig. (3): Effect of line on SL at different ages (SL_1-SL_{35}=Shank length at one day-old to 35 days old, SLFE=Shank length at first egg) of Japanese quail, *=Significant difference ($P < 0.05$).



	SL1	SL7	SL14	SL21	SL28	SL35
Male	18.35	20.8	25.89	34.05	38.91	40.99
Female	18.2	21.19	26.74	34.74	39.39	41.49

Fig. (4): Effect of sex on SL at different ages (SL_1-SL_{35}=Shank length at one day-old to 35 days old) of Japanese quail.

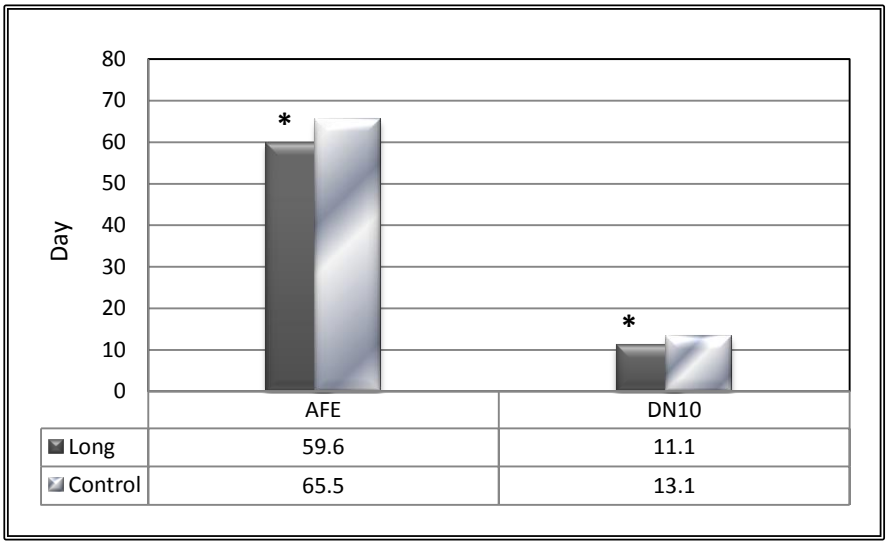


Fig. (5): Effect of line on age at first egg (AFE) and number of days needed to produce the first 10 eggs (DN_{10}) of Japanese quail, *=Significant difference ($P < 0.05$).

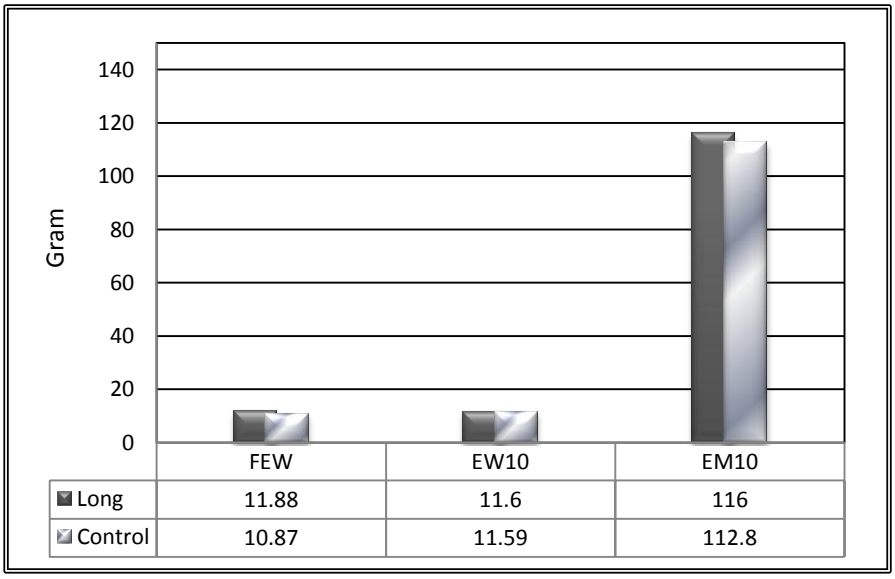


Fig. (6): Effect of line on first egg weight (FEW), egg weight of the tenth egg (EW_{10}) and egg mass of the first 10 eggs (EM_{10}) of Japanese quail.

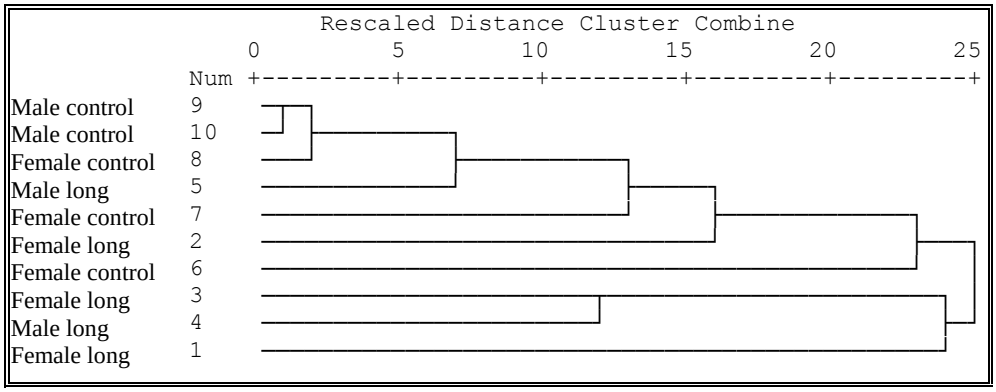


Fig. (7): Dendrogram for the 10 individual quails constructed from the RAPDs data using UPGMA and similarity matrix computed according to Dice coefficient.

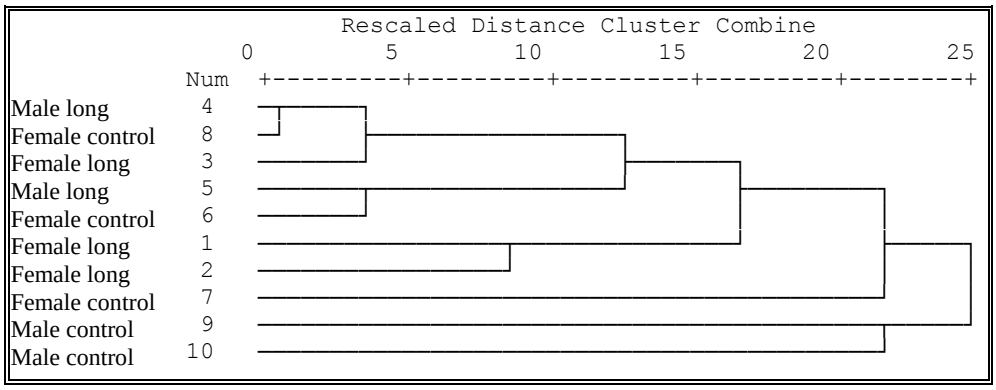


Fig. (8): Dendrogram for the 10 individual quails constructed from the ISSRs data using UPGMA and similarity matrix computed according to Dice coefficient.

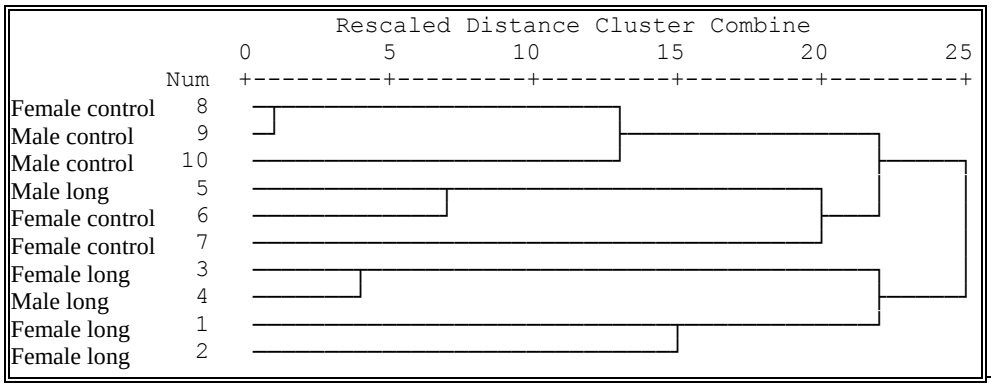


Fig. (9): Dendrogram for the 10 individual quails constructed from the RAPDs and ISSRs data using UPGMA and similarity matrix computed according to Dice coefficient.