Cellular and Molecular Biology

Asadi *et al. Cell. Mol. Biol.*2016, 62 (12): 51-55
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.12.9

Original Research

Genetic diversity of the Dwarf honeybee (*Apis florea* Fabricius, 1787) populations based on microsatellite markers

N. Asadi[1, 2], A. Rahimi[3,4], M. Ghaheri[5], D. Kahrizi[5,6], M. Bagheri Dehbaghi[4], S. Khederzadeh[7], M.H. Banabazi[8], S. Esmaeilkhanian[8], B. Veisi[9], M. Geravandi[5], H. Karim[10], S. Vaziri[11], F. Daneshgar[12], J. Zargooshi[13*]

[1] Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Razi Street, P.O. Box: 381351551. Khoramabad, Iran
[2] Animal Science Research Institute of Jihad-e- Agriculture Ministry
[3] Department of Plant Protection, Razi University, Kermanshah, Iran
[4] Zagros Bioidea Co. Razi University Incubator, Razi University, Kermanshah, Iran
[5] Department of Agronomy and Plant Breeding, Razi University, Kermanshah, Iran
[6] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[7] Natural History Museum and Genetic Resources, Department of Environment, Pardisan Eco-Park, Tehran, Iran
[8] College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[9] Department of Soil Science, Razi University, Kermanshah, Iran
[10] Department of Cardiology, Kermanshah University of Medical Sciences, Kermanshah, Iran
[11] Department of Infectious Diseases. Kermanshah University of Medical Sciences, Kermanshah, Iran
[12] Department of Ophthalmology. Kermanshah University of Medical Sciences, Kermanshah, Iran
[13] Department of Sexual Medicine, The Rhazes Center for Research in Family Health and Sexual Medicine; Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract: *Apis florea* is one of two species of small, wild honeybee. The present study was conducted to evaluate the genetic diversity of *Apis florea* honeybee from 48 nests (colonies) using microsatellite markers in the South of Iran. All honeybee samples were analyzed for six microsatellite loci (A88, A107, A7, B124, A113 and A35). The six loci had different numbers of alleles in the sampled colonies ranging from 7 (loci A107) to 3 (loci A7, A35). Gene diversity in *Apis florea* ranged from 0.491 to 0.595. This range probably reflects the spreading of nests in a large region with a varied climate. Phylogenetic tree showed two distinct clusters including a) Minab region samples and b) Bandar Abbas, Bandar Khamir and Qeshm Island regions. All of these regions are geographically rich, having varied vegetation and climate conditions. Our findings are an important contribution to the methods of studying distribution and conservation of *Apis florea*.

Key words: *Apis florea,* Genetic diversity, SSR, Polymorphism, Phylogenetic tree.

Introduction

Apis florea are found in Southeastern Asian countries, especially Thailand, Iran, Oman, India, Myanmar, some part of china, Cambodia and Vietnam (1). This species also called as "Dwarf Honeybee". They live in forests. In the Southern Iran they are excellent pollinators for the tropical fruit crops. *Apis florea* are well established in Iraq, Oman and Yemen (2,3) and have recently been detected in Sudan (4) and in central Saudi Arabia (5). *Apis florea* build exposed nests and there is always a single comb (usually less than 25 cm wide) on a single branch. This small nest contains a crown above the branch, which is used for honey storage and as a platform for the foragers leaving from and arriving to the nest. Even in a single nest, there is high genetic diversity among the *Apis florea* bees. Since honeybee queens are polygamous, wide genetic variability exists. The tendency of this species to perform certain tasks is dependent on this variation. For example fanning of the nest is performed by a specific colony, when the nest reaches a specific temperature threshold (6). Studies confirmed that genetic diversity is enormously important in honeybee colonies (7-11). Currently advanced molecular techniques can to clarify the genetic diversity of honeybee colonies (7,11). Of these techniques, microsatellites are extremely useful new tools for examining taxonomy and population biology (12-15). High mutation rate of *Apis florea*, and large number of alleles in microsatellites, make microsatellites particularly useful for genome mapping and paternity analysis. The number of loci required to study the population in question is high (16). Several genetic structure studies of *Apis mellifera* honeybee populations using microsatellite markers have been conducted in Slovenia, Spain, Canary Islands, Balearic Islands, continental Italy, Sicily Island, Africa continent and Iran (17-21). In addition, microsatellites have been successfully used in studies of *Apis mellifera* and other spices, at population level (22-24). All studies of genetic diversity in Dwarf honeybee focused on morphological characteristics, rather than molecular ones.

So far no information on genetic diversity of *Apis florea* using microsatellite markers have been published in Iran and neighboring countries. The objective of our

Received May 22, 2016; **Accepted** October 14, 2016; **Published** October 31, 2016

*** Corresponding author:** Javaad Zargooshi, Professor of Urology. Chair, Department of Sexual Medicine, Muhammad Zakariya Razi (Rhazes) Boulevard, Kermanshah University of Medical Sciences, Kermanshah, Iran. Email: zargooshi@gmail.com

Table 1. Collecting data of 4 populations of *Apis florea* in the South of Iran.

Region cod	Collection locality	Position	Number of Sample
A	Minab	27°11′53″N 54°22′7″E	15
B	Bandar Abbas	27°11'11"N 56°16'38"E	12
C	Bandar Khamir	26 56' 40"N 55 35' 04"E	13
D	Qeashm Island	26°41′43″N 55°37′06″E	8

Table 2. Microsatellite Core sequences and Primer sequences (5'-3').

Locus	Core sequence	Primer sequences (5'-3')
A88	(CT)10 TC (CCTT)2 (CTTT)3… (GGA)	F: 5'CGAA TTAACC5G5A TTTGTCG3' R:5'GATCGCAATTATTGAAGGAG3'
A107	(GCTC)2 (GCT)2 (CT)23	F: 5'CCGTGGGAGGTTTATTGTC3' R:CCTTCGTAACGGATGACACC3'
A7	(CT)3 (T)7 CCTTCG (CT)24	F: 5'GTTAGTGCCCTCCTCTTGC3' R: 5'CCCTTCCTCTTTCATCTTCC3'
B124	(CT) 8 TCCTCTTC....(CT)14 CCTC (GC)3… (GGCT)8	F: 5'GCAACAGGCGGGTTAGAG3' R: CAGGATAGGGTAGGTAACAG3'
A113	(TC)2 C (TC)2 TT (TC)5 TT (TC)8 TT (TC)5	F: 5'CTCGAATCGTGGCGTCC3' R: 5'CCTGTATTTTGCAACCTCGC3'
A35	(GT)14	F: 5'GTACACGGTTGCACGGITTG3' R: 5'CTTCGATGGTCGITTGTACCC3'

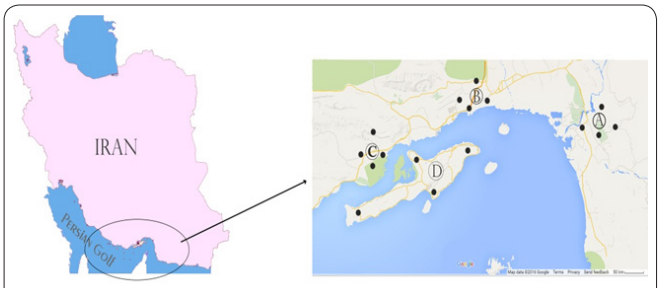

Figure 1. The geographical distribution of *Apis florea* populations collected in this study.

study was to determine the genetic diversity of *Apis florea* honey bee colonies from the Southern Iran using microsatellite markers.

Materials and Methods

Sampling

Adult *Apis florea* workers were collected from 48 nests (colonies) in four different regions covering the species' distribution in the Southern Iran: A) Minab, B) Bandar Abbas, C) Bandar Khami, and D) Qeshm Island (Fig. 1, Table 1). Only one bee per colony was subject to genetic analysis. Bee samples were kept in absolute ethanol at –20°C until DNA extraction.

Molecular analysis

Genomic DNA extracted from whole body tissues of *Apis florea* using the method described by Asadi (25). DNA samples amplified using multiplex PCR with six microsatellite loci. The core regions of these microsatellite loci were already known (B124, A107, A35, A88, A113 and A7) (20-23). Primer sequences conditions are given in the Table 2.

A standard PCR was performed on an eppendorf thermo cycler (MJ research Inc, USA) following the published protocol for each marker. Each 15 µl reaction mixture contained 50 ng template DNA, 200 µM of each dNTP, 10X PCR buffer, 1 µM of each primer pair, 5 units/µl *Taq* DNA polymerase and $MgCl_2$ (0.7–1.5 mM). Amplification profiles consisted of one cycle at 94ºC during 10 min, followed by 35 cycles at 94°C for 30s. Appropriate annealing temperature was at 55-58 °C for 30 s, extension at 72°C for 30s and finally extension step at 72°C for 10 minutes. PCR products were electrophoresed on an 8% non-denaturing polyacrylamide-bis acrylamide gel and stored for 20 ± 2 hours at 40 V. Gels were silver-stained according to Bassam (15). Two standard size markers, (stepladder; Roche, Germany) were included in each run.

Statistical analyses

Microsatellite allele sizes scored by comparing the length of the PCR fragments to the standard 100 bp size markers, stepladder (Roche, Germany). Population parameters and estimates of gene diversity calculated with the POPGENE software version 1.31 (26). The exact test for Hardy-Weinberg equilibrium, genotypic linkage disequilibrium, and genetic structure (genotypic differentiation) were computed with POPGENE version 1.31. Microsatellite variation within and between populations was analysis with FSTAT (27). The exact test for Hardy-Weinberg equilibrium and genotypic differentiation performed using POPGENE. Unbiased estimates and standard deviations of heterozygosity calculated according to Nei (28). Polymorphic information content (PIC) for each locus was estimated using PIC 1.80 software (29).

Results

All of the six microsatellite loci were polymorph. Sixty seven alleles were found for six microsatellite loci in *Apis florea* colonies from four regions in the Southern Iran. The number of alleles per locus varied from 3 (locus A7 and A35) to 7 (loci A107). Samples from Minab showed a higher level of polymorphism, with an average of 3.3 alleles per locus. In contrast, colonies from the Qeshm Island had the lowest average alleles per locus (average, 2.2). Average observed heterozygosity (Ho) per locus ranged from 0.833 (A107) to 0.000

Table 3. Number of alleles (Na), heterozygosity observed (Ho) and expected (He) per locus for *Apis florea.*

Population		Locus						
		A88	**A107**	**A7**	**B124**	**A113**	**A35**	**Ave**
Minab(A)	Na	4	7	2	3	1	3	3.33
	Ho	0.755	0.788	0.000	0.111	0.000	0.000	0.277
	He	0.899	0.815	0.494	0.568	0.000	0.593	0.562
Bandar Abbas(B)	Na	2	4	2	4	2	2	3
	Ho	1.000	0.556	0.000	0.000	0.000	0.000	0.259
	He	0.500	0.691	0.346	0.716	0.480	0.219	0.492
Bandar Khamir(C)	Na	4	5	2	2	3	2	3
	Ho	0.889	0.571	0.000	0.111	0.000	0.000	0.2618
	He	0.623	0.724	0.198	0.745	0.642	0.444	0.563
Qeshm Island(D)	Na	2	2	2	3	2	2	2.17
	Ho	0.667	0.666	0.000	0.000	0.000	0.000	0.222
	He	0.444	0444	0.444	0.647	0.500	0.444	0.487

Table 4. Allele frequencies at the microsatellite studied loci in *Apis florea* populations.

Locus	Allele	Minab	B.Abbas	B.Khamir	Qeshm
A88	135	0.278	0.000	0.056	0.000
	140	0.556	0.500	0.500	0.667
	150	0.056	0.000	0.111	0.000
	158	0.111	0.500	0.333	0.333
A107	Allele	Minab	B.Abbas	B.Khamir	Qeshm
	107	0.167	0.222	0.143	0.000
	109	0.222	0.444	0.429	0.667
	111	0.278	0.000	0.143	0.000
	113	0.056	0.000	0.000	0.000
	117	0.111	0.222	0.000	0.000
	119	0.056	0.111	0.214	0.333
	121	0.111	0.000	0.071	0.000
A7	Allele	Minab	B.Abbas	B.Khamir	Qeshm
	114	0.000	0.000	0.111	0.667
	119	0.556	0.778	0.889	0.333
	124	0.444	0.222	0.000	0.000
B124	Allele	Minab	B.Abbas	B.Khamir	Qeshm
	225	0.333	0.333	0.389	0.333
	228	0.556	0.333	0.611	0.333
	231	0.111	0.222	0.000	0.333
	234	0.000	0.111	0.000	0.000
A113	Allele	Minab	B.Abbas	B.Khamir	Qeshm
	193	0.000	0.400	0.000	0.000
	212	1.000	0.000	0.000	0.000
	224	0.000	0.600	0.222	0.500
	227	0.000	0.000	0.333	0.500
	232	0.000	0.000	0.444	0.000
A35	Allele	Minab	B.Abbas	B.Khamir	Qeshm
	58	0.222	0.000	0.667	0.333
	60	0.556	0.875	0.333	0.667
	62	0.222	0.125	0.000	0.000

(A7, A13 and A35). The highest and lowest HE were estimated for A107 (0.669) and A7 (0.370) loci, respectively. Among *Apis florea* colonies, the average observed heterozygosity ranged from 0.222 (Qeshm) to 0.262 (Bandar Khamir). As seen in the table 3, heterozygosity values, as criteria for diversity within population, had a range from 0.491 in Qeshm Island to 0.595 in Minab (Table 3).

The number of alleles specific for *Apis florea* in the Southern Iran is presented in the Table 4.

The Shannon indices were also in accordance with heterozygosity values. Polymorphism information content (PIC) that may be used as a guide for applying of loci in the next studies was estimated (Table 5).

Based on the result of distance matrices, *Apis florea* colonies in Minab and Qeshm Island had the highest genetic distance. In contrast, the colonies of Bandar Khamir and Qeshm Island showed the lowest genetic distance (Table 6). Multi locus *Fst* values for *Apis florea* colonies in different regions are presented in the Table 7.

Phylogenetic relationships among the *Apis florea* populations showed two distinct clusters. Dwarf honeybee populations of Bandar Abbas, Bandar Khamir and Qeshm Island were located in the first group. The second group included dwarf honeybee population of Minab (Fig. 2).

Table 5. Average value of PIC microsatellite in *Apis florea*.

Loci	A35	A113	B124	A7	A107	A88
PIC	0.35	0.32	0.52	0.29	0.61	0.48

Table 6. The genetic distance among *Apis florea* populations.

	A	B	C	D
A(Minab)	0.000			
B(Bandar Abbas)	0.471	0.000		
C(Bandar Khamir)	0.475	0.341	0.000	
D(Qeshm Island)	0.666	0.295	0.289	0.000

Discussion

This is the first report on genetic diversity of *Apis florea* in Iran. Genetic variability or diversity is an essential characteristic of any population for the fitness of individuals as well as survival of the whole population,

Table 7. Average value of F_{ST} of *Apis florea* populations using microsatellite analysis.

	Minab(A)	Bandar abbas(B)	Bandar khamir(C)	Qesh Island(D)
F_{ST} Value	- 0.135	0.011	0.036	0.063

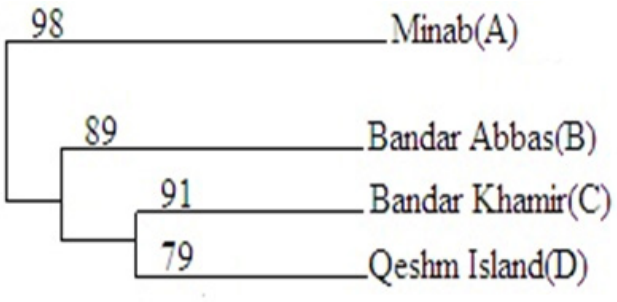

Figure 2. Phylogenetic relationships of *Apis florea* populations in the South of Iran.

permitting adaptation to the changing environmental conditions. Therefore, the degradation of genetic diversity of a species reduces its capability for adaptation and increases the risk of its extinction (30, 31). The polymorphism indices of *Apis florea* in Minab region were higher than other regions. This may reflect the unique climate in Minab (high annual precipitation rate, rich vegetation including extensive citrus gardens, and a moderate climate). Climate and altitude are likely two main factors affecting body characteristics of honeybee (32- 36). In contrast, low polymorphism indices in Qeshm Island colonies may have been resulted from their isolation in this island of Persian Gulf, with no genetic connection with other regions. It is likely that the bees in Minab have been imported by humans, who traveled by ship. Our results are similar within the islands and coincide with the results observed in the endemic Canarian, bumblebee Bombus canariensis (22, 37) and in honeybee populations from Crete and Sicily (23, 24). In this context, genetic diversity among honeybee colonies may be caused by random mating of a queen with many males from the neighboring apiaries. Another factor affecting the genetic diversity within each population may be the continued migration of colonies into some areas with the same climate. The *Fst* values indicate inbreeding in a population level in a range from -1 as lowest to +1 as highest inbreeding. Our results showed the lowest inbreeding within Minab colonies (-0.135) and the highest value (+0.063) in the Qeshm Island. *Fst* values were related to population size and genetic connections among the studied populations. For instance, the limited number of nests (colonies) and low number of bees per nest, especially the number of male and geographically isolation of Qeshm Island colonies caused a higher inbreeding among the bees of this region than the bees in other regions. In contrast, in Minab region, rich vegetation, *A. florea* importing from other regions, and connections between Minab colonies and other populations, lent to a negative *Fst*. According to the likelihood ratio test, all loci showed a significant deviation from Hardy-Weinberg (HW) equilibrium. It seems that this is mainly due to 1) limited number of nests of the studied regions and 2) sampling method that are two disturbance factors for HW equilibrium. Our research provides new information concerning the genetic variability of *A. florea*. It can be used for conservation purposes (38).

Acknowledgements

The authors wish to thank the beekeepers kindly helped with useful information about *Apis florea* honeybee colonies in different region and also animal science research institute personals. This study has been supported by the projects 2-020-210000-03-0000-84052 (Animal science research institute (ASRI) of Karaj, Iran). The authors thank the colleagues and staffs in Razi University Incubator, Zagros Bioidea Co., Biotechnology Lab (Kermanshah, Iran) for their helpful advices.

References

1. Deowanish S, Wattanachaiyingcharoen W, Wongsiri S, Oldroyd BP, Rinderer TE, Sylvester HA. Biodiversity of dwarf honey bees in Thailand. Proc. 7th Int. Conf. Trop. Bees, Chiang Mai, Thailand 2001; 97-103.
2. Wongsiri S, Lekprayoon C, Thapa R, Thirakupt K, Rinderer TE, Sylvester HA, Oldroyd BP, Booncham U. Comparative biology of *Apis andreniformis* and *Apis florea* in Thailand. BeeWorld 1996; 77: 23–35.
3. Parechehreh SH, Farshineh Adl M.B, Fallahzadeh M, Babaei M. Determining phenotypic diversity of little bee (*Apis florea*) in Iran by use of clustering analysis. Prec. 7th honey bee Congr, 11- 12 January, Karaj, Iran 201; 54-55.
4. Mogga GB, Ruttner F. *Apis florea* in Africa; source of the founder population. Bee World 1988; 69: 0–103.
5. Hepburn HR, Radlo SE, Otis GW, Fuchs S, Verma LR, Tan K, Chaiyawong T, Tahmasebi GH, Wongsiri S. *Apis florea*: morphometric, classification and biogeography. Apidologie 2005; 36: 359-376.
6. Jones C, Piyamas N, Benjamin PO. The role of genetic diversity in nest cooling in a wild honey bee, *Apis florea*. J Comp Physiol 2007; 5: 159-65.
7. Rahimi A. Study of the genetic diversity of Iranian honey bee (*Apis mellifera meda* Skorikow, 1829) populations using the mtDNA COI–COII intergenic region. *Biologija* 2015; 61: 54-59.
8. Saraithong P, Li Y, Saenphet K, Chen Z, Chantawannakul P. Bacterial community structure in *Apis florea* larvae analyzed by denaturing gradient gel electrophoresis and 16S rRNA gene sequencing. *Insect science* 2015; 22: 606-618.
9. Bashir S.M.S.M. Cytogenetic study and allozyme varians of two species of the honeybee *Apis*: (*Apis Mellifera* L. and *Apis Florea* Fab.) From Sudan (Doctoral dissertation, UOFK).
10. Biewer M, Lechner S, Hasselmann M. Similar but not the same: insights into the evolutionary history of paralogous sex-determining genes of the dwarf honey bee *Apis florea*. *Heredity* 2016; 116: 12-22.
11. Rahimi A, Mirmoayedi A, Kahrizi D, Zarei L, Jamali J. Genetic diversity of Iranian honey bee (*Apis mellifera meda* Skorikow, 1829) populations based on ISSR markers. Cell Mol Biol 2016; 62: 53-58.
12. Takezaki N, Nei M. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 1996; 144: 389-399.
13. Sinacori A, Rinderer TE, Lancaster V, Sheppard WS. A morphological and mitochondrial assessment of *Apis mellifera* from Palermo, Italy. Apidologie 1998; 29: 481–490.
14. Hall HG. PCR amplification of a locus with RFLP allele specific to African honeybees. Biochem Genet 1998; 36: 351–361.
15. Bassam BJ, Caetano AG. Silver staining of DNA in polyacrylamide gels. Applied Biochemistry and Biotechnology 1993; 42: 181-188.
16. Barker JSF. A global protocol for determining genetic distances among domestic livestock breeds. Proc. 5th World Cong. Genetics Appl. Lives. Prod. University of Guelph, Canada 1994; 21: 501-508.
17. De La Rua P, Galian J, Serrano J, Moritz RFA. Genetic structure and distinctness of *Apis mellifera* L. populations from the Canary Islands. Mol Ecol 2001; 10: 1733-1742.

18. De La Rua P, Galian J, Serrano J, Moritz RFA. Microsatellite analysis of non-migratory colonies of *Apis mellifera iberica* from South-Eastern Spain. J. Zool. Syst. Evol. Res 2002; 40:164-168.
19. De La Rua P, Galian J, Serrano J, Moritz RFA. Genetic structure of Balearic honeybee populations based on microsatellite polymorphism. Genet. Sel. Evol 2003; 35: 339-350.
20. Rahimi A, Miromayedi A, Kahrizi D, Abdolshahi R, Kazemi E, Yari KH. Microsatellite genetic diversity of *Apis mellifera meda* skorikov. Mol Biol Rep 2014; 41: 7755-7761.
21. Frank P, Garnery L, Loiseau A, Oldroyd BP, Hepburn HR, Solignac M, Cornuet JM. Genetic diversity of the honeybee in Africa: Microsatellite and mitochondrial data. Heredity 2001; 86:420-430.
22. Estoup A, Garnery L, Solignac M, Cornuet JM. Microsatellite variation in honeybee (*Apis mellifera* L.) population: Hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics 1996; 140: 679-695.
23. Garnery L, Solignac M, Celebrano G, Cornuet JM. A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera* L. Experientia 1993; 49: 1016–1021.
24. Franck P, Garnery L, Solignac M, Cornuet M. Molecular confirmation of a fourth lineage in honeybees from the Near East. Apidologie 2000; 31: 167–180.
25. Asadi N, Esmaeilkhanian S, Nejati javaremi A, Gharahdaghi AA, Mirhadi A. Genomic DNA extraction from honeybee using a modified procedure in Iran. Proc. 5th Iranian Honeybee conference Karaj, Iran 2004; 5: 45-51.
26. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. POPGENE, the User-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta, 1997.
27. Goudet J. FSTAT (version 1.2) a Computer Program to Calculate F-Statistics. J. Hered 1995; 86: 485,486.
28. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 1978; 89: 583-590.
29. Ott J. Program HET version 1.80, Utility programs for analysis of genetic linkage. Rockefeller University New York, USA 2001.
30. Frankham R. Conservation genetics. Annu Rev Genet 1995; 29: 305–327.
31. Meixner MD, Ruttner F, koeniger N, koeniger G. The mountain bees of the Kilimanjaro region and their relation to nighbouring bee populations. Apidologie 1986; 20: 165–174.
32. Meixner DM, Miroslaw W, Jerzy W, Fuchs S, Nikolaus K. *Apis mellifera mellifera* range in eastern Europe morphometric variation and determination of its limits. Apidologie 2007; 38:1-7.
33. Rahimi A, Asadi M. Morphological characteristics of *Apis mellifera meda* (Hymenoptera: Apidae) in Saghez (west of Iran). Nature Montenegro 2010; 10:101-107.
34. Rahimi A, Mirmoayedi M. Evaluation of morphlogical characteristics of honey bee *Apis mellifera meda* (Hymenoptera: Apidae) in Mazandaran (North of Iran**).** TJEAS 2013; 3: 1280-1284.
35. Rahimi A, Mahdavi V. A study of the morphological variety of honey bees in Kordestan. TJEAS 2013; 3: 608-613.
36. Rahimi A, Asadi M, Mahdavi V, Abdulshahi R. Morphological characteristics of *Apis mellifera meda* (Hym.: Apidae) in Kerman (South of Iran). TJEAS 2013; 3: 614-624.
37. Widmer A, Schmid-Hempel P, Estoup A, Scholl A. Popula-tion genetic structure and colonization history of Bombus terrestris (Hymenoptera: Apidae) from the Canary Islands and Madeira. Heredity 1998; 81: 563–572.
38. Motamedi J, Zebarjadi AR, Kahrizi D, Salmanian. AH. In vitro propagation and Agrobacterium-mediated transformation of safflower (*Carthamus tinctorius* L.) using a bacterial mutated aroA gene. Aust J Crop Sci 2011; 4(5): 479-486.