GENETIC DIVERSITY ASSESSMENT AMONG SIX RABBIT BREEDS USING RAPD AND SRAP MARKERS

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Currently, there are several domesticated rabbit breeds have been developed world wide with many efforts are exerting to develop new ones. Basically, most of these breeds are rearing as important source for human consumption, and some are kept for fur production which consider quite valuable today. Also, rabbit has many advantages make it a good animal for experimental research (Brem *et al*., 1994; Coulibaly *et al*., 2002; Li *et al*., 2006; Schmitt and Barrow 2017) and suitable model for studying different types of human diseases (Peng, 2012; Fan *et al*., 2015).

The variation among rabbit breeds offers different materials to be used for different breeding and production programs and even in experimental research and these variations may represent a genetic variability in their gene pool (Rangoju *et al*., 2007). Therefore, genetic diversity assessment among rabbit breeds is very essential for breeders in further genetic improvement programs. The higher genetic variability presented in animal breeds and populations introduce valuable material for breeders to develop new ones with superior characteristics regard to environmental changes.

Presently, there are numerous DNA-based markers have been developed and widely used as powerful tools for estimating genetic diversity among and within animal breeds and populations. Random amplified polymorphic DNA (RAPD) marker which developed for the first time by Williams *et al*. (1990), now is one of the most frequently markers that are used in genetic diversity estimation. RAPD marker was used extensively to study and analyze the population genetic structure and genetic diversity with different rabbit breeds (Rangoju *et al*., 2007; Keliang *et al*., 2008; Osman *et al*., 2010; El-Bayomi *et al*., 2013; Galal *et al*., 2013; El-Sabrout and Aggag 2014; El-Sabrout and El-Raffa 2015) and other animal such as sheep (Kantanen *et al*., 1995; Gwala *et al*., 2015), buffalo (Barwar *et al*., 2008), cattle (Mufti *et al*., 2009), fish (Revaldaves *et al*., 2016), geese and duck (Abdo Basha *et al*., 2016) and chicken (Mollah *et al*., 2009). Also, sequence-related amplified polymorphism (SRAP) marker is another efficient molecular marker with high reproducibility which depends on amplifying the coding regions of genomic DNA with two primers targeting the open reading frames (Li and Quiros, 2001). SRAP marker has been

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widely used for analyzing population structures and genetic diversity in different living organisms (Sun *et al*., 2006; Yu *et al*., 2008; Li *et al*., 2009; Song *et al*., 2011; Moghaieb *et al*., 2017; Abouzaid *et al*., 2016). However, there is no previous information about using SRAP marker to study genetic diversity in rabbits.

The present study aimed to evaluate the genetic diversity and similarity among six rabbit breeds namely, Bouscat, California, Chinchilla, Flemish, Gabali and New Zealand and to investigate the efficiency of RAPD and SRAP markers in assessment of genetic diversity among these breeds.

MATERIALS AND METHODS

Animals

A total of sixty rabbit individuals belong to six different rabbit breeds viz Buskat (BSK), California (KLF), Chinchilla (CHN), Flemish (FLM), Gabali (GBL) and New Zealand (NZL) (10 individuals, five males and five females, for each breed) were used in this study. All used animals were normal and healthy with proven fertility.

Blood collection and DNA extraction

For each individual, about 2 ml of blood was collected from the central ear artery in 15 mL conical centrifuge Falcon tube containing EDTA as anticoagulant. Genomic DNA was isolated from the whole blood according to the standard protocol described by Shams *et al*. (2011). The concentration and quality of the genomic DNA were checked by spectrophotometer and agarose gel electrophoresis. Equal amount of DNA isolated from ten individuals of each breed were mixed to prepare the bulked DNA sample for each rabbit breed.

RAPD and SRAP Assays

RAPD was performed as previously described by Williams *et al*. (1990) and SRAP was carried out according to Li and Quiros (2001). Nine 10-mer RAPD primers (Operon Technologies Inc., USA) and seven SRAP primer combinations (Bioneer, Inc., South Korea) were randomly selected and used for the analysis of genetic diversity in the present study (Table 1). PCR products of RAPD and SRAP were separated on 1.5 and 2.5% agarose gel, respectively and visualized under UV light after staining with ethidium bromide.

Data scoring and analysis

The banding patterns of RAPD and SRAP were scored and used to create binary data matrix of (1) and (0) for the presence and absence of bands, respectively. The data matrix and genetic similarities among rabbit breeds were analyzed and calculated using NTSYSpc software, ver. 2.20s according to Jaccard's coefficient (1908). The cluster analysis of genetic distance and dendrograms creation were performed using UPGMA in the module SHAN in NTSYSpc program. The robustness of the dendrograms was evaluated by bootstrapping with 1,000 replicates using the Free Tree program. The differences

between RAPD and SRAP similarity values were tested using *t*-test. To evaluate the informativeness and discriminatory power of the RAPD and SRAP primer sets, number of marker features was estimated. These marker features are: percentage of polymorphism (P%) which calculated as the percentage of polymorphic bands, polymorphic information content (PIC), resolving power (Rp), marker index (MI), and diversity index (DI) which calculated according to the formulas presented by Mandal *et al*. (2016).

RESULTS AND DISCUSSIONS

In the present study nine RAPD primers and seven different combinations of SRAP primers were chosen randomly and used to investigate the genetic diversity among the six rabbit breeds under investigation. All these primers successfully generated a number of bands with DNA samples extracted from the six rabbit breeds. Figure (2) showed an example of banding pattern generated by RAPD (OPA-2) and SRAP (Me10-Em5) markers. All RAPD and SRAP primers tested could generate polymorphic patterns among the tested rabbit breeds. The total number of bands generated by RAPD primers was 102 of which 51 (50%) polymorphic bands and the number of bands per primer ranged from 8 bands (OPY-05) to 15 bands (OPA-08) with an average of 11.33 bands per primer. This range of band numbers which generated by this set of primers were similar with those generated by other RAPD primers with rabbits (Rangoju *et al*., 2007; El-Bayomi *et al*., 2013; Galal *et al*., 2013). While the total number of bands generated by SRAP primers was 67 of which 16 (23.9%) polymorphic bands and the number of bands per primers combination ranged from 4 bands (Me10-Em10) to 14 bands (Me10-Em5) with an average of 9.6 bands per primers combination. To the best of our knowledge this is the first time to investigate the genetic diversity among rabbit breeds using SRAP marker. Therefore, there are no previous data about using SRAP marker with rabbit to compare. However this range of band numbers and average number of bands per primers combination which generated by these combinations of SRAP primers were comparable with those generated by different combinations of SRAP primers with other organisms such as fish (Zhu *et al*., 2014; El Fadly *et al*., 2016) and Silene species (Bargish and Rahmani, 2016).

Herein, the percentages of polymorphism (P%) were estimated for both RAPD and SRAP markers. The maximum percentages of polymorphism detected by RAPD and SRAP primers were generated by primers OPA-08 (80%) and Me10-Em10 (50%), respectively. However, it is clear that estimation of the polymorphism percentage only is not enough to clarify the informativeness and discriminative power for primers and markers tested because it considers only the number of polymorphic bands generated by the primer regardless how many genotypes among the tested genotypes are responsible for this polymorphism. Therefore the values of different marker features were estimat-

ed in the present study, these values of a primer features help in determining its effectiveness in genetic diversity analysis (Saini *et al*., 2010; Heikrujam *et al*., 2015). The polymorphic information content (PIC) of RAPD primers ranged from 0.05 (OPC-18 and OPI-02) to 0.35 (OPA-08) with an average of 0.19, while the PIC of SRAP primers ranged from 0.06 (Me2-Em3) to 0.22 (Me10-Em10) with an average of 0.11. Also, the resolving power (Rp) was calculated for both RAPD and SRAP primers; it ranged from 0.67 (OPC-18 and OPI-02) to 8.34 (OPA-08) with an average of 3.6 and from 0.67 (Me2-Em3) to 2 (Me3-Em2) with an average of 1.48, respectively. In addition, the highest values of MI and DI for RAPD primers were 4.2 and 4.1, respectively which generated by primer OPA-08, while the highest values of MI and DI for SRAP primers were 0.44 and 1.03 which generated by primers Me10-Em10 and Me10-Em5, respectively. The details of marker features obtained by the two molecular markers are presented in Table (2). From our data, based on PIC, Rp, MI and DI values, it seems that some RAPD primers (OPA-08, OPC-07, OPE-07, OPM-07 and OPK-17) and SRAP primers combinations (Em3-Me2, Em2-Me5, Em10-Me5 and Em10-Me10) were more informativeness and have higher discriminative power than other primers in regard to investigate the genetic diversity among rabbit breeds and may be useful in future studies.

Furthermore, the genetic similarities among the six rabbit breeds were calculated from RAPD and SRAP banding profiles using Jaccard's coefficient (1908). Significant differences between the similarity coefficient values were observed (P= 0.00003, *t-test*); these values were lower in case of RAPD. These differences may result from the higher percentage of polymorphism generated by RAPD as compared to that generated by SRAP (Table 2), in addition to the different regions of genome which RAPD and SRAP analyze (Williams *et al*., 1990; Li and Quiros, 2001). Moreover, the relationship among rabbit breeds in this study was demonstrated differentially by the two used markers. In this regard, the highest similarity coefficient value generated by RAPD was observed between Bouscat and Gabali (0.791) and the lowest value was observed between Bouscat and Chinchilla (0.649), while in case of SRAP, the highest similarity coefficient value was observed between New Zealand and Chinchilla (0.917) and between Bouscat and Gabali (0.917), while the lowest value was observed between Bouscat and New Zealand (0.810). Additionally, the combined data of RAPD and SRAP showed similarity coefficient value ranged from 0.739 between Bouscat and Chinchilla to 0.842 between Bouscat and Gabali (Table 3).

The UPGMA cluster analysis of RAPD marker separated the six rabbit breeds into two main clusters at a similarity coefficient of 0.710 (Fig. 2a). The first main cluster has two subclusters branched at 0.733 similarity level, the first subcluster gathered Bouscat and Gabali at 0.791 level of similarity, while the second subcluster gathered California and New

Zealand grouped together at 0.787 level of similarity. These findings were similar to the results of El-Bayomi *et al*., (2013) who found that California and New Zealand were more related which placed them in one cluster separated from Flander. And the second main cluster gathered breeds of Chinchilla and Flemish at 0.758 level of similarity. On the other hand, the SRAP based cluster analysis separated the rabbit breeds into two main clusters at similarity coefficient of 0.850 (Fig. 2b), where Bouscat and Gabali breeds were grouped together at 0.917 level of similarity and gathered with California in one cluster at 0.880 level of similarity, while Chinchilla and New Zealand were grouped together at 0.917 level of similarity and gathered with Flemish in one cluster at 0.909 level of similarity. These findings were somewhat in accordance with phenotypic data based phylogenetic tree presented by Khaled *et al*., (2018), they reported that Chinchila and New Zealand were most related species and gathered with California in the same cluster which separated from Bouscat and Gabali. Moreover, in accordance with the present study they found that the most related breed to Gabali was Bouscat.

Interestingly, the combined data of RAPD and SRAP based dendrogram separated the rabbit breeds into two main clusters at a similarity coefficient of 0.770 (Fig. 2c). The first cluster grouped Bouscat and Gabali breeds together at 0.842 level of similarity and gathered them with California breed at 0.806 level of similarity in the same cluster; and these three breeds were gathered in one cluster by the same way like the first cluster in SRAP based dendrogran. It seems that Gabali and Bouscat are the most closely related breeds where they showed the highest similarity values and grouped together in the dendrograms generated by RAPD, SRAP and combined data, also these two breeds and Californa revealed more similar morphometric characters (Khaled *et al*., 2018). The second cluster grouped Chinchilla and Flemish breeds together at 0.820 level of similarity and gathered them with New Zealand breed at 0.806 level of similarity in the same cluster; while these three breeds are grouped in one cluster like the second cluster in SRAP based dendrogram but the breed of Chinchilla was more related to Flemish than New Zealand and this was similar to the second cluster in RAPD based dendrogram. Khaled *et al*. (2018) found that some morphometric characters of New Zealand breed e.g. body weight at maturity and body tall were more similar to those of Chinchilla breed and higher than those of Bouscat, Gabali and California breeds and this may explain why these breeds (New Zealand and Chinchilla) were separated and grouped together with Flemish which consider one of the large sized rabbit in one cluster in the present study (Fig. 2b and 2c).

As mentioned previously both of RAPD (Williams *et al*., 1990) and SRAP (Li and Quiros, 2001) markers analyzing different regions of the genome and as shown in Table (2) that RAPD marker are more informative and has higher discrimi-

native power than SRAP primers. However, SRAP marker has some advantages like the higher reproducibility compared to RAPD and it is correlated with the coding areas of the genome (Li and Quiros, 2001; Bargish and Rahmani 2016). It seems that the dendrogram generated from combined data of RAPD and SRAP is more effective than those generated from the data of one marker only and this was confirmed with the high values of bootstrapping which refer to the robustness of the clustering in the dendrograms (Lai *et al*., 2012; Abouzaid *et al*., 2016) (Fig. 2). Dendrogram analysis and genetic similarities among the rabbit breeds presented in this study is very useful for breeders when use some of these breeds in any genetic improvement programs (Ceron and Angel, 2001).

Moreover, both of RAPD and SARP markers successfully generated number of unique bands specific for a particular rabbit breed. In case of RAPD, there were two specific bands (110 and 130 bp) for Chinchilla breed which generated by one primer (OPE-07). And in case of SRAP, one primers combination (Me2-Em5) generated one unique band specific for California (965 bp) and another unique band specific for New Zealand (1130 bp) breeds. Another primers combination (Me10-Em5) generated one specific band for California breed (990 bp). These specific bands may become an objective for breeders as rapid, useful and easy tool for identification and characterization of these breeds in further breeding and genetic improvement programs (Saengprajak and Saensouk, 2012). However, the presence of these specific bands in all breed individuals may need to be validated by using larger sample size (Barwar *et al*., 2008).

SUMMARY

Rabbits consider one of the important animal farm species as a source for healthy and nutrient meat in Egypt and studying the genetic diversity among rabbit breeds is very important key for breeders to develop new breeds with superior economic traits. In the present study nine RAPD and seven combinations of SRAP primers were used to analyze the genetic diversity among six rabbit breeds namely Bouscat, Gabali, California, Chinchilla, Flemish, and New zealand. Also, the efficiency of the markers and the primers sets were investigated. The used primers of RAPD and SRAP generated 102 and 67 bands, respectively. Some of RAPD primers (OPA-08, OPC-07, OPE-07, OPM-07 and OPK-17) and SRAP primers combinations (Em3-Me2, Em2-Me5, Em10-Me5 and Em10-Me10) were more informativeness and showed higher discriminative power or generated specific bands for some rabbit breeds; these primers could be useful in further studies. The cluster analysis of combined data generated form RAPD and SRAP banding profile patterns revealed similarity coefficient values ranged from 0.739 between Bouscat and Chinchilla to 0.842 between Bouscat and Gabali. Additionally, from genetic similarity matrix and dendrogram analysis it appears that Bouscat, Gabali and California are more closely related

and differ from Chinchilla, Flemish and New Zealand which are more closely related.

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Table (1): List of RAPD and SRAP primers codes and sequences used for molecular analysis.

RAPD			SRAP		
No	Code	Sequence (5′-3′)	No	Code	Sequence (5′-3′)
1	OPA-02	TGCCGAGCTG	1	Me-02	TGAGTCCAAACCGGAGC
2	OPA-08	GTGACGTAGG		Em-03	GACTGCGTACGAATTGAC
3	OPC-07	GTCCCGACGA	2	Me-03	TGAGTCCAAACCGGAAT
4	OPC-18	TGAGTGGGTG		Em-02	GACTGCGTACGAATTTGC
5	OPE-07	AGATGCAGCC	3	Me-03	TGAGTCCAAACCGGAAT
6	OPI-02	GGAGGAGAGG		Em-05	GACTGCGTACGAATTAAC
7	OPK-17	CCCAGCTGTG	4	Me-05	TGAGTCCAAACCGGAAG
8	OPM-07	CCGTGACTCA		Em-02	GACTGCGTACGAATTTGC
9	OPY-05	GGCTGCGACA	5	Me-09	TGAGTCCAAACCGGAGG
				Em-05	GACTGCGTACGAATTAAC
			6	Me-10	TGAGTCCAAACCGGTAC
				Em-05	GACTGCGTACGAATTAAC
			7	Me-10	TGAGTCCAAACCGGTAC
				Em-10	GACTGCGTACGAATTTAG

Table (2): Efficiency parameters values of RAPD and SRAP primers used for molecular analysis.

Marker	Primer	TNB	MB	PB	SB	P%	PIC	Rp	MI	DI
RAPD	OPA-02	10	5	5		50.00	0.22	3.67	1.10	1.39
	OPA-08	15	3	12		80.00	0.35	8.34	4.20	4.10
	OPC-07	11	3	8		72.73	0.29	5.00	2.32	2.35
	OPC-18	10	9	1		10.00	0.05	0.67	0.05	0.27
	OPE-07	14	9	5	2	35.71	0.12	2.00	0.60	1.17
	OPI-02	9	8	1		11.11	0.05	0.67	0.05	0.27
	OPK-17	14	5	9		64.29	0.25	5.34	2.25	2.35
	OPM-07	11	3	8		72.73	0.30	5.34	2.40	2.13
	OPY-05	8	6	2		25.00	0.10	1.34	0.20	0.50
	Total	102	51	51	Average	46.84	0.19	3.60	1.46	1.61
SRAP	Me2-Em3	7	6	1		14.29	0.06	0.67	0.06	0.37
	Me2-Em5	13	10	3	2	23.08	0.08	1.34	0.24	0.87
	Me3-Em2	9	6	3		33.33	0.14	2.00	0.42	0.77
	Me3-Em5	10	8	2		20.00	0.10	1.67	0.20	0.71
	Me9-Em5	10	8	2		20.00	0.09	1.67	0.18	0.71
	Me10-Em5	14	11	3	1	21.43	0.08	1.67	0.24	1.03
	Me10-Em10	4	2	2		50.00	0.22	1.34	0.44	0.64
	Total	67	51	16	Average	26.02	0.11	1.48	0.25	0.73

TNB: Total number of bands, MB: Number of monomorphic bands, PB: Number of polymorphic bands, SB: P%: Specific bands, percentage of polymorphism, PIC: polymorphic information content, Rp: resolving power, MI: marker index, and DI: Diversity index.

Table (3): Genetic similarity matrix for 6 rabbit breeds obtained from combined RAPD and SRAP banding profiles.

Genotypes	BSK	FLM	GBL	CLF	NZL
FLM	0.781				
GBL	0.842	0.804			
CLF	0.795	0.759	0.817		
NZL	0.747	0.792	0.769	0.805	
CHN	0.739	0.820	0.750	0.763	0.820

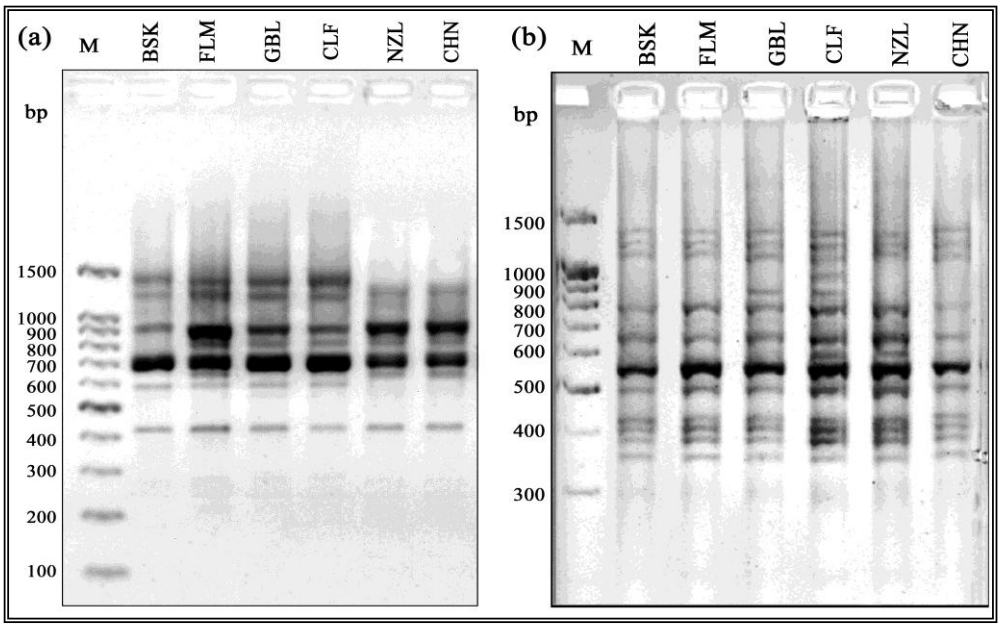

Fig. (1): Examples of banding profile pattern of six rabbit breeds generated by primers (a) OPA-2 and (b) Me10-Em5 of RAPD and SRAP markers.

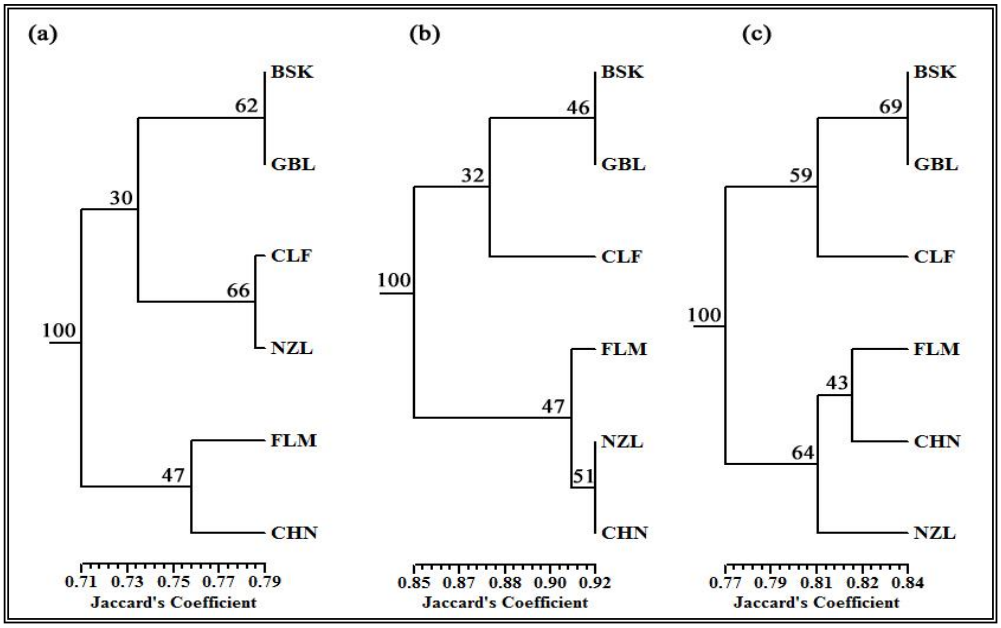

Fig. (2): UPGMA dendrogram analysis of six rabbit breeds based on (a) RAPD, (b) SRAP and (c) combined data using Jaccard's coefficient, numbers over branches indicate the bootstrapping values.