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CHARACTERIZATION OF SELECTED JAPANESE QUAIL AND RANDOMBRED CONTROL LINES IN THE 4th GENERATION BASED ON PRODUCTIVE PERFORMANCE AND RAPD-PCR ANALYSIS

E. A. Eissa*; Bothaina Y. Mahmoud; E. M. El-Komy***and Ensaf A. El-Full****

****Genet. Dept., Fac. of Agric., Fayoum Univ., 63514 Fayoum, Egypt***
*****Poult. Prod. Dept., Fac. of Agric., Fayoum Univ., 63514 Fayoum, Egypt***
******Anim. Prod. Dept., Agric. and Bio. Res. Div., Nat. Res. Cent., 12612 Dokki, Giza, Egypt***

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ABSTRACT: Productive performance and RAPD analysis were used to find the genetic variations and relatedness among two selected, maternal (line1) and long shank length (line3), and randombred (line2) males and females of Japanese quail in the 4th generation of selection. Body weight (BW) and shank length (SL) were measured at 1, 7, 14, 21, 28 and 35 days of age and BW at sexual maturity. Age at first egg (AFE), Age at 10 eggs (Age_{10}), Age at 30 eggs (Age_{30}), number of days needed to produce the first 10 eggs (DN_{10}), number of days needed to produce the first 30 eggs (DN_{30}), egg mass of the first 10 eggs (EM_{10}) and egg mass of the first 30 eggs (EM_{30}) were recorded individually for each female. Individual blood samples were collected from 60 birds [20 birds (10 males and 10 females/line)] to extract DNA. RAPD-PCR amplification was performed using 10 random primers that succeeded to generate informative polymorphic bands. The results indicated that, line3 had higher BW and SL than line1 and line2 at all studied ages except at one day old, while the line2 had the significant lowest values at all ages. The line1 matured at earlier age than the line2 and line3, and had shorter DN_{10} and DN_{30}. Also, line1 had lower Age_{10} and Age_{30} than the line2 and line3, and had heavier EM_{10} and EM_{30} than the line2 and line3. The differences among lines and sexes were due to the genetic changes resulted

Key Words: Quail; Selection; Maternal; Shank; Randombred; RAPD analysis; Similarity

Corresponding author: eissahmd@yahoo.com

from selection for 4 consecutive generations. Ten primers were examined, produced 286 bands and the number of bands amplified with each primer ranged from 6-53, within a mean of 28.6. The number of polymorphic bands varied from 11.11-100% and the mean of polymorphism percentage across 10 primers was 73.28. The total number of polymorphic amplicons obtained by the primers was 39, and an average number of polymorphic fragments/primer was 3.9. The results of polymorphism demonstrate the efficiency of the used primers to assess the genetic specificity and reflect the genetic diversity in the lines and sexes. The primers also detected 28 unique band specific for lines and sexes. The overall mean genetic similarity between the three lines across 10 primers on the basis of RAPD marker was 0.52 and 0.54, respectively in males and females. The highest similarity (0.86) was recorded within line1 males and females, and the lowest similarity (0.47) was recorded between line1 males and line2 females, and between line1 females and line3 males. The dendrogram clustered the lines into two groups. The first group consisted of line1 males and females were delimited in separate group, while line3 males and females, and line2 males and females were delimited in other separate group. Line3 males and females were separated in one sub-group, from the rest of sub-group line2 males and females. This result seems to be reliable since it goes with the expectation of clustering males and females in the same line in one cluster. Gathering both line3 and line2 in one cluster even though, line1 delimited in separate group. They might share some genes between selected lines and randombred line through successive selections during four generations of selection which started in randombred population. The randombred population was the original from which the maternal and long shank length lines had selected. The line1 males and females were delimited in separate group, while the line3 males and females, and line2 males and females were clustered in the same group form another cluster. That means line1 were selected with high pressure of selection more than line3. The same trend was observed for the productive performance and indicated that the line1 has better for all studied egg production-related traits than the other two lines.

INTRODUCTION

Poultry breeds are a national capital in developing countries, their conservation and utilization are important for economy. The Japanese quails are a well-established animal model in biology and used for intensive egg and meat production (Minvielle, 2004). The skeletal development is an important measurement at reaching optimum live weight and uniformity for hens. In addition, shank length is an important measurement of skeletal development (North and Bell, 1990). Shank length has generally high heritability values during the growing period. This suggests that this trait can be improved through individual selection. The researches for relationships between some body measurements and production traits of other animals are used in animal production, to put on the agenda about a relationship between shank length and production traits (Gulinski et al., 1997 and Baco et al., 1998). Several investigators used shank length to predict live body weight in poultry (Amao et al., 2010 and Ojo et al., 2014). However, for improving growth and egg production traits, selection indices are of interest to breeders to select for more than a single trait. To maximize

genetic progress simultaneously in all the traits, it has been suggested that a desirable proposition would be to combine them into an index when the information are available on all the traits. Selection index is the most efficient method of selection for improving genetically antagonistic traits. Index of total performance involving many traits may not cause many changes in body weight and egg size. The ultimate goal of a poultry breeder is to improve the overall genetic economic worth of the bird through multi-trait selection by considering maximum number of traits at a time (Devi and Gupta, 2012).

During the past decades, molecular genetics can be applied with classical breeding in several farm animals including poultry. DNA markers are powerful tools in characterization and estimation of relatedness between genotypes. The random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990) was the first polymerase chain reaction (PCR)-based marker system used in genetic analysis and showed high level of polymorphism among closely related genotypes. The potential applications of RAPD marker reveal its importance as a powerful tool in genetics and breeding in animals. DNA fingerprinting of individuals quail could be distinguishable even within a line (Mannen et al., 1993 and Ye et al., 1998). RAPD markers were found to be effective to detect polymorphism and genetic diversity in quail selected lines (Kumar et al., 2000, Sharma et al., 2000 and Karabağ and Balcioğlub, 2010). The estimation of genetic variability of a species is an important for its conservation and genetic improvement (Rahimi et al., 2005). RAPD analyses have been used for estimating genetic similarity and diversity in chickens (Singh and Sharma, 2002, Ahlawat et al., 2004, El-Gendy et al., 2006, Chatterjee et al., 2009 and Mollah et al., 2009), in Polish goose breeds (Maciuszonek et al., 2005), and genetic diversity in ducks (El-Gendy et al., 2005 and Alyethodi et al., 2010), and in poultry research (Salem et al., 2005). The use of molecular information in selection programmes has the potential to increase productivity and maintain genetic diversity (Naqvi, 2007). Dehghanzadeh et al. (2009) showed that RAPD technique is a useful tool for evaluation of genetic variation among domesticated animals and the ability to detect polymorphisms at the DNA level has led to new approaches for the genetic analysis. Recently using RAPD phylogenetic relationship and diversity established by some authors in chickens (Monira et al., 2011,Yap and Kumaran, 2011, Tamara et al., 2012 and Alatafi et al., 2013). RAPD-PCR method was used also to genetically analyze in cattle (Thiagarajan and Thangaraju, 2011), in rabbit breeds (El-Bayomi et al., 2013). Limited studies have been performed to assess the genetic polymorphism among selected and randombred control lines in Japanese quail using DNA fingerprinting technique.

The aim of the present study was to distinguish among the selected maternal (line1), selected long shank length (line3) and a randombred (line2) of Japanese quail based on productive traits and DNA markers. The lines have been formed by an ongoing selection breeding program aiming to develop Japanese quail productive performance. In addition, the genetic relationship among them was determined using RAPD-PCR analysis.

MATERIALS AND METHODS

Productive performance studies:

The experimental work was carried out on the flock of Japanese quail maintained by the Poultry Research Center,

Faculty of Agriculture, Fayoum University. Three quail lines were established, in maternal (line1), a selection index was applied to select a female line according to the aggregate breeding values of age at first egg, body weight at sexual maturity and days needed to produce the first 10 eggs selected for four successive generations with selection pressure of 19%. Long shank length (line3) at four weeks of age, individual phenotypic selection was carried out separately for each sex for four generations, higher shank length (one male and two females) were selected according to their deviation from the mean of their sexes and randombred control (line2) which maintained as non-selected pedigreed population. The control line originated from the unselected base population from which the two selected lines 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. Environment and management practices were at conventional levels through the whole study. Feed and water were provided ad-libitum. The same diets were provided to birds on the selection process across various generations. The following traits were measured, body weight (BW) and shank length (SL) at 1, 7, 14, 21, 28 and 35 days of age and BW at sexual maturity. Age at first egg (AFE), Age at 10 eggs (Age_{10}), Age at 30 eggs (Age_{30}), number of days needed to produce the first 10 eggs (DN_{10}), number of days needed to produce the first 30 eggs (DN_{30}), egg mass of the first 10 eggs (EM_{10}) and egg mass of the first 30 eggs (EM_{30}) were recorded individually for each female.

Statistical analysis:

Statistical analysis was conducted using General Linear Model's procedure of SPSS software (SPSS, 2008). The model used was $Y_{ijk}=\mu+L_i+S_j+LS_{ij}+e_{ijk}$, where Y_{ijk}=observed value in the i^{th} line in the j^{th} sex of the k^h individual, μ=overall mean, L_i=line effect (i=1-3), S_j=sex effect (k=1, 2), LS_{ij}=interaction of line by sex and e_{ijk} is the error associated with Y_{ijk}. While, data of productive 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 (j=1-3), and e_{ij}=random error term. Means were compared for main effects and their interaction by Duncan's new multiple range tests (Duncan, 1955) when significant F values were obtained.

Molecular genetics studies:

Extraction of DNA:

Individual blood samples were collected from 60 birds [20 birds (10 males and 10 females/line)], highest performance index for line1, randomly assigned for line2 and the longest shank length for line3. Blood sample was 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. Upon use, the blood samples were thawed and 300 µl of each sample was used to extract genomic DNA according to Wizard Genomic DNA Purification Kit.

PCR conditions and RAPD-PCR analysis:

Equal concentrations of DNA of the individual samples within sex and line were drawn and mixed together to get a pooled 2 DNA samples (males and females). RAPD-

PCR analysis was then applied to the pooled samples. Samples were screened with 10-mer arbitrary sequenced primers of Kit C. Ten primers were used, the base sequences and GC contents of the primers are presented in Table (1). The PCR reaction mixture consisted of 3.0 µl (75 ng) of genomic DNA, 3.0 µl (30 ng) of random primer synthesized by Operon Technologies, USA, 15.0 µl of master mix and 4.0 µl sdH_2O, total volume 25.0 µl. Amplification of DNA fragments was carried out in a (Techne, TC3000). The PCR program included an initial denaturation step at 95ºC/10 min followed by 35 cycles with 95ºC/30 sec for DNA denaturation, 37ºC/30 sec for annealing with each primer, extension at 72ºC/45 sec and final extension at 72ºC/5 min were carried out. The amplified DNA fragments were separated on 2.5% agarose gel and stained with ethidium bromide and the molecular weight (bp) of amplified fragments were estimated with the ladder marker. The amplified patterns were visualized on an ultraviolet light transilluminator and photographed. PCR amplification was performed using 10 random primers that succeeded to generate informative polymorphic bands.

Molecular data analysis:

The bands of RAPD-PCR products on agarose gel were scored and data were processed to determine the band sizes using the computer software: Lab. image V2.7. The presence and absence of band was recorded as (1) and (0), respectively. The binary coded characters (1, 0) that were processed to generate a molecular data set were used for the genetic analysis. Band sharing level (BS) was used to estimate the genetic similarity for each primer (Lynch, 1990) and a simple expression of similarity measured in terms of sharing bands between lines and sexes. The BS between lines and sexes x and y was calculated as $BS_{xy}=2N_{xy}/N_x+N_y$ where N_{xy} is the number of common fragments observed in lines and sexes x and y, N_x and N_y are the total number of fragments scored in x and y lines and sexes, respectively. The genetic distance indices between lines and sexes were used to construct a dendrogram graph for the lines and sexes, using PhyloDraw software package established by Choi et al. (2000).

RESULTS AND DISCUSSION

Productive traits:

Means and standard errors of body weight (BW) and shank length (SL) at different ages which studied for the three lines are presented in Table (2). Line3 had higher BW and SL than line1 and line2 at all ages studies except for BW at one day old, while the line2 had the significant lowest values at all ages. Neither the effect of sex nor the interaction (sex by line) was significant for BW at 28 and 35 days of age and SL at all ages. The interaction (sex by line) effect was significant for BW at 1, 7, 14 and 21 days of age as shown in Table (3). Line had significant effects on all egg production-related traits studied (Table 4). Line1 was better for all traits than other lines, it matured at earlier age than the line2 and line3 by 18.08 and 10.27days, respectively. Similar results were reported for age at first egg (Nath et al., 2011). Line1 had shorter days needed to produce the first 10 and 30 eggs by 3.15 and 5.46 days than the line2 and 2.36 and 4.68 days than the line3, lower age at 10 and 30 eggs by 21.23 and 23.54 days than the line2 and 12.63 and 13.95 days than the line3. The line1 had heavier egg mass for the first 10 and 30 eggs by 12.45 and 34.66g than the line2 and 12.23 and 28.34g than the line3. Similar results were reported for DN_{30} and EM_{30} (Farrag, 2011). Bahie El-Deen and

El-Sayed (1999) reported that the period needed to produce the first 10 eggs for the control and selected line (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. These results are in agreement with Bahie El-Deen (1994), Nestor et al. (1996), Shalan (1998) and Ali et al. (2002). They reported that the quail line selected for high egg production were better for egg production traits than other lines in this respect. In the present study, it can be observed that the differences among different lines favoring the maternal line (line1) and sexes were due to the genetic changes resulted from selection for 4 generations. The lines are diverse and specific in their performance traits.

Molecular genetics analyses:

Molecular markers are efficient tools for genotype identification, characterization and estimation of relatedness through DNA fingerprinting. RAPD-PCR technique was employed in this study to find out genetic variations and relatedness within and among two selected lines, 1 and 3 and randombred control line2 males and females of Japanese quail. Using mixed DNA samples was proven to be an effective approach when comparing among lines and sexes patterns. All the 10 primers (Table 1) examined produced different RAPD-PCR fragment patterns (Plate 1 and Table 5). In total, the 10 RAPD primers produced 286 scorable bands as shown in Table (6). The number of bands amplified with each primer ranged from 6-53 bands, within a mean of 28.6. While, the number of polymorphic fragments ranged from 1-8, a maximum number of 53 amplicons were amplified with primer OPC-20, while the minimum number of fragments (6) was amplified with primer OPC-17 as shown in Table (6). The highest number of polymorphic bands (8) was obtained with primer OPC-02, and the lowest number of polymorphic bands (1) was obtained with primer OPC-16 as shown in Table (7). Primers OPC-10, -17 and -18 exhibited the highest percentage (100%) of polymorphism (Table 7). Table (7) also revealed that the primer OPC-16 exhibited the lowest percentage (11.11%) of polymorphism. The number of polymorphic bands varied from 11.11-100% of the total bands. The mean of polymorphism percentage across all 10 primers was 73.28. Primers OPC-10, -17 and -18 produced amplification products of polymorphic bands for a given three lines (Table 7).

Furthermore, primer OPC-10 detected polymorphic bands (PB) only specific for sexes and lines. Primer OPC-10 amplified PB at 957.87 bp and 803.10 bp specific for control (C) males and line3 females, PB at 585.41 bp specific for line1 males and females and C males, PB at 473.33 bp specific for line1 males and females and C males and line3 females, PB at 424.52 bp specific for C males and line3 females, and PB at 345.03 bp specific for C males and line3 females. Table (7) also revealed that the total number of polymorphic amplicons obtained by the 10 studied primers was 39, and an average number of polymorphic fragments/primer of 3.9. As shown in Plate (1), all the tested primers exhibited intralines polymorphisms as well as interline variations. Six primers (OPC-02, -08, -10, -17, -18 and -20), resulted in highly polymorphic markers for quail genome. However, each of the 10

primers used was effective in amplifying polymorphic bands for a given three lines. The polymorphic nature of many bands amplified by the 10 primers was also obvious within lines and sexes. The results of polymorphism demonstrate the efficiency of the used primers to assess the genetic specificity and reflect the genetic diversity in Japanese quail lines and sexes. According to results of parallel studies, where the same and other RAPD primers were tested, higher values were received than reported in the chicken, 5-12 (Singh and Sharma, 2002) and 4-19 (Dehghanzadeh et al., 2009), in ducks, 4-13 (El-Gendy et al., 2005), in goose, 0-8 (Maciuszonek et al., 2005). According to their reports, the results of the present study were adequate. The size of scorable amplified fragments ranged from 95-2642 bp as shown in Table (6).

Some of the primers produced no amplification products of monomorphic bands (OPC-10, -17 and -18), and for some primers a monomorphic product was obtained (OPC-02, -08, -11, -14, -15, -16 and -20). When OPC-16 primer was used for the study, most of the bands were monomorphic (8). The primers also detected monomorphic bands (MB) specific for the two sexes of each line (Table 5). Primer OPC-02 amplified 3 MB at 724.94, 559.10 and 451.60 bp. Primer OPC-08 amplified 1 MB at 665.98 bp. Primer OPC-11 amplified 3 MB at 1986.97, 1738.96 and 667.20 bp. Primer OPC-14 amplified 4 MB at 2641.68, 1669.37, 1229.32 and 1068.46 bp. Primer OPC-15 amplified 1 MB at 457.03 bp. Primer OPC-16 amplified 8 MB at 1255.48, 905.58, 772.33, 701.40, 634.32, 545.54, 499.60 and 436.94 bp. Primer OPC-20 amplified 4 MB at 726.99, 571.45, 483.73 and 94.82 bp as shown in Table (7). In chickens, Sharma et al. (2001) demonstrated the presence of monomorphic bands characterizing different breeds.

Some of the primers produced amplification products of unique bands (UB) (OPC-02, -08, -11, -14, -17, -18 and -20), and for some primers no amplification products of UB (OPC-10, -15 and -16). The primers also detected UB specific for line and sex as shown in Table (5) and (7). Primer OPC-02 detected 4 UB (positive markers) at 1176.29 bp specific for control females, UB at 1050.67 bp specific for line1 males, UB at 923.44 bp specific for control females and UB at 610.32 bp specific for line1 females. Primer OPC-08 detected 5 UB (positive markers) at 2045.46 bp specific for line1 males, UB at 1654.87 bp specific for line1 males, UB at 1631.13 bp specific for line3 males, UB at 1615.49 bp specific for line3 females and UB at 1502.91 bp specific for line1 males. Primer OPC-11 detected 1 UB (positive marker) at 1236.86 bp specific for control males. Primer OPC-14 detected 3 UB (positive markers) at 771.91 bp specific for line1 males, UB at 429.41 bp specific for control females and UB at 418.60 bp specific for line3 males. Primer OPC-17 detected 2 UB (positive markers) at 1588.30 bp specific for control males and UB at 1563.61 bp specific for control females. Primer OPC-18 detected 6 UB (positive markers) at 474.64 bp specific for line3 males, UB at 455.34 bp specific for line3 females, UB at 415.22 bp specific for control females, UB at 405.75 bp specific for control males, UB at 387.46 bp specific for line3 males and UB at 375.15 bp specific for line3 females. Primer OPC-20 detected 7 UB (4 positive and 3 negative markers) at 929.15 bp specific for line1 males, UB at 641.57 bp specific for line3 males, UB at 612.55 bp specific for line1 males and UB at 417.12 bp specific for line1 males, and UB at 1072.53 bp specific

for control males, UB at 924.86 bp specific for line1 males and UB at 411.37 bp specific for line1 males.

Summary of the polymorphic information between and within lines is presented in Table (8). Accordingly, primers OPC-10, -17 and -18 resulted in highly polymorphic markers, where they amplified 6, 4 and 11 bands with average of 0.417, 0.250 and 0.273, respectively as shown in Table (7). The high percentages of polymorphic bands are expected since two sexes, males and females of three different lines were tested. Polymorphism in distant species was reported by Ahlawat et al. (2004), El-Gendy et al. (2005), Alyethodi et al. (2010) and El-Gendy et al. (2006), where DNA bands were produced by RAPD procedure and successfully used to differentiate between Nicobari fowls, ducks, chickens, and goose (Maciuszonek et al., 2005). Also, Alatafi et al. (2013) reported polymorphic patterns in DNA bands of male and female breeds of chickens by RAPD procedure to distinguish the male and female bird's accessions.

Table (9) showed that the overall mean genetic similarity between the three lines across 10 primers on the basis of RAPD-PCR marker was 0.52 and 0.54, respectively in males and females. The results of RAPD-PCR are in harmony with Ye et al. (1998) reported that the genetic variation within and between selected and randombred lines of quail using DNA fingerprinting and BS. Within lines, BS was ranged from 0.384-0.525 and 0.230-0.308 between selected lines. Also, to detect polymorphism in various quail lines, RAPD markers were tested (Sharma et al., 2000) and found to be effective. Six decamer primers generated distinct polymorphic patterns between the quail lines, 19 bands (31.7%) out of 60 amplified bands were found to be polymorphic, genetic similarity within the lines ranged from 0.762-0.836. The present results are in line with Karabağ and Balcioğlu (2010) genetic diversity among selected and a control quail lines were investigated also by RAPD-PCR. Seven males-7 females from each line were analyzed using 24 primers, and 196 polymorphic loci were amplified and polymorphism rate was 99.49%, and genotypic polymorphism rates were 63.45%, 31.47%, 42.13%, 35.53% and 36.55%. They concluded that the genetic variation within line and the genetic relationships among lines could be estimated successfully using RAPD in selected quail lines.

Phylogenetic relationship among quail lines based on RAPD marker:

On the basis of RAPD-PCR marker as shown in Table (10), the highest similarity (0.86) was recorded within line1 males and females as expected in the same selected line. The lowest similarity (0.47) was recorded between line1 males and control females as expected in the two different lines (selected and control), and between line1 females and line3 males as expected in the two different selected lines (maternal and long shank length). Genetic variation, both within and between lines, is essential for the genetic improvement of quail. Loss of variation will restrict the selection for desirable characteristics within lines. The data obtained from the analysis of RAPD were used to draw precise relationships among the three tested quail lines, and the resultant dendrograms are shown in Fig. (1a, b and c). Dendrograms of phylogenetic relationships were constructed based on the genetic similarity indices. Cluster analysis was conducted to generate a dendrogram illustrating possible relationships among the studied three quail lines based on molecular attributes. These dendrograms

clustered the quail lines into two clusters (groups). The first group consisted of selected line1 males and females was delimited in separate group form one cluster from the rest of studied quail lines males and females. Selected line3 males and females, and control line2 males and females were delimited in other separate group form another cluster. Selected line3 males and females were separated in one sub-group, from the rest of sub-group control line2 males and females. Based on RAPD analysis, the selected line1 males and females were delimited in separate group, while the selected line3 males and females, and control line2 males and females were clustered in the same group form another cluster. This result seems to be reliable since it goes with the expectation of clustering males and females in the same line in one cluster. Gathering both selected line3 and control line2 in one cluster even though, selected line1 delimited in separate group. They might share some genes between selected lines and control line through successive selections during four generations of selection for maternal and long shank length lines which started in control population. The randombred control population was the original from which the maternal and long shank length lines had selected. The line1 males and females were delimited in separate group, while the line3 males and females, and line2 males and females were clustered in the same group form another cluster. That means line1 were selected with high pressure of selection more than line3. The same trend was observed for the productive performance and indicated that the line1 has better for all studied egg production-related traits than the other two lines.

GENERAL CONCLUSION

The productive performance and molecular genetic analysis used in the present study successfully distinguished among different Japanese quail lines and sexes. The result of molecular genetic analysis is in agreement with the result of ANOVA for the productive traits. The study showed that RAPD fingerprinting analysis is an effective method for generating polymorphic DNA markers in two selected lines and randombred control line. 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 will provide Japanese quail breeders with environment independent DNA markers, which should be regarded as essential tools for selection. RAPD fingerprinting analysis will be practical in Japanese 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 10 primers used for RAPD-PCR analysis and their GC content.

Primer code	Sequence (5´- 3´)	GC Content %
OPC-02	GTG AGG CGT C	70
OPC-08	TGG ACC GGT G	70
OPC-10	TGT CTG GGT G	60
OPC-11	AAA GCT GCG G	60
OPC-14	TGC GTG CTT G	60
OPC-15	GAC GGA TCA G	60
OPC-16	CAC ACT CCA G	60
OPC-17	TTC CCC CCA G	70
OPC-18	TGA GTG GGT G	60
OPC-20	ACT TCG CCA C	60

Table (2): Means and standard errors for line and sex as main effects on body weight (BW) and shank length (SL) at different ages of Japanese quail.

Item	Line1	Line2	Line3	Sex	
				Males	Females
BW_1	9.48^{a} ±0.18	7.84^{b}±0.19	9.21^{a} ±0.18	8.90±0.16	8.84±0.14
BW_7	31.51^{b}±1.09	19.72^{c}±1.16	35.52^{a}±1.08	29.27 ±0.96	28.94±0.85
BW_{14}	93.51^{b}±1.96	67.26^{c}±2.06	106.45^{a}±1.91	88.74 ±1.69	89.40±1.53
BW_{21}	149.62^{b}±2.54	125.98^{c}±2.71	171.50^{a}±2.52	150.53 ±2.23	148.90±1.99
BW_{28}	203.70^{a}±3.36	162.50^{b} ±3.59	214.76^{a}±3.33	190.85 ±2.96	196.53±2.63
BW_{35}	220.31^{a} ±3.96	181.16^{b} ±4.15	230.71^{a}±3.86	209.84 ±3.42	211.43±3.09
SL_1	17.22^{b} ±0.12	17.70^{a}±0.13	18.05^{a} ±0.12	17.57±0.10	17.75±0.09
SL_7	25.81^{b}±0.39	23.87^{c}±0.42	27.16^{a}±0.38	29.27 ±0.34	28.94±0.31
SL_{14}	31.51^{b}±0.42	28.27^{c}±0.44	33.11^{a}±0.41	30.83 ±0.36	31.16±0.32
SL_{21}	36.75^{b}±0.44	33.86^{c}±0.47	38.66^{a}±0.43	36.51 ±0.39	36.41±0.34
SL_{28}	40.21^{b}±0.41	37.79^{c}±0.44	41.84^{a}±0.41	39.93 ±0.36	40.00±0.32
SL_{35}	41.45^{b}±0.37	40.36^{c}±0.39	43.03^{a}±0.36	41.52 ±0.32	41.72±0.29

BW_1-BW_{35}=Body weight at one day-old to 35days of age, SL_1-SL_{35}=Shank length at one day-old to35days of age and $^{a, b \text{ and } c}$=Means within the same effect in the same row with different letters are significantly different ($P \leq 0.05$).

Table (3): Means and standard errors for the significant line by sex interaction effect on body weight (BW) at different ages of Japanese quail.

Item	Line1		Line2		Line3	
	Males	Females	Males	Females	Males	Females
BW_1	9.81^{a} ±0.28	9.25^{ab} ±0.23	8.04^{b} ±0.28	7.67^{b} ±0.25	8.85^{b} ±0.24	9.61^{ab} ±0.25
BW_7	30.63^{b} ±1.63	32.18^{b}±1.43	22.89^{c}±1.83	17.77^{d} ±1.43	34.27^{ab} ±150	36.87^{a} ±1.56
BW_{14}	91.20^{b} ±2.89	95.82^{b}±2.64	76.20^{c}±3.23	58.33^{d} ±2.54	98.83^{b} ±2.64	114.07^{a} ±2.76
BW_{21}	144.70^{b} ±3.82	153.40^{bc}±3.35	138.80^{c}±4.27	118.12^{d} ±3.35	168.10^{ab} ±3.49	175.22^{a} ±3.64

BW_1-BW_{21}=body weight at day-old to 21 days of age and $^{a, b \text{ and } d}$=Means in the same row with different letters are significantly different ($P \leq 0.05$).

Table (4): Means and standard errors for line effect on egg production-related traits for different lines of Japanese quail.

Item	Line1	Line2	Line3
Age at sexual maturity, day	43.46^{c} ±0.85	61.54^{a} ±0.85	53.73^{b} ±0.93
Age at 10 egg, day	54.00^{c}±1.35	75.23^{a} ±1.37	66.63^{b}±1.49
Age at 30 egg, day	75.38^{c}±1.73	98.92^{a} ±1.74	89.33^{b}±1.88
Body weight at sexual maturity, gram	241.00 ±5.64	235.71 ±5.64	246.78 ±6.13
Number of days needed to produce the first 10 eggs, day	10.54^{b} ±0.95	13.69^{a} ±3.14	12.90^{a} ±1.04
Number of days needed to produce the first 30 eggs, day	31.92^{b} ±1.43	37.38^{a} ±3.89	35.60^{a} ±1.56
Egg mass of the first 10 eggs, gram	120.54^{a} ±1.60	108.09^{b} ±1.59	108.3^{b} ±1.73
Egg mass of the first 30 eggs, gram	363.86^{a} ±5.41	329.20^{b} ±5.40	335.52^{b} ±5.87

$^{a, b \text{ and } c}$=Means within the same effect with different letters are significantly different ($P \leq 0.05$).

Table (5): Banding patterns of RAPD-PCR by different primers for three lines, two selected lines (1=Maternal line1, 3=Long shank length line3) and 2=Control line2 (♂♂ and ♀♀) of Japanese quail.

Primer code	Band No.	R F	Size of bands (bp)	1 ♂♂	1 ♀♀	2 ♂♂	2 ♀♀	3 ♂♂	3 ♀♀
OPC-02	1	0.387	1282.00	1	0	0	0	1	1
	2	0.389	1268.28	1	1	0	0	0	0
	3	0.403	1176.29	0	0	0	1	0	0
	4	0.424	1050.67	1	0	0	0	0	0
	5	0.448	923.44	0	0	0	1	0	0
	6	0.462	856.46	1	1	1	0	1	1
	7	0.493	724.94	1	1	1	1	1	1
	8	0.525	610.32	0	1	0	0	0	0
	9	0.541	560.00	1	1	1	1	1	1
	10	0.581	451.60	1	1	1	1	1	1
	11	0.643	323.55	0	0	0	0	1	1
	12	0.732	200.48	0	0	0	0	1	1
	13	0.733	199.40	0	0	1	1	1	0
	14	0.764	168.78	0	0	0	0	1	1
	15	0.804	136.11	1	1	1	0	1	1
OPC-08	1	0.294	2045.46	1	0	0	0	0	0
	2	0.299	1996.80	0	0	0	0	1	1
	3	0.303	1958.70	1	1	1	0	0	0
	4	0.323	1778.84	0	0	0	0	1	1
	5	0.336	1670.88	0	1	1	0	0	0
	6	0.338	1654.87	1	0	0	0	0	0
	7	0.341	1631.13	0	0	0	0	1	0
	8	0.343	1615.49	0	0	0	0	0	1
	9	0.358	1502.91	1	0	0	0	0	0
	10	0.403	1210.08	0	1	1	0	0	0
	11	0.527	665.98	1	1	1	1	1	1
	12	0.580	515.95	1	1	0	0	0	0
OPC-10	1	0.435	957.87	0	0	1	0	0	1
	2	0.469	803.10	0	0	1	0	0	1
	3	0.530	585.41	1	1	1	0	0	0
	4	0.571	473.33	1	1	1	0	0	1
	5	0.592	424.52	0	0	1	0	0	1
	6	0.632	345.03	0	0	1	0	0	1
OPC-11	1	0.301	2139.73	1	0	1	0	1	0
	2	0.316	1986.97	1	1	1	1	1	1
	3	0.343	1738.96	1	1	1	1	1	1
	4	0.365	1559.95	1	0	1	0	1	1
	5	0.412	1236.86	0	0	1	0	0	0
	6	0.537	667.20	1	1	1	1	1	1

RF=Relative mobility

Table (5): Cont.

Primer code	Band No.	R F	Size of bands (bp)	1 ♂♂	1 ♀♀	2 ♂♂	2 ♀♀	3 ♂♂	3 ♀♀
OPC-14	1	0.411	2641.68	1	1	1	1	1	1
	2	0.459	1945.34	0	0	0	0	1	1
	3	0.483	1669.37	1	1	1	1	1	1
	4	0.531	1229.32	1	1	1	1	1	1
	5	0.553	1068.47	1	1	1	1	1	1
	6	0.604	771.92	1	0	0	0	0	0
	7	0.612	733.54	0	0	1	1	1	0
	8	0.691	443.32	1	1	1	0	0	0
	9	0.696	429.41	0	0	0	1	0	0
	10	0.700	418.60	0	0	0	0	1	0
OPC-15	1	0.494	1017.10	1	1	0	1	1	1
	2	0.654	457.03	1	1	1	1	1	1
	3	0.756	274.45	0	0	0	1	1	0
OPC-16	1	0.353	1255.48	1	1	1	1	1	1
	2	0.373	1154.59	1	0	0	0	1	0
	3	0.431	905.58	1	1	1	1	1	1
	4	0.469	772.33	1	1	1	1	1	1
	5	0.492	701.40	1	1	1	1	1	1
	6	0.516	634.32	1	1	1	1	1	1
	7	0.552	545.54	1	1	1	1	1	1
	8	0.573	499.61	1	1	1	1	1	1
	9	0.605	436.94	1	1	1	1	1	1

RF=Relative mobility

Table (5): Cont.

Primer code	Band No.	R F	Size of bands (bp)	1 ♂♂	1 ♀♀	2 ♂♂	2 ♀♀	3 ♂♂	3 ♀♀
OPC-17	1	0.289	1691.05	1	1	0	0	0	0
	2	0.301	1588.31	0	0	1	0	0	0
	3	0.304	1563.61	0	0	0	1	0	0
	4	0.307	1539.30	0	0	0	0	1	1
OPC-18	1	0.397	1177.46	1	1	0	0	0	0
	2	0.433	997.33	1	1	0	0	0	0
	3	0.568	535.10	1	1	1	1	0	0
	4	0.594	474.64	0	0	0	0	1	0
	5	0.603	455.34	0	0	0	0	0	1
	6	0.618	424.90	1	1	0	0	0	0
	7	0.623	415.22	0	0	1	0	0	0
	8	0.628	405.75	0	0	0	1	0	0
	9	0.638	387.46	0	0	0	0	1	0
	10	0.645	375.15	0	0	0	0	0	1
	11	0.660	350.08	1	1	0	0	0	0
OPC-20	1	0.254	1966.93	1	0	0	0	1	0
	2	0.283	1719.82	1	0	0	0	1	0
	3	0.331	1377.14	1	0	0	0	1	1
	4	0.351	1255.36	1	1	0	0	1	0
	5	0.385	1072.53	1	1	0	1	1	1
	6	0.416	929.15	1	0	0	0	0	0
	7	0.417	924.86	0	1	1	1	1	1
	8	0.469	726.99	1	1	1	1	1	1
	9	0.496	641.57	0	0	0	0	1	0
	10	0.506	612.55	1	0	0	0	0	0
	11	0.521	571.45	1	1	1	1	1	1
	12	0.557	483.73	1	1	1	1	1	1
	13	0.589	417.13	1	0	0	0	0	0
	14	0.592	411.37	0	1	1	1	1	1
	15	0.909	94.82	1	1	1	1	1	1

RF=Relative mobility

Table (6): Number of amplified fragment markers by different primers of two selected and control lines and sex detected based on RAPD-PCR analysis.

Primer code	Molecular weight (bp)	Band NO	Line1		Line2		Line3		Total bands	Mean
			♂♂	♀♀	♂♂	♀♀	♂♂	♀♀		
OPC-02	136-1282	15	8	7	6	6	10	9	46	7.67
OPC-08	516-2046	12	6	5	4	1	4	4	24	4.00
OPC-10	345-958	6	2	2	6	0	0	5	15	2.50
OPC-11	667-2140	6	5	3	6	3	5	4	26	4.33
OPC-14	444-2642	10	6	5	6	6	7	5	35	5.83
OPC-15	274-1017	3	2	2	1	3	3	2	13	2.17
OPC-16	437-1256	9	9	8	8	8	9	8	50	8.33
OPC-17	1539-1691	4	1	1	1	1	1	1	6	1.00
OPC-18	350-1178	11	5	5	2	2	2	2	18	3.00
OPC-20	95-1967	15	12	8	6	7	12	8	53	8.83
Total		91	56	46	46	37	53	48	286	47.66
Mean		9.1	5.6	4.6	4.6	3.7	5.3	4.8	28.6	4.77

Line1=Maternal selected, Line2=Control and Line3=Long shank length selected.

Table (7): The polymorphism percentage produced by different primers on the studied three lines.

Primer code	BNO	MB	UB	PB	PB+UB	Polymorphism %	Mean of band frequency
OPC-02	15	3	4	8	12	80.00	0.511
OPC-08	12	1	5	6	11	91.67	0.333
OPC-10	6	0	0	6	6	100.00	0.417
OPC-11	6	3	1	2	3	50.00	0.722
OPC-14	10	4	3	3	6	60.00	0.583
OPC-15	3	1	0	2	2	66.67	0.722
OPC-16	9	8	0	1	1	11.11	0.926
OPC-17	4	0	2	2	4	100.00	0.250
OPC-18	11	0	6	5	11	100.00	0.273
OPC-20	15	4	7	4	11	73.33	0.589
Total	91	24	28	39	67	73.63	5.326
Mean	9.1	2.4	2.8	3.9	6.7	73.28	0.533

BNO=Band numbers, MB=Monomorphic bands, UB=Unique bands and PB=Polymorphic bands

Table (8): Polymorphic information content between and within lines and sexes.

Variable	Line1		Line2		Line3		Total	Mean
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀		
NB	56	46	46	37	53	48	286	47.67
NB/marker	5.6	4.6	4.6	3.7	5.3	4.8	28.6	4.77
NPB	24	21	19	8	24	21	117	19.50
PB %	42.86	45.65	41.30	21.62	45.28	43.75	240.46	40.08
NUB	8	1	5	5	5	4	28	4.67
UB %	14.29	2.17	10.87	13.51	9.43	8.33	58.60	9.77
NPB+UB	32	22	24	13	29	25	145	24.17
PB+UB %	57.14	47.83	52.17	35.14	54.72	52.08	299.08	49.85
Homogeneity%	42.86	52.17	47.83	64.86	45.28	47.92	300.92	50.15
NMBADL	24							
MBADL%	26.37							

Line1=Maternal, Line2=Control and Line3=Long shank length. NB=Number of bands, NPB=Number of polymorphic bands, NUB=Number of unique bands and NMBADL=Number of monomorphic bands across different lines

Table (9): Similarity between the lines and sexes on the basis of RAPD-PCR marker.

Primer code	Males			Females			Overall mean		Over-all mean
	1*2	1*3	2*3	1*2	1*3	2*3	♂♂	♀♀	
OPC-02	0.71	0.67	0.75	0.46	0.63	0.40	0.71	0.50	0.61
OPC-08	0.40	0.20	0.25	0.33	0.22	0.40	0.28	0.32	0.30
OPC-10	0.50	0.00	0.00	0.00	0.29	0.00	0.17	0.10	0.14
OPC-11	0.91	1.00	0.91	1.00	0.86	0.86	0.94	0.91	0.93
OPC-14	0.83	0.62	0.77	0.73	0.80	0.73	0.74	0.75	0.74
OPC-15	0.67	0.80	0.50	0.80	1.00	0.80	0.66	0.87	0.76
OPC-16	0.94	1.00	0.94	1.00	1.00	1.00	0.96	1.00	0.98
OPC-17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OPC-18	0.29	0.00	0.00	0.29	0.00	0.00	0.10	0.10	0.10
OPC-20	0.44	0.75	0.67	0.93	0.88	0.93	0.62	0.91	0.77
Total	5.69	5.04	4.79	5.54	5.68	5.12	5.17	5.45	5.32
Mean	0.57	0.50	0.48	0.55	0.57	0.51	0.52	0.54	0.53

1=Line1 maternal, 2=Line2 control, 3=Line3 long shank length and
*=Similarity between the lines.

Table (10): Similarity matrix between the lines and sexes of two selected line1 and line3 and control line2 Japanese quail on the basis of RAPD-PCR marker.

Line and Sex	Line1 males	Line1 females	Line2 males	Line2 females	Line3 males	Line3 females
Line1 males	1.00					
Line1 females	0.86	1.00				
Line2 males	0.57	0.64	1.00			
Line2 females	0.47	0.55	0.55	1.00		
Line3 males	0.50	0.47	0.48	0.50	1.00	
Line3 females	0.55	0.57	0.57	0.51	0.59	1.00

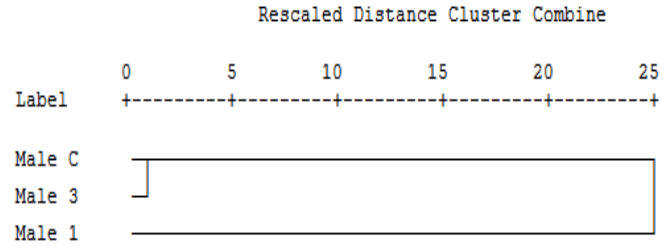

Fig. (1a): Dendrogram tree analysis for the three lines of Japanese quail males (1=Maternal, C=Control and 3=Long shank length) based on RAPD-PCR analysis.

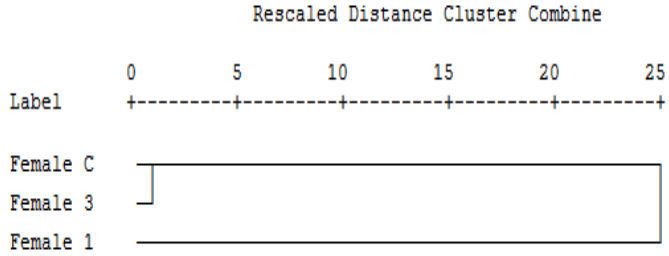

Fig. (1b): Dendrogram tree analysis for the three lines of Japanese quail females (1=Maternal, C=Control and 3=Long shank length) based on RAPD-PCR analysis.

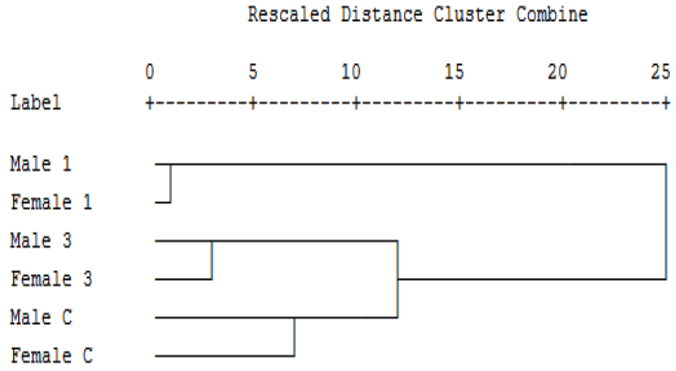

Fig. (1c): Dendrogram tree analysis for the three lines of Japanese quail males and females (1=Maternal, C=Control and 3=Long shank length) based on RAPD-PCR analysis.

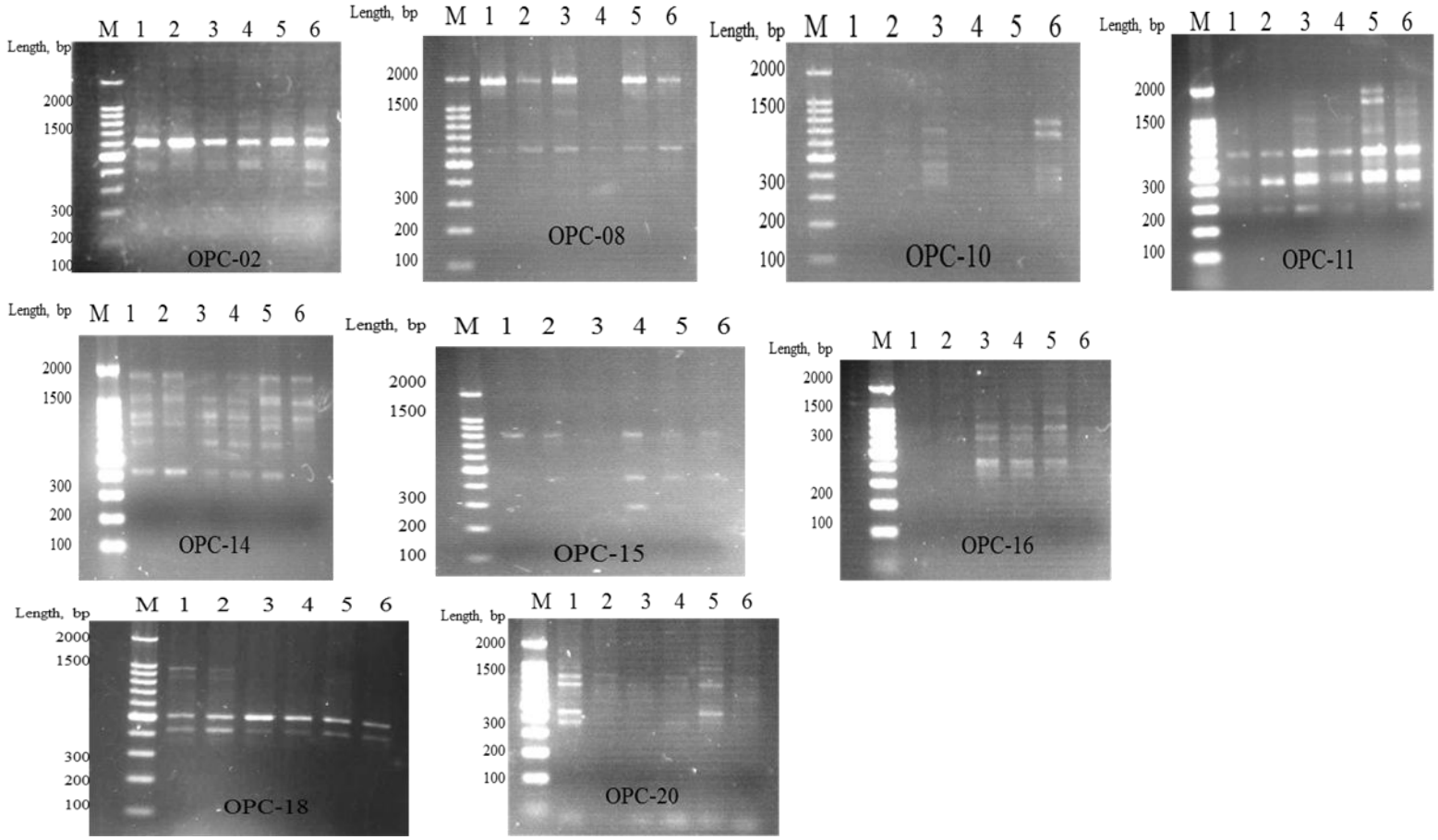

Plate (1): RAPD fingerprints profiles of the two selected and control Japanese quail lines amplified with 9 different RAPD primers. M=Ladder marker, 1=Selected line1 males, 2=Selected line1 females, 3=Control males, 4=Control females, 5=Selected line3 males and 6=Selected line3 females.

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الملخص العربي

توصيف خطوط منتخبة ومتزاوجة عشوائيا في الجيل الرابع من السمان الياباني على أساس الأداء الإنتاجي وتحليل التباين الوراثى الناتج من التكبير العشوائى لمقاطع الحمض النووى بتفاعل إنزيم البلمرة المتسلسل

عيسي احمد عيسي* وبثينة يوسف محمود واستفتاح محمد الكومي*** وانصاف احمد الفل****

*قسم الوراثة - كلية الزراعة - جامعة الفيوم - ٦٣٥١٤ الفيوم - مصر

**قسم إنتاج الدواجن - كلية الزراعة - جامعة الفيوم - ٦٣٥١٤ الفيوم - مصر

***قسم الانتاج الحيواني - شعبة البحوث الزراعية والبيولوجية - المركز القومي للبحوث - ١٢٦١٢ الدقي - الجيزة - مصر**

استخدم فى هذه الدراسة الأداء الإنتاجي وتحليل التباين الوراثى الناتج من التكبير العشوائي لمقاطع من الحمض النووى د ن ا لتوصيف وتحديد العلاقات الوراثية داخل وبين ثلاثة خطوط من السمان اليابانى وهم خطين منتخبين خط منتخب أمي (الخط الأول) وخط منتخب لطول قصبة الساق (الخط الثالث) والكنترول المتزاوج عشوائيا (الخط الثاني) في الجيل الرابع فى كلا من الذكور والإناث. تم قياس وزن الجسم وطول قصبة الساق اسبوعيا من عمر يوم الى ٣٥ يوم وكذلك وزن الجسم عند عمر النضج الجنسي وتسجيل العمر عند وضع أول بيضة وعدد الأيام اللازمة لإنتاج أول ١٠ و٣٠ بيضة والعمر عند إنتاج أول ١٠ و٣٠ بيضة ووزن أول ١٠ و٣٠ بيضة. وتم سحب عينات دم منفرده من عدد ٦٠ طائر [٢٠ طائر (١٠ ذكور و١٠ إناث لكل خط)] لإستخلاص ال د ن ا. وتم إستخدام تقنية تفاعل إنزيم البلمرة المتسلسل بإستخدام ١٠ بوادىء عشوائية التي مكنت من الحصول على بصمات وراثية مميزة للخطوط الثلاث فى الذكور والإناث. أظهرت النتائج أن الخط الثالث كان أعلى بفروق معنوية في صفتي وزن الجسم وطول قصبة الساق مقارنة بالخط الأول والثاني عند كل الاعمار فيما عدا عمر يوم بينما كان الخط الثاني أقل الأوزان بفروق معنوية عند كل الأعمار. تفوق الخط الأول بفروق معنوية مقارنة بالخط الثاني والثالث في كل صفات إنتاج البيض المدروسة حيث كان أقل في العمر عند وضع أول بيضة وعدد الأيام اللازمة لإنتاج أول ١٠ و٣٠ بيضة والعمر عند إنتاج أول ١٠ و٣٠ بيضة وأعلى في وزن أول ١٠ و٣٠ بيضة. وقد أمكن الحصول على بصمات وراثية مميزة للخطوط الثلاث فى الذكور والإناث بإستخدام ١٠ بوادىء عشوائية التى أنتجت ٢٨٦ حزمة حيث أظهرت التحليلات وجود تباينات واضحة فى طرز الحزم بين الخطوط المختلفة وتراوح عدد الحزم المتباينة بين ٦-٥٣ بمتوسط ٢٨,٦. وتراوحت النسبة المئوية للحزم المتباينة بين ١١,١١-١٠٠% حيث كان متوسط النسبة المئوية للتباين الوراثي للعشرة بوادىء ٧٣,٢٨. وكان العدد الكلى للحزم المتباينة التي تم الحصول عليها بإستخدام البادئات ٣٩ بمتوسط ٣,٩ حزمة متباينة وراثيا لكل بادئ. ونتائج تحليل التباين الوراثي توضح كفاءة البوادىء المستخدمة في التحليل الوراثي وتعكس التباين الوراثي في الخطوط الثلاث للذكور والإناث. كما أمكن تمييز وتحديد الإختلافات الوراثية بين الذكور والإناث فى الخطوط الثلاث بواسطة ٢٨ واسم جزيئى فريد ومتخصص نتج من البوادىء العشوائية المستخدمة. وتم أيضا دراسة التشابه الوراثي بين الخطوط الثلاثة في الجنسين حيث تراوح متوسط التشابه الوراثي الكلى بين الثلاث خطوط للبوادىء العشرة ٠,٥٢ في الذكور و٠,٥٤ فى الإناث. تم تسجيل أعلى نسبة تشابه وراثي بين ذكور وإناث الخط الاول (٠,٨٦) وأقل نسبة بين ذكور الخط الأول وإناث الخط الثاني (٠,٤٧) وكذلك بين إناث الخط الأول وذكور الخط الثالث. وأظهرت صورة شجرة القرابة أن الخطوط الثلاث كانت مجمعة في مجموعتين منفصلتين. المجموعة الأولى احتوت على ذكور وإناث الخط الأول والأخرى انفصلت فيها ذكور وإناث الخط الثاني والثالث. كما انفصلا ذكور وإناث الخط الثالث معا في تحت مجموعة عن إناث وذكور الخط الثاني اللذان انفصلا في تحت مجموعة اخرى. وهذه النتائج تتوافق مع المتوقع في تجميع ذكور وإناث نفس الخط في مجموعة واحدة. على الرغم من أن كل من الخط الثالث والثاني قد تجمعا معا في مجموعة واحدة فإن الخط الأول قد انفصل في مجموعة اخرى. وربما يرجع ذلك الى بعض الجينات المشتركة بين الخطوط المنتخبة والكنترول خلال أربعة أجيال متتالية من الإنتخاب بداية من العشيرة القاعدية التي نشا منها الخطوط الثلاث. وعلى الرغم من أن ذكور وإناث الخط الأول قد انفصلا في مجموعة واحدة بينما ذكور وإناث الخط الثاني والثالث قد تجمعت في نفس المجموعة. يفسر ذلك أن الخط الأول قد تم انتخابه بشدة إنتخاب أعلى من الخط الثالث بالنسبة لصفات إنتاج البيض. وهذه النتائج اتفقت مع نتائج الأداء الإنتاجي التى دلت على أن الخط الأول قد تفوق بفروق معنوية في كل صفات إنتاج البيض مقارنة بالخطين الاخرين. وتوضح الدراسة أن استخدام طرق الوراثية

Quail; Selection; Maternal; Shank; Randombred; RAPD analysis; Similarity.

الجزيئية هى وسائل ذات كفاءة عالية للتمييز بين خطوط السمان المختلفة فى برامج التربية تحت ظروف الإنتخاب أو التزاوج العشوائى كما أنها تلعب دورا هاما فى حفظ الأصول الوراثية.