Genetic variability in mite-resistant honey bee using ISSR molecular markers

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ABSTRACT

One of the major problems facing the beekeeping industry in Saudi Arabia is the infestation of honeybee colonies with parasitic mite Varroa destructor. Bee breeders strive to reduce exposure of bees to pesticides by developing stocks of bees that resist the mites. The goal of this study was to investigate the genetic variability of eleven colonies representing three honeybee races namely; Apis mellifera yementica (Indigenous) tolerant to mites, Apis mellifera carnica (Carniolan) sensitive to mites and hybrids between Indigenous and Carniolan moderately tolerant. These colonies were collected from the gene pool of the Bee Research Unit of King Saud University through ISSR analysis. The ten ISSR primers used generated 34 polymorphic fragments and the average heterozygosity for each primer was 0.244. These data showed that most of the detected loci were polymorphic reflecting the fact that the eleven colonies under-study came from wide diverse races. Also, choosing the similar and the dissimilar fragments among all genotypes indicated clearly that the ISSR primers used in this study were sufficient to fingerprint the 11 colonies under-study. In addition, the average similarity coefficient among the 11 colonies was calculated and it was 0.561 indicating wide range of genetic diversity within the current gene pool of the Bee Research Unit of King Saud University germpl.asm. Finally, a dendogram was generated by UPGMA method which divided the colonies into three groups according to their origin. The first group included the indigenous colonies, the second included the carniolan colonies and the third group was the hybrid group and it was between the two groups. The dendrogram demonstrated clearly the ability of the ISSR marker technique to detect the genetic variability among and within the honey bee colonies used in this study.

Key words: *Genetic variability, Mite-resistance bees, ISSR markers.*

INTRODUCTION

Honey bee (*Apis mellifera* L.) is an important agricultural insect for honey production, for the pollination of more than 90 crops and for other products. The natural range of *A. mellifera* extends worldwide from northern Europe to southern Africa and from the British Isles to the Ural Mountains, western Iran, and the Arabian Peninsula (Ruttner, 1988). Honey world production is around 1.4 million tons per year (FAO, 2007). Intraspecific taxonomy of the honey bee *Apis mellifera* L. is based mainly on morphology. At present, 26 subspecies of *A. mellifera* are recognized on the basis of morphometric characters (Ruttner, 1988; 1992; Sheppard *et al.*, 1997). However, in Saudi Arabia, there are about one million bee colonies owned by approximately 5000 beekeepers and the total production of honey is about 9000 tons. About 70- 80 % of the bees kept in Saudi Arabia are Carniolan (*Apis mellifera carnica*) and Egyptian X Carniolan

F1 hybrid. The rest is typically indigenous bees *Apis mellifera yementica*, whereas Italian bees *A. m. ligustica* are less found due to less information available for beekeepers about this race and its performance under arid conditions.

One of the major problems facing the beekeeping industry in Saudi Arabia is infestation of honeybee colonies with parasitic mite *Varroa destructor*. The ectoparasitic mite *Varroa destructor* (Anderson and Trueman, 2000) was discovered by Jacobson in 1904. He described it as a natural ectoparasitic mite of eastern honeybee. But later, the mite switched to host the Western honeybee and has now become a serious pest of that bee worldwide. In Saudi Arabia, the mite was discovered in 1987 and now has become a major pest throughout the Kingdom (Al Ghamdi, 1990). It is threatening the survival of managed and feral honeybees, the beekeeping industry and the future of many agricultural crops due to the role of bees in pollination. Therefore, it is the most significant production problem for the nation's beekeeping industry. Mites spread rapidly between colonies, and infested colonies historically died. Infestation debilitates colonies to the degree that production losses occur in the first year of infestation. Losses include the colonies themselves, lost honey production and pollination, costs associated with the time and labor required to replace colonies, and costs of acaricides and their application. In addition, mites develop resistance to chemicals quickly, and few chemicals are registered and available for mite control. Although acaricides control various mites in colonies of honey bees, use of chemicals endangers bees and hive products. Bee breeders strive to reduce exposure of bees to chemicals by developing stocks of bees that resist the mites.

However, producing suitable stocks has proved daunting since most of the variation in resistant colonies originates from environmental sources (Kulincevic *et al.*, 1997, Kulincevic *et al.*, 1992, de Guzman *et al.*, 1996, Harbo and Harris, 1999). Therefore, most of the breeding programs have encountered insufficient genetic variability to produce resistant commercial stocks. Hence, the effective methods for controlling parasitic mites, should integrate traditional breeding methods for development of mite-resistant stocks with modern molecular technologies. The emphasis on identification and the use of molecular markers for mite resistance selection will offer the opportunity to find genetically resistant stocks. Also, it will transform beekeeping from an industry that has become dependent on a growing number of expensive pesticides and antibiotics into one that is free of chemical inputs and that is economically viable in today's competitive global marketplace.

A variety of molecular marker methods were used in honey bee, however PCR-based markers are more suitable for fingerprinting studies such as Random Amplified Polymorphic DNA (RAPD), Simple sequence repeats (SSR) and the inter simple sequence repeat (ISSR). Inter-simple sequence repeat (ISSR) markers were originally devised for differentiating among closely related plant cultivars but have become extremely useful for studies of populations of different organisms including insects. ISSR markers involve PCR amplification of DNA using a single primer composed of a microsatellite sequence. Therefore, it does not require prior knowledge of DNA sequence for primer design. The markers are easily generated using minimal equipment and are hypervariable, yielding a reasonable amount of data for a reasonable cost to the researcher (Sheppard and Smith, 2000). Therefore, using molecular markers such as ISSR might be useful for identification of the location of target genomic region.

The goal of the present study was to investigate the genetic variability of honey bee population collected from the gene pool of the Bee Research Unit of King Saud University through ISSR analysis. The specific objectives of this research were: (1) Detecting the level of polymorphism among the honey bee colonies under-study, (2) DNA genotyping of these colonies using ISSR markers and (3) Finding the genetic relationships between these colonies.

MATERIALS AND METHODS

Materials

Eleven colonies representing three honeybee races were used namely; *Apis mellifera yementica* (Indigenous), *Apis mellifera carnica* (Carniolan) and hybrids between Indigenous and Carniolan to study and compare the resistance to *Varroa destructor*. The eleven colonies were four *Apis mellifera yementica* (Indigenous), three *Apis mellifera carnica* (Carniolan) and four hybrids between Indigenous and Carniolan. Carniolan bees were imported from Egypt whereas the Indigenous bees were brought from the south of the kingdom.

Methods

1. Isolation of DNA

A modified method based on the protocol of Dellaporta *et al.* (1983) was conducted for isolation of total genomic DNA from the 11 colonies. 0.5 gm live adults from each colony homogenized with 320 µl of extraction buffer (10 Mm Tris-Hcl pH 8.0, 10 mM EDTA, 350 Mm NaCl, 0.5% SDS). The eppendorf tubes were incubated at 50°C for 20 min, followed by an equal volume of a standard phenol / chloroform / isoamyl alcohol extraction, vortexed and spun for 2 min. The aqueous phase was transferred to a fresh 1.5 ml tube, then centrifuged in a microfuge at a maximum speed for 10 min. Then the aqueous phase was transferred to a fresh tube and 120 µl of 3 M sodium acetate and absolute ethanol were added. For precipitation, microfugation was carried out at a maximum speed for 15 min, and then the pellet was washed with 70% ethanol and was dried in a speed vacuum drier. The dried pellet was dissolved in TE buffer depending on the size of the pellet. The concentration of the DNA was measured using UV-Spectrphotometer and a suitable dilution was adjusted. Also, the genomic DNA was electrophorased in agarose gel to evaluate its quality for further experiments. Finally, genomic DNA from each colony was adjusted at the concentration of 100ng/µl for PCR analysis.

2. Inter Sequence Simple Repeats (ISSR) molecular marker technique

a) Primers used in ISSR analysis: For ISSR analysis, 17 primers were used (Table 1).

b) PCR Reaction: Pre-made PCR reaction Master Mix (Fermentas®, Lithuania) was used. The final total volume of each reaction was 25 ul containing 0.625 Units of Taq DNA polymerase, 2 mM $MgCl_2$ and 0.2 mM of each dNTPs. 50 ng of DNA and 1000 pmol of primer were added to the reaction

c) Thermocycler program and temperature profiles: The thermocycler was programmed by an initial standard denaturation cycle at 94°C for 7 min. The following 45 cycles were composed of: denaturation step at 94°C for 1 min, annealing step was programmed at different temperatures according to the primer used for 1 min and elongation step at 72°C for 2 min. The final cycle was polymerization cycle performed at 72°C for 7 min.

d) Electrophoresis of PCR products: The PCR products of each reaction were analysed by electrophoretic separation in 2 % agarose gel. DNA marker of Fermentas (100 bp DNA ladder marker) was added on one side of the gel to determine the DNA patterns. Gel was

stained by ethidium bromide (0.5 mg/ml). A mixture of 50 ml reaction and 10 ml loading buffer were loaded into the wells of the gel. Electrophoresis was run at 40 volts for 5 hr.

e) Visualization and photography: After electrophoresis, the ISSR patterns of the PCR products were visualized under UV light using UV-transilluminator and photographed.

f) Estimation of sizes of ISSR fragments: Sizes of ISSR fragments were determined by generating standard curve of DNA marker for each gel using Fermentas 100 bp ladder. DNA ladder size marker produced fragments of precisely known molecular weights namely: 1500, 1400, 1300, 1200, 1100, 900, 800, 700, 500, 400, 300, 200 and 100. The mobility of each fragment, was measured and recorded and a standard curve was drawn. Also, the mobility of each band produced from primer amplification of each sample was measured. The size in base pair of each fragment product was estimated using the standard curve. Microsoft excel 2003 (Microsoft corporation, USA) computer program was used to draw the standard curves and to estimate sizes.

g) Data analysis: Firstly, ISSR markers derived from 10 primers were scored with the 11 honey bee colonies under-study. Data were scored as (1) and (0) which stands for the presence and the absence of PCR product in all primers used. The output was analyzed using an agglomerative hierarchical clustering method with complete linkage strategy. Secondly, the data were subjected to analysis to produce a matrix of similarity values according to Jaccard's (1908). Thirdly, cluster analysis was conducted on the genetic similarity matrix with unweighted pair group method based on arithmetic average (UPGMA) to develop a dendrogram using computer program NTSYS- pc ver 2.1 (Rolhf, 1992).

RESULTS AND DISCUSSION

Eleven honey bee genotypes were used as materials for DNA genotyping. These colonies were selected according to their tolerance characters to *Varroa destructor*. Al Ghamdi (2002) showed that the indigenous bees were the most tolerant to *Varroa destructor*, the hybrid bees were moderately tolerant and the Carniolan bees were sensitive. The 11 colonies under-study were representative samples of bee genotypes from the gene pool of the Bee Research Unit of King Saud University. Currently these colonies are used as selecting material to produce tolerant stocks of honey bee in the Bee Research Units of King Saud University. The genotyping study in this report was conducting using 17 ISSR primers and also a relationship study was carried out. Among the molecular markers the ISSR analysis was chosen because of many advantages such as; it targets simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome and evolve rapidly and it includes amplification of specific and repeatable fragments (Sheppard and Smith, 2000).

Only ten out of the seventeen primers used generated multiple fragments in the group of 11 colonies namely; 7, 8, 9, 10, 11, 12, 13, 14, 16 and 17 (Table 2). The DNA fragment profiles of the ten primers are represented in Figs. (1, 2 and 3). Primer No. 7 showed the highest number of polymorphic fragments (5 fragments) (Fig. 1A). Primers No. 16 and 17 showed the lowest number of polymorphic fragments (Figs. 3A and 3B). The total number of detected fragments was 41 with an average of 4.1 fragments per primer and the range was between 2 to 5 fragments per primer (Table 3). The total number of detected fragments per primer was expected because it depends on the number of ISSR loci in each colony. While, the total number of polymorphic fragments

was 34 with an average of 3.4 per primer and the range was between 2 to 5 per primer. The percent of polymorphic fragments ranged between 67 to 100 with an average of 81.6 per primer. These data showed that most of the detected loci were polymorphic, reflecting the fact that the eleven colonies under-study came from wide diverse races imported for mite-resistance breeding. These data were in line with the results of Paplauskiene et al. (2006) where they detected 4 polymorphic fragments per ISSR primer in honey bee. The range of fragment sizes was between 171 and 491 bp which are relatively narrow. This may be due to the type of primers used in this study. Thereafter, variability for each locus was measured. Each polymorphic fragment was scored as a locus with two allelic classes, therefore, the maximum heterozygosity value of an ISSR locus was 0.5. Finally, the average heterozygosity values for all detected loci for each primer were estimated. The average heterozygosity was 0.244 and the range was between 0.209 and 0.320. This is another indication that the colonies under-study was relatively diverse.

A comparison among different races was established by calculating the number of polymorphic loci for each race (Table 4). The colonies of the indigenous race revealed the highest total number of detected polymorphic loci while, the carniolan was the lowest. This means that the primers were able to identify more variable loci in the indigenous race than in the others. This result is indicative of recent introgression events apparently caused by the local beekeepers. This introgression is most probably due to massive losses of colonies due to a *Varroa* infection and followed by the recovery of the population from outsider colonies, according to the information given by the local beekeepers. Also, the low level of polymorphic loci among carniolan colonies is an indication that these colonies were imported specifically for breeding for mite-resistance purposes only.

Table (1): DNA sequence of ISSR primers.

No	Primer	Sequence 5'→3'
1	$(GA)_8CG$	5'-GAGAGAGAGAGAGAGACG-3'
2	$(GA)_8TG$	5'-GAGAGAGAGAGAGAGATG-3'
3	$(AC)_7CGCG$	5'-ACACACACACACACCGCG-3'
4	$(AC)_8TA$	5'-ACACACACACACACACTA-3'
5	$(AT)_9GC$	5'-ATATATATATATATATATGC-3'
6	$(AT)_9GAG$	5'-ATATATATATATATATATGAG-3'
7	$(AGAC)_4GC$	5'-AGACAGACAGACAGACGC-3'
8	$AC(GACA)_4$	5'-ACGACAGACAGACAGACA-3'
9	$(GACA)_4GT$	5'-GACAGACAGACAGACAGT-3'
10	$(GACA)_4CT$	5'-GACAGACAGACAGACACT-3'
11	$(ATG)_5GA$	5'-ATGATGATGATGATGGA-3'
12	$(TCC)_5GT$	5'-TCCTCCTCCTCCTCCGT-3'
13	$(CTC)_5GT$	5'-CTCCTCCTCCTCCTCGT-3'
14	$(AC)_8G$	5'-ACACACACACACACACG-3'
15	$(CT)_8A$	5'-CTCTCTCTCTCTCTCTA-3'
16	$(AC)_8AT$	5'-ACACACACACACACACAT-3'
17	$(AC)_8T$	5'-ACACACACACACACACT-3'

Table (2): ISSR markers detected in the 11 honey bee colony races under-study.

Primer No	Fragment molecular weights (bp)	Race of honey bees: Indigenous bees				Hybrid bees				Carniolan bees		
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 6	Colony 7	Colony 8	Colony 9	Colony 10	Colony 11
7	120	+	-	-	-	-	-	-	-	-	-	-
	170	+	-	-	-	-	-	-	-	-	-	-
	180	-	+	+	+	+	+	+	+	+	+	+
	240	-	-	-	-	+	+	+	+	+	+	+
	700	-	-	-	-	-	-	+	-	-	-	
8	100	+	+	+	+	+	+	+	+	+	+	+
	170	+	-	-	-	-	-	-	-	-	-	-
	220	-	+		+	-	-	-	+	+	-	+
	250	+	-	-	-	-	-	-	-	-	-	-
	300	+	-	+	-	-	-	-	-	-	-	-
9	150	+	+	+	+	+	+	+	+	+	+	+
	210	-	-	-	-	+	+	+	-	-	-	-
	260	+	-	+	-	-	-	+	-	+	+	-
	850	+	-	-	-	-	-	-	-	-	-	-
10	100	-	+	+	-	-	-	-	-	-	-	-
	210	-	+	+	+	+	-	-	-	+	+	+
	250	+	-	-	-	-	-	-	-	-	+	-
	340	+	+	-	+	+	+	+	+	+	+	+
11	260	+	+	-	+	+	+	+	+	+	+	+
	300	+	-	+	-	-	-	-	-	-	-	-
	400	+	-	-	-	-	-	-	-	-	-	-
12	200	-	+	+	+	+	+	+	+	+	+	+
	250	-	+	+	+	+	+	+	-	-	-	-
	300	+	-	-	+	+	+	+	+	+	+	+
	450	+	-	-	-	-	-	-	-	-	-	-
13	180	-	+	-	+	+	+	+	+	-	-	-
	220	-	+	+	+	+	+	+	+	+	+	+
	300	+	-	-	+	+	+	+	+	+	+	-
	400	+	+	+	+	+	+	+	+	+	+	+
	500	+	-	-	-	-	-	-	-	-	-	-
14	200	+	+	+	+	+	+	+	+	+	+	+
	260	-	-	-	-	-	+	+	-	+	+	+
	300	-	-	-	-	-	+	+	-	+	+	-
	320	+	-	-	-	-	-	-	-	-	-	-
	420	-	-	-	-	+	+	+	-	-	-	-
16	250	-	-	-	+	+	+	+	+	+	+	+
	300	-	-	-	-	+	+	+	+	-	-	-
	500	+	+	+	+	+	+	+	+	+	+	+
17	150	-	-	-	+	-	-	-	+	-	-	-
	250	+	+	+	-	+	+	+	+	+	+	+
	450	+	+	-	-	-	+	-	+	+	+	-

(+present, — absent)

The obtained result showed that ISSR technique is a useful method for detecting polymorphism in honey bee. The simple inheritance of ISSR markers combined with

the easiness of detecting polymorphic markers gave that system great potential in studies of honey bee genotyping. Therefore, for the genotyping purpose, the 41 ISSR markers (Table 2) were tracked to discriminate among the colonies. For example ISSR 120 bp and 140 bp fragments of the primer No. 7, 170 bp and 250 bp fragments of the primer No. 8, 850 bp fragment of primer No. 9, 400 fragment of primer No. 11, 450 bp fragment of primer No. 12, 500 bp fragment of primer No. 13 and 320 bp fragment of primer No. 14 were found to be unique for colony number 1 (Table 2). While, with primer No. 7, the fragment 700 bp was unique for colony No. 7. Also, by calculating the similar and the dissimilar fragments among all genotypes, the detected 41 ISSR fragments indicated that primers tested were able to discriminate between all 11 colonies. These results showed clearly that the ISSR primers used in this study were sufficient to genotype and fingerprint the 11 colonies under-study.

Table (3): Levels of polymorphism detected by the 10 ISSR primers in 11 honey bee colonies.

No	Primer	Total number of fragments	Number of polymorphic fragments	Percent of polymorphic fragments	Range of fragment sizes (bp)	Heterozygosity
7	$(AGAC)_4GC$	5	5	100	120-700	0.225
8	$AC(GACA)_4$	5	4	80	100-300	0.225
9	$(GACA)_4GT$	4	3	75	150-850	0.264
10	$(GACA)_4CT$	4	4	100	100-340	0.306
11	$(ATG)_5GA$	3	2	67	260-400	0.209
12	$(TCC)_5GT$	4	4	100	200-450	0.281
13	$(CTC)_5GT$	5	4	80	180-500	0.245
14	$(AC)_8G$	5	4	80	200-420	0.304
16	$(AC)_8AT$	3	2	67	250-500	0.287
17	$(AC)_8T$	3	2	67	150-450	0.320
	Mean	4.1	3.4	81.6	171-491	0.244

Table (4): Comparison of polymorphic loci among the three honey bee races using 10 ISSR primers.

Honey bee race	Total number of detected loci	Number of polymorphic loci	Percent of polymorphic loci
Indigenous bees	35	31	88.57
Hybrid bees	31	16	51.61
Carniolan bees	23	8	34.78

Table (5): Similarity matrix for the 11 honey bee colonies based on Jaccard's coefficient obtained from 41 ISSR fragments.

	Colony1	Colony2	Colony3	Colony4	Colony5	Colony6	Colony7	Colony8	Colony9	Colony10	Colony11
Colony1	1										
Colony2	0.281	1									
Colony3	0.300	0.600	1								
Colony4	0.273	0.667	0.435	1							
Colony5	0.286	0.583	0.440	0.696	1						
Colony6	0.306	0.538	0.357	0.577	0.833	1					
Colony7	0.297	0.464	0.393	0.556	0.800	0.880	1				
Colony8	0.333	0.609	0.346	0.727	0.708	0.720	0.630	1			
Colony9	0.364	0.583	0.440	0.625	0.615	0.692	0.667	0.708	1		
Colony10	0.406	0.520	0.440	0.560	0.615	0.692	0.667	0.640	0.909	1	
Colony11	0.281	0.619	0.455	0.667	0.652	0.600	0.570	0.682	0.810	0.727	1

The data of this report were in accordance with data of Paplauskiene *et al.* (2006). From these results, it can be concluded that ISSR is a powerful marker system, under our study conditions because it produces enough polymorphism even using small

number of primers. Also, it should be possible to establish a set of high polymorphic ISSR markers for honey bee race identification and for detection of mite-tolerant genotypes. These results were in harmony with the results obtained by Paplauskiene *et al.* (2006) where they found that ISSR banding profiles were very repeatable on duplicate samples and that different honey bee races have very different fingerprint patterns, even among the closely related races being difficult to distinguish by other molecular-marker techniques. Also, Roux *et al.* (2007) demonstrated that the ISSR-PCR was a good tool for discrimination and genetic structure analysis of *Plutella xylostella* populations native to different geographical areas. de Leon *et al.* (2006) were able to uncover distinct banding patterns in *Gonato-erus* species three individuals emerging from different host tribes by using ISSR-PCR fingerprinting. Dušinsk *et al.* (2006) used inter-simple sequence repeat (ISSR) markers successfully for discrimination between and within species of blackflies. Meena *et al.* (2005) used ISSR analysis for molecular characterization of *Tospovirus* transmitting Thrips populations from India. de Leon *et al.* (2004) indicated that The ISSR markers were geographic-specific in *Gonato-erus morrilli* and can therefore be considered diagnostic since there was no band sharing between different populations. Philips *et al.* (2002) showed that amplification of inter simple sequence repeats regions of *Medicago aethiopoides* DNA demonstrated clear genetic differences between French and New Zealand *M. aethiopoides*. Luque *et al.* (2002) have shown that some ISSR amplifications are possible and demonstrate their applicability in studying intra- and inter-specific variation in some Noctuid populations.

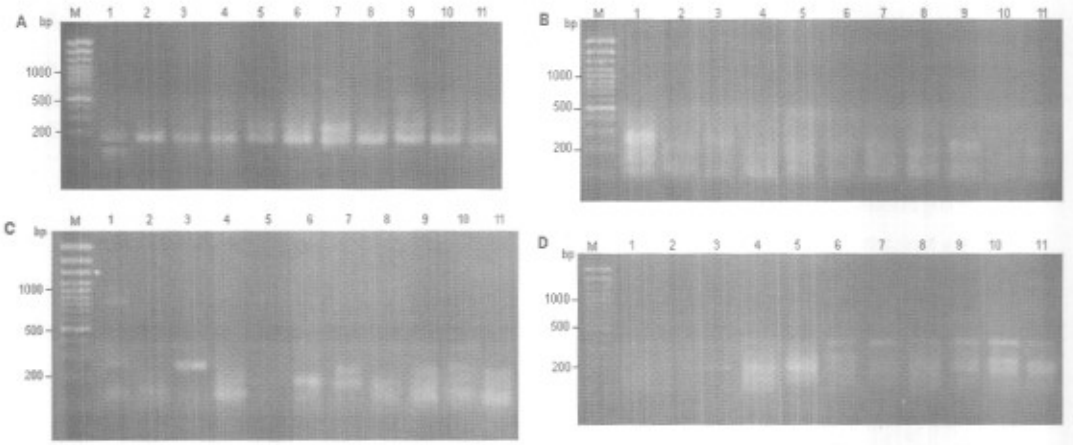

Fig. (1): DNA fragments profile of the 11 honey bee Apis mellifera genotypes amplified with ISSR primers namely; A) $(AGAC)_4GC$ B) $AC(GACA)_4$ C) $(GACA)_4GT$ and D) $(GACA)_4CT$. (M) size molecular marker 100 base-pair ladder (1-4) yementica, (5-7) hybrid and (8-11) carnica.

Similarity coefficients were calculated among the 11 colonies based on the 41 ISSR fragments. Similarities between all pairs of the 11 colonies are presented in Table (5). The obtained similarities ranged from 0.273 between colony 1 and colony 4 to 0.909 between 9 and 10 with an average of 0.561. These values indicated a wide range of genetic diversity among these 11 colonies and consequently within the current gene pool of

the Bee Research Unit of King Saud University germplasm. This wide range of genetic diversity can be explained by the fact that many foreign germplasms were introduced to the germplasm of the Bee Research Units of King Saud University. Also, this range can be considered as a good potential source for selection and genetic improvement which may lead to increase in tolerance to mites.

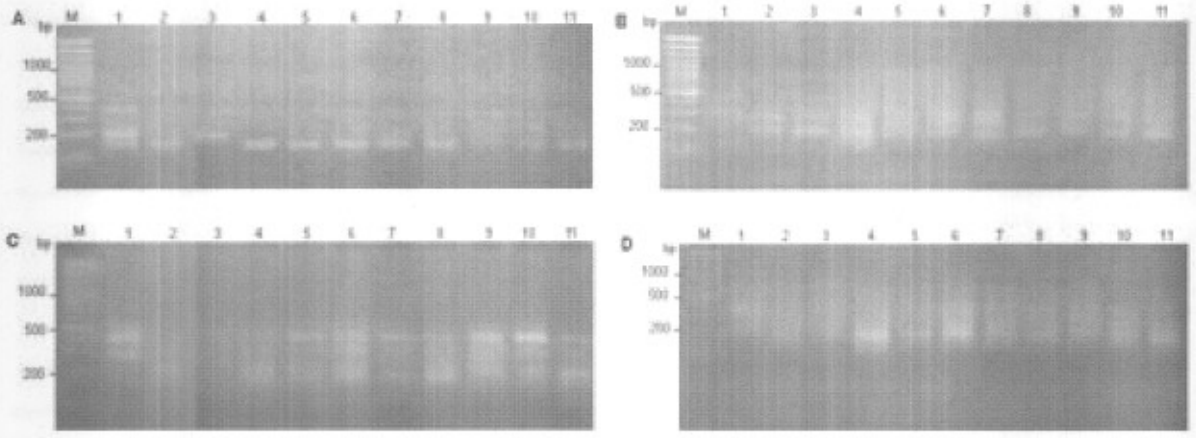

Fig. (2): DNA fragments profile of the 11 honey bee Apis mellifera genotypes amplified with ISSR primers namely; A) $(ATG)_5GA$ B) $(TCC)_5GT$ C) $(CTC)_5GT$ and D) $(AC)_8G$ (M) size molecular marker 100 base-pair ladder (1-4) yementica, (5-7) hybrid and (8-11) carnica.

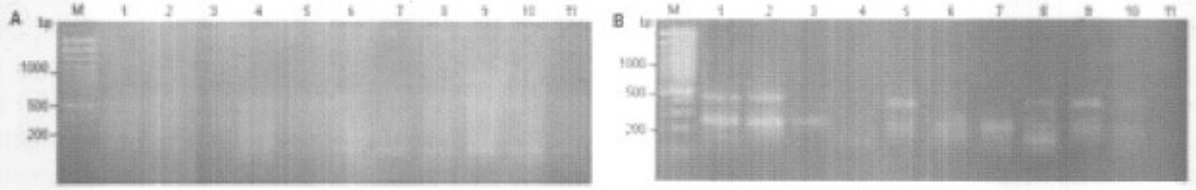

Fig. (3): DNA fragments profile of the 11 honey bee Apis mellifera genotypes amplified with ISSR primers namely; A) $(AC)_8AT$ and B) $(AC)_8T$. (M) size molecular marker 100 base-pair ladder (1-4) yementica, (5-7) hybrid and (8-11) carnica.

A dendogram was generated by UPGMA to show the genetic relationship of the colonies under-study and was presented in Figure (4). Colony number 1 formed a distinct group that was separated from all other colonies (higher most) at similarity coefficient of 0.31. Therefore, according to this result this colony was different from the rest of the colonies, may be because it was developed directly from the old indigenous races. The rest of the colonies formed two distinct groups at the similarity coefficient level of 0.49 (Fig. 4). The first was a small group, which included two colonies and separated at similarity coefficient of about 0.60. These two colonies are indigenous colonies, therefore they formed one group. Although, colony 1 formed a unique branch, it was very close to the other indigenous colonies. The second group included eight colonies and separated at similarity coefficient of about 0.64. This group is divided into two subgroups. The first sub-group included three colonies and the second sub-group included five colonies. The first

sub-group included three Carniolan colonies at similarity coefficient of about 0.77. According to this result, these three colonies were very similar to each other. The second sub-group is divided into two branches, one included three hybrid colonies at similarity coefficient of about 0.80 and the other included two colonies; one indigenous (colony 4) and the other is hybrid colony (number 8) at similarity coefficient of about 0.73.

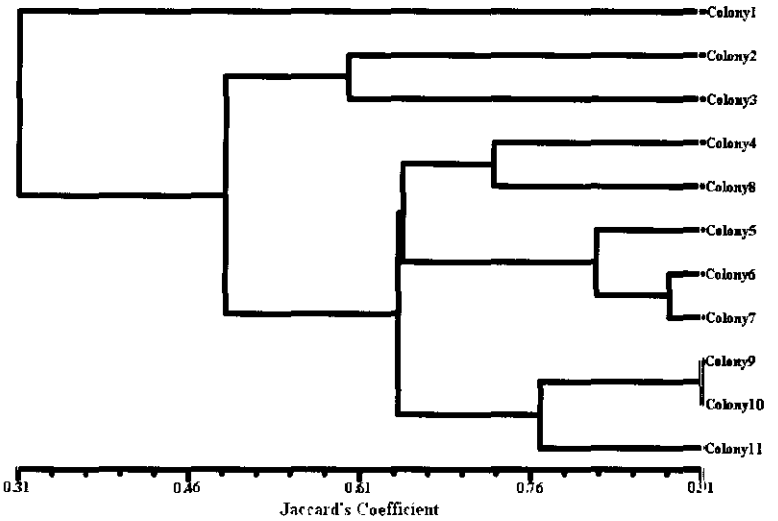

Fig. (4): Dendogram generated by UPGMA cluster analysis based on Jaccard's coefficient using 41 ISSR fragments.

This branch included two colonies from different origin; colony 4 and colony 8. Although, colony 8 is a hybrid colony, it was closer to the other indigenous colonies. This result may indicate that colony 8 is the closest colony to the indigenous colonies. In conclusion, the dendogram divided the colonies into three groups according to their origin. The first group included the indigenous colonies, the second included the Carniolan colonies and the third group was the hybrid group and it was between the two groups. The dendrogram demonstrated clearly the ability of the ISSR markers to detect the genetic variability between and within the honey bee colonies used in this study and to identify groups and subgroups with different levels of genetic distance. Also, the results have shown that it is possible to distinguish the honey bee races to carry out phylogenetic studies and to select lines for highest genetic diversity using this type of marker. Finally, ISSR markers can be used in molecular marker-assisted breeding programs for mite-resistance. Vijayan *et al.* (2006) indicated that genetic diversity and differen-tiation among populations of the Indian eri silkworm, *Samia Cynthia ricini,* can be revealed by ISSR markers. Khemakhem *et al.* (2005) indicated that ISSR can be useful as DNA-based molecular markers for studying genetic diversity and phylogenetic relationships of *Mayetiola* haplotypes. Chatterjee and Mohandas (2003) identified ISSR markers associated with productivity traits in silkworm, *Bombyx mori* L.

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الملخص العربي

التغيرات الوراثية في نحل العسل المقاوم للحلم باستخدام الواسمات الجزيئية ISSR

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احد أهم المشاكل التي تواجه صناعة تربية النحل بالمملكة العربية السعودية هو إصابة خلايا نحل العسل بالحلم المتطفل *Varroa destructor* و يحاول مربوا النحل بالمملكة جاهدين تقليل تعرض النحل للمبيدات عن طريق إنتاج سلالات من النحل مقاومة لهذا الحلم. والهدف من هذه الدراسة هو فحص التغيرات الوراثية بإستخدام تقنية ISSR لإحدى عشر طائفة من النحل تم جمعها من وحدة بحوث النحل بجامعة الملك سعود و هي تمثل ثلاث سلالات من نحل العسل و هي السلالة المحلية *Apis mellifera yementica* و هي سلالة تتحمل الإصابة بالحلم و السلالة المستوردة كارنيولي *Apis mellifera carnica* و هي سلالة حساسة للإصابة بالحلم و السلالة الثالثة هجين بين السلالتين السابقتين و هي سلالة متوسطة التحمل للإصابة و قد أوضحت نتائج تلك الدراسة أن العشرة بوادئ الISSR المستخدمة أعطت ٣٤ شظية مختلفة من الدنا و أن متوسط الإختلافات على مستوى كل بادئ هي ٠,٢٤٤ مما أوضح أن معظم المواقع التي تم التعرف عليها بها نسبة من الإختلافات و هذا يعكس حقيقة هامة وهي أن الإحدى عشر طائفة تحت الدراسة إنحدرت من سلالات بينها تنوع شديد ، و أيضا عند تتبع الشظايا المتشابهة وغير المتشابهة الناتجة من بوادئ الISSR بين الطوائف الإحدى عشر أوضحت أن العشرة بوادئ المستخدمة في تلك الدراسة كافية لعمل بصمة وراثية لإحدى عشر طائفة ، و بالإضافة إلى ذلك فعند حساب متوسط معامل التشابه بين تلك الطوائف وجد أنه ٠,٥٦١ و الذي أعطى مؤشرا آخر عن المدى الواسع من التنوع الوراثي للمحتوى الحالي من الطوائف الممثلة للسلالات الثلاث المتواجدة في وحدة بحوث النحل بجامعة الملك سعود و في النهاية تم عمل دندوجرام لتوضيح العلاقة بين تلك السلالات بإستخدام طريقة الUPGMA و بناءا عليه فقد أظهرت نتائج الدندوجرام أن هذه الطوائف قد قسمت إلى ثلاث مجاميع كل منها يمثل سلالة من السلالات، الأصلية فالمجموعة الأولى شملت طوائف السلالة المحلية و الثانية شملت طوائف السلالة كارنيولي و الثالثة طوائف السلالة الهجين و كان موقعها في الدندوجرام بين السلالتين السابقتين مما أوضح قدرة الISSR على تقدير الإختلافات الوراثية داخل و بين الطوائف تحت الدراسة .