MOLECULAR FINGERPRINTING OF CERTAIN CEREAL APHIDS IN EGYPT (HEMIPTERA: STERNORRHYNCHA: APHIDIDAE) USING RAPD AND ISSR MARKERS

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ABSTRACT: Cereal aphids are one of the most important insect pests limiting cereal production worldwide**.** Classical morphological criteria for aphid species identification may be affected by environmental factors such as climatic conditions and physiological status of the host plant**.** So, two modern molecular techniques; Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSRs) were used to find diagnostic markers for fingerprinting eleven cereal aphids those collected from different cereal plants and from different localities in Egypt. Eight RAPD and five ISSRs primers were successively proven 97 and 69 markers that could be used to differentiate the eleven different cereal aphid species. Also these molecular techniques with 26 diagnostic morphological characters were used to find the Phylogenetic relationship among the different collected species; that divided into two clusters with similarity matrix percentages of 73% and 82%. From these results it could concluded that these techniques could be used successively to fingerprint and identify these aphid species and differentiate among them.

KEY WORDS: Cereal aphids, Fingerprinting, RAPD, ISSRs, Identification, Markers, Microsatellites, Phylogeny.

Aphids are one of the most wide spread groups of pests in agricultural systems and may cause crop losses in forest trees, field crops and horticultural crops. Plants in these production systems may be affected directly or indirectly as a result of the presence of aphid populations (Minks & Harrewijn, 1989). There are approximately 5,000 described species of aphids (Remaudiere & Remaudiere, 1997). Cereal aphids are one of the most important insect pests limiting cereal production worldwide (Vickerman & Wratten, 1979; Dixon, 1987; D'Arcy & Mayo, 1997). Proper identification of agronomical important species of aphids is necessary in order to establish properly their diversity and population dynamics in a crop. Classical morphological criteria for aphid species identification suffer from at least two drawbacks: they depend on adult individuals since in many cases nymphal instars do not lend themselves to an accurate determination, and they may be affected by environmental factors such as climatic conditions and physiological status of the host plant (Cenis et al., 1993; Figueroa et al., 1999). Thus, identification based on morphological traits has been problematic for several closely related species (Loxdale & Brookes, 1989). Random Amplified Polymorphic DNA (RAPD) has proven to be invaluable source of markers for many sap-sucking insect pests identification such as aphid species (Cenis et al., 1993; Lushai et al., 1997; Figueroa et al*.,* 2005 and Shahadi-Fatima et al., 2007). Another genetic technique is Simple Sequence Repeats (ISSRs or 'microsatellites') which has been used by plant biologists for a variety of applications (Wolfe & Liston, 1998) and rarely used in animals (Kostia et al.,

2000; Reddy et al., 1999). This technique used for population-level studies in two species of cyclically parthenogenetic aphids, *Acyrthosiphon pisum* and *Pemphigus obesinymphae* (Abbot, 2001). Also used to identify different biotypes of greenbug, *Schizaphis graminum* (Weng et al., 2007). The ISSRs technique is used here for the first time to differentiate among different separate insect species.

This study aimed to differentiate among eleven cereal aphid species found in Egypt, and also to establish molecular genetic fingerprint for these species using RAPD and ISSRs polymorphism and elucidate relationships among these species.

MATERIALS AND METHODS

Survey of certain cereal aphid species:

Field survey of aphid species was carried out during the period 2008-2009 in different localities of Egypt. Eleven cereal aphid species were collected from different localities allover the years. The species are listed alphabetically by scientific name in Table (1).

Samples from the infested plants were transferred to the laboratory and alate individuals of aphid were mounted on slides for identification by using available keys.

Identification of these species was conducted according to the key of Fathi and El-Fatih (2009). The eleven species were reared on their host separately, where one apterous adult was transferred by a brush to a healthy plant. Nymphs were leaved to feed and developed to adults. This method was repeated for two off-springs. The adults of the second offspring of each species were put in tubes and preserved at -20 ºC until use in the biochemical and molecular studies.

RAPD-PCR Analysis

a. DNA Extraction

DNA were extracted from eleven different species of adult Aphid.Animal tissues were ground under liquid nitrogen to a fine powder, then bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

b. Polymerase Chain Reaction (PCR)

PCR amplification was performed using eight random 10 mer arbitrary primers synthesized by (Operon biotechnologies, Inc.Germany).

Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl $MgCl_2$ (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 ng / µl), 1 µl of *Taq* polymerase (1U/ µl) and 10.5 µl of sterile dd H_2O. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 36º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min. Amplified products were size-fractioned using ladder marker (100bp +1.5 Kbp) by electrophoresis in 1.5 % agarose gels in TBE buffer at 120 V for 1 h. the bands were visualized by ethidium bromide under UV florescence and photographed.

ISSR-PCR Analysis

a. DNA Extraction

Eleven different species of adult Aphids samples were collected and extracted DNA from them. Animal tissues were ground under liquid nitrogen to a fine powder, and then bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

b. Polymerase Chain Reaction (PCR)

PCR amplification was performed using five Inter Simple Sequence Repeat (ISSR) Table, 3.

Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl $Mgcl_2$ (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 ng / µl), 1 µl of *Taq* polymerase (1U/ µl) and 10.5 µl of sterile dd H_2O. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) The PCRs were programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94 °C, 1 min at 57 °C, and 2 min at 72 °C. The reaction was finally stored at 72 °C for 10 min. Amplified products were size-fractioned using ladder marker100bp (1000, 900, 800, 700, 60, 500, 400, 300, 200 and 100bp) by electrophoresis in 1.5 % agarose gels in TBE buffer at 120 V for 30 min. the bands were visualized by ethidium bromide under UV florescence and photographed.

Densitometry Scanning and Analysis:

All gels resulted from DNA fingerprints, were scanned using Bio-Rad GelDoc2000 to calculate the pair-wise differences matrix and plot the dendrogram among different aphid species.

Phylogenetic relationship among the eleven species:

To study the Phylogenetic relationship among different aphid species based on three different criteria; eight RAPD primers, five ISSR primers and twenty-six diagnostic morphological characters were used. In this method all employed characters were regarded as being of equal importance. The morphological characters were selected to be of most taxonomic importance according to Fathi and El Fatih 2009. The analysis data of the three criteria were taken as basis for assessing the relationships between each species and the others. Statistical soft ware program (SPSS program) was used to calculate the similarity matrix. The degree of similarity was shown as numbers between 0 and 1 for the most dissimilar and the most similar pair of species, respectively. The performing and drawing of the phylogenetic tree was done by Statistical Package for Social Science (SPSS ver. 10) computer program.

RESULTS

Random Amplified Polymorphic DNA (RAPD):

Eight RAPD primers were tested against the eleven cereal aphid species to find markers for identification of these species. These primers generated 167 fragments, 97 bands of them were considered as markers for different species (58.1%), the highest number of markers was 20 bands generated by OPC-01 and OPC-13, while the lowest number of markers was 4 bands generated by OPD-07. the highest number of markers was 12 bands detected for *S. graminum*, while the lowest number of markers was 5 bands detected for *M. dirhodum* (Table, 5). Thirty-seven common bands (22.2%) were detected among the eleven species found by the eight tested primers.

OPC-01 primer:

This primer generated 23 bands with molecular weight ranged from 903 to 111bp. and the average number of bands generated in different species ranged from 8 bands in *S. avenae* and 4 bands in *M. dirhodum*. This primer showed 87.1% polymorphism whereas three common bands among the eleven species were detected (784, 378 and 250bp.). Twenty marker bands were detected for the eleven species; the highest number of markers was three bands for *S. graminum*

and *S avenae*, while the lowest number was one band in *R. maidis*, *S. minuta, M. dirhodum* and *H. pruni*. While the other five species, each of them has two marker bands.

OPC-13 primer:

This primer generated 30 bands with molecular weight ranged from 1360 to 105bp. and the average number of bands generated in different species ranged from 11 bands in *H. pruni* and 4 bands *R. maidis*. This primer showed 90% polymorphism whereas three common bands were detected among the eleven species with molecular weights 764, 431 and 355bp. Twenty marker bands were detected for the eleven species, the highest number of markers was five bands for *H. pruni*, while the lowest number was one band for six species; *A. corni*, *T. africana*, *R. maidis*, *S. graminum*, *S. avenae* and *S. scirpus*. Three marker bands were detected for two species; *S. rotundiventrus* and *S. minuta,* two marker bands for *R. padi* and *M. dirhodum*.

OPD-07 primer:

This primer generated 16 bands with molecular weight ranged from 1231 to 223bp. and the average number of bands generated in different species ranged from 10 bands in six species; *R. maidis*, *S. rotundiventrus*, *S. avenae*, *M. dirhodum*, *H. pruni* and *S. scirpus* to 5 bands in *S. graminum* and *S. minuta*. This primer showed 68.75% polymorphism whereas five common bands (1231, 750, 520, 392 and 328bp.) among the eleven species were detected. Only four marker bands; 886, 639, 795 and 935bp. were detected for *A. corni*, *R. maidis*, *R. padi* and *M. dirhodum*, respectively.

OPE-03 primer:

This primer generated 24 bands with molecular weight ranged from 1186 to 208bp. and the average number of bands generated in different species ranged from 12 bands in *A. corni* to 5 bands in *S. rotundiventrus*. This primer showed 83.3% polymorphism whereas four common bands (737, 387, 356 and 208bp) among the eleven species were detected. Thirteen marker bands were detected for eight species, three marker bands for *S. scirpus*; two marker bands for three species; *A. corni*, *T. africana* and *S. avenae*. While only one marker band was detected for four species; *R. maidis*, *R. padi, S. minuta* and *H. pruni*.

OPE-06 primer:

This primer generated 19 bands with molecular weight ranged from 1132 to 190bp. and the average number of bands generated in different species ranged from 8 bands in *A. corni* to 4 bands in *S. graminum*. This primer showed 79 % polymorphism whereas four common bands (421, 332, 222 and 190bp) among the eleven species were detected. Ten marker bands were detected for seven species, two marker bands for three species; *A. corni*, *S. minuta* and *S. scirpus*. While one marker band (monomorphic) was detected for four species; *R. padi*, *S. rotundiventrus*, *S. avenae* and *H. pruni*.

OPI-17 primer:

This primer generated 21 bands with molecular weight ranged from 1343 to 162bp. and the average number of bands generated in different species ranged from 10 bands in *T. africana* and *S. graminum* to 7 bands in two species; *A. corni* and *R. padi*. This primer showed 66.7% polymorphism whereas seven common bands (988, 841, 656, 557, 486, 419 and 343bp.) among the eleven species were detected. fourteen marker bands (monomorphic) were detected for nine species, the highest number of markers was five bands detected for *S. graminum,* while the lowest number was one band was detected for seven; *A. corni*, *R. maidis*, *S. minuta*, *S. rotundiventrus*, *S. avenae*, *M. dirhodum* and *H. pruni*. While two

marker bands were detected for *T. africana*. No marker bands for two species; *R. padi* and *S. scirpus*.

OPL-20 primer:

This prime generated 15 different fragments with molecular weight ranged from 1240 to 202bp, the average number of bands generated in different species ranged from 9 bands in *R. maidis* to 7 bands in *S. rotundiventrus*, *H. pruni* and *S. scirpus*; while the other eight species have 8 bands. This primer showed 60% polymorphism whereas, six common bands were detected by this primer with molecular weights 1240, 731, 662, 507, 400 and 202bp. Six marker bands in six different species were detected (one marker band for each species); *A. corni, T. africana, R. maidis, R. padi*, *S. rotundiventrus* and *S. avenae*. No marker bands for the other six species.

OPQ-15 primer:

This primer generated 19 bands with molecular weight ranged from 1162 to 202bp. and the average number of bands generated in different species ranged from 8 bands in three species; *T. african, R. maidis*, *R. padi*, to 5 bands in *M. dirhodum*. This primer showed 73.7% polymorphism whereas five common bands among the eleven species were detected (683, 410, 341, 238 and 202bp.). Ten marker bands (monomorphic) were detected for nine species; the highest number of markers was two bands detected for *S. graminum*. While just one marker band was detected for eight species; *A. corni*, *T. africana, R. maidis, R. padi*, *S. rotundiventrus*, *S. minuta*, *H. pruni* and *S. scirpus*. While no marker bands were detected for *S. avenae* and *M. dirhodium*.

Inter simple sequence repeats (ISSRs):

Five ISSRs primers were tested against the eleven cereal aphid species to find markers for identification of these species. These primers generated 122 fragments, 69 bands of these fragments were considered as markers for different species (56.6%), the highest number of markers was 15 bands generated by two primers; HP-09 and HP-13, while the lowest number of markers was 12 bands generated by HP-14 primer. *A. corni* had the highest number of marker bands (10 bands), while the lowest number of markers was 3 bands detected for *S. scirpus* (Table, 6). Twenty-one common bands (17.2%) were detected among the eleven species by these five tested primers.

HP-09 Primer:

This primer generated 23 bands with molecular weight ranged from 1109 to 117bp. and the average number of bands generated in different species ranged from 8 bands in *A. corni* to 5 bands in *R. padi* and *S. avenae*. This primer showed 82.6% polymorphism whereas 4 common bands (422, 349, 254 and 172bp) were detected among the eleven species. Fifteen marker bands (monomorphic) were detected for ten species; the highest number of markers was three bands were detected for *A. corni*, while the lowest number was one marker band was detected for six species; *R. padi*, *S. graminum*, *S. minuta, S. avenae*, *H. pruni* and *S. scirpus*. While no marker bands were detected for *M. dirhodium*.

HP-11 Primer:

This primer generated 30 bands with molecular weight ranged from 1301 to 124bp. This primer showed 73.3 % polymorphism, where eight common bands (930, 780, 685, 516, 436, 288, 242 and 174bp) were detected among the eleven species. The average number of bands generated in different species ranged from 15 bands in *S. rotundiventrus* to 10 bands in *S. scirpus*. Fourteen marker bands were detected for the eleven species; the highest number of markers was two bands were detected for three species; *A. corni, S. graminum* and *S.*

rotundiventrus, while the lowest number was one marker band was detected for the other eight species.

HP-12 Primer:

This primer generated 22 bands with molecular weight ranged from 842 to 95bp. This primer showed 90.9% polymorphism, where two common bands (323 and 230bp) were detected among the eleven species. The average number of bands generated in different species ranged from 9 bands in *S. avenae* to 2 bands in *T. africana*. Thirteen marker bands were detected for eight species; the highest number of markers was three bands were detected for *S. avenae*, while the lowest number was one marker band was detected for four species; *A. corni*, *R. maidis, R. padi* and *S. rotundiventrus*. Two marker bands were detected for three species; *S. graminum*, *M. dirodium* and *H. pruni*. While no marker bands were detected in *T. africana*, *S. minuta* and *S. scirpus*.

HP-13 Primer:

This primer generated 25 bands with molecular weight ranged from 1016 to 123bp. This primer showed 88% polymorphism, where three common bands (640, 398 and 174bp) were detected among the eleven species. The average number of bands generated in different species ranged from 10 bands in *S. avenae* to 5 bands in *H. pruni*. Fifteen marker bands were detected for the eleven species; the highest number of markers was two bands were detected for four species; *A. corni*, *T. africana*, *R. maidis* and *S. graminum*. While the lowest number was one marker band was detected for the other seven species.

HP-14 Primer:

This primer generated 22 bands with molecular weight ranged from 963 to 32bp. This primer showed 81.8% polymorphism, where four common bands (261, 192, 153 and 101bp) were detected among the eleven species. The average number of bands generated in different species ranged from 8 bands in *S. avenae, H. pruni* and *S. scirpus* to 5 bands in *S. minuta* and *M. dirhodium*. Twelve marker bands were detected for nine species; the highest number of markers was two bands were detected for three species; *A. corni, S. rotundiventrus* and *S. avenae*. While the lowest number was one marker band was detected for six species; *T. africana*, *R. maidis, R. padi*, *S. graminum*, *S. minuta* and *M. dirhodium*. While no marker bands were detected for two species; *H. pruni* and *S. scirpus*.

Phylogenetic relationship among the eleven species:

Genetic similarities and genetic relatedness amongst the eleven cereal aphid species were based on data obtained of three different criteria; eight RAPD primers and five ISSRs primers as molecular markers and as well as twenty-six diagnostic morphological characters. These data were subjected to using SPSS computer program to support the existence of high level of genetic relatedness amongst the eleven cereal aphid species. The relatedness dendrogram was indicated two main clusters. The first cluster included ten aphid species while, the second included *A. corni* only with similarity matrix percentage of 73%. The first cluster was divided into two sub-clusters with similarity matrix percentage of 82%; the first one included six aphid species with similarity matrix percentage of 88%; *H. pruni, S. scirpus, S. avenae, M. dirhodum, S. rotundiventrus* and *S. minuta*. While the second sub-cluster contained four aphid species with similarity matrix percentage of 84%; *S. graminum, R. maidis, R. padi* and *T. africana*. The highest similarity matrix percentage in this phylogenetic relationship was 98% between *H. pruni* and *S. scirpus*, while the lowest similarity matrix percentage found between *A. corni* and *H. pruni* (73%) (Fig. 3). These results indicated the success of these techniques with morphological characters to draw the

Phylogenetic relationship of these aphids' species whereas the species those belonging to the same genus are closest to each other.

DISCUSSION

Some of the insect species are easy to identify and categorize, while for others, such as aphids species are difficult because of their small size and morphological similarity. Moreover, it is further difficult to identify morphological variation due to environmental factors by available traditional methods (Loxdale and Brookes, 1989; Cenis et al., 1993; Figueroa et al., 1999). To overcome these problems, the advanced molecular techniques, viz., randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and simple sequence repeats (ISSRs) have been useful tools in assessing insect genetic diversity (Black et al., 1992; Cenis, 2003; Gobbi et al., 2003; Sartor et al., 2008; Sharma et al., 2008; Perumal et al., 2009; Qiu et al., 2009). RAPD markers have become the most common yardsticks for measuring genetic differences between individuals, within and between related species or population (Jain et al. 2010). This technique also used to distinguish different geographical and/or host associated populations of some cryptic complex species (Zitoudi et al., 2001; Bulman et al., 2005; Helmi, 2011). In Egypt RAPD-PCR technique is successively used to fingerprint of some sap-sucking insect species belonging into the same taxonomic category such as fingerprinting of ten aphids species belonging to Genus *Aphis* (Shahadi-Fatima et al., 2007) and also used to fingerprint fifteen whitefly species (Amin et al., 2009).

Inter simple sequence repeats (ISSRs) is a valuable addition to the inventory of PCR-based methods for rapid, large-scale screening of genetic variations. The vagaries of PCR and the chosen method of band detection limit any PCR-based marker (Wolfe & Liston, 1998). But ISSRs markers are typically highly reproducible, due to stringent annealing temperatures, long primers, and low primer-template mismatch (that is, the primers are not 'arbitrary', but designed *a priori* to anchor onto anonymous SSR loci; Wolfe et al., 1998) and ISSRs can reveal polymorphisms without more elaborate detection protocols (Esselman et al., 1999).

ISSRs method has shown much promise for the study of the population biology of plants (Clausing et al., 2000; Hess et al., 2000), but rarely used in animals (Reddy et al., 1999; Kostia et al., 2000 and Abbot, 2001). There are two available known studies in use of ISSRs in aphids; one of them was on population-level studies in two species of cyclically parthenogenetic aphids; *Acyrthosiphon pisum* and *Pemphigus obesinymphae* (Abbot et al., 2001) who reported that IISSRs are suitable for invertebrate populations those have small size bodies and low levels of within-population variation. While the other study was on host-associated genetic differences and regional differences among the green bug, *Schizaphis graminum* biotypes (Weng et al., 2007) who cited that the use of ISSRs would be useful for aphid genetic, ecological, and evolutionary studies and can potentially shorten the time and cost for biotype identification.

CONCLUSION

Molecular fingerprinting of eleven cereal aphid species collected from Egypt were carried out using two modern genetic techniques; RAPD-PCR and ISSRs. These techniques successively generated many molecular markers for different studied species, those could be used to identify these aphid species and differentiate among them. Also these two techniques in addition to some

diagnostic morphological characters were used to determine the Phylogenetic relationship among the eleven aphid species.

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Table 1. List of certain eleven cereal aphid species with their host plants and localities in Egypt.

Aphid species	Locality	Host plants
Anoecia corni (Fabricius)	Qalyubiya	*Cyperus longus* roots
Tetraneura africana Van der Goot	Giza	*Cynodon dactylon* roots
Rhopalosiphum maidis (Fitch)	Qalyabiya	*Triticum* spp.
Rhopalosiphum padi (Linnaeus)	Beni-Suif	*Triticum* spp.
Schizaphis graminum (Rondani)	Giza	*Avena fatua*
Schizaphis rotundiventris Signoret	Beni-Suif	*Hordeum vulgare*
Schizaphis minuta (van der Goot)	Sharqiya	*Cyperus rotundus*
Sitobion avenae (Fabricius)	Sohag	*Sorghum virgatum*
Metopolophium dirhodum (Walker)	Qalyabiya	*Lolium temulentum*
Hyalapterus pruni (Geoffroy)	Behaira	*Arundo donax*
Saltusaphis scirpus Theobald	Qalyabiya	*Cyperus rotundus*

Table 2. List of the RAPD primers and their nucleotide sequences:

No	Name	Sequence	No	Name	Sequence
1	OP-C01	5` TTC GAG CCA G 3`	5	OP-E06	5` TCG CAG AAC G 3`
2	OP-C13	5` AAG CCT CGT C 3`	6	OP-Q15	5` CAA ACA GCG T 3`
3	OP-D07	5` GGA CCC AAC C 3`	7	OP-I17	5` AAG AGA GGG G 3`
4	OP-E03	5` GGG CGG TAC T 3`	8	OP-L20	5 ` TGG TGG ACC A 3`

Table 3. List of the ISSRs primers and heir nucleotide sequences:

No	Name	Sequence
1	HB-9	5` GTG TGT GTG TGT GG 3`
2	HB-11	5` GTG TGT GTG TGT CC 3`
3	HB-12	5` CAC CAC CAC GC 3`
4	HB-13	5` GAG GAG GAG GC 3`
5	HB-14	5` GTG TGT GTG TGT GC 3`

Table 4. Profile of diagnostic morphological characters, to identify the eleven aphid species, under consideration, expressed as Zero and one values.

No.	Aphid Character	*A. corni*	*T. africana*	*R. maidis*	*R. padi*	*S. graminum*	*S. rotundiventrus*	*S. minuta*	*S. avenae*	*M. dirhodum*	*H. pruni*	*S. scirpus*
1	Supra Caudal Process	0	0	0	0	0	0	0	0	0	0	1
2	Siphunculi on hairy cone	1	0	0	0	0	0	0	0	0	0	1
3	Siphunculi ring like	0	1	0	0	0	0	0	0	0	0	0
4	Cauda rounded	1	0	0	0	0	0	0	0	0	0	0
5	Cauda knobbed	0	0	0	0	0	0	0	0	0	0	1
6	Antennae 5 segmented	1	0	0	0	0	0	0	0	0	0	0
7	Frontal tubercles diverging	0	0	0	0	0	0	0	1	1	0	0
8	Siphunculi with polygonal reticulated apex	0	0	0	0	0	0	0	1	0	0	0
9	post-siphunclar sclerite	0	0	0	0	0	0	0	1	0	0	0
10	Siphunculi shorter than the cauda	0	0	0	0	0	0	0	0	0	1	0
11	Siphunculi slightly swollen	0	0	1	0	0	0	0	0	0	0	0
12	Cauda elongate with 4 long hairs	0	0	0	0	1	1		0	0	0	0
13	Cauda elongate with 6 long hairs	0	0	0	0	0	0	1	0	0	0	0
14	Unigus twice the basal part	0	0	1	0	0	0		0	0	0	0
15	Unigus 3 times the basal part	0	0	0	0	1	0	0	0	0	0	0
16	Unigus 4 times the basal part	0	0	0	1	0	0	0	0	0	0	0
17	Unigus 5 times the basal part	0	0	0	0	0	1	1	0	0	0	0
18	Infesting cereal roots only	1	1	0	0	0	0	0	0	0	0	0
19	Infesting cereal weeds only	0	0	0	0	0	0	1	0	0	1	1
20	Frontal tubercles not well developed	0	0	1	1	1	1	1	0	0	1	0
21	Siphunculi longer than the cauda	0	0	1	1	1	1	1	0	0	0	0
22	Siphunculi with distinct flange	0	0	1	1	0	0	0	0	0	0	0
23	Siphunculi without distinct flange	0	0	0	0	1	1	1	0	0	0	0
24	Cauda elongate	0	0	1	1	1	1	1	1	1	1	0
25	Cauda short	1	1	0	0	0	0	0	0	0	0	1
26	Siphunculi without polygonal reticulated apex	1	1	1	1	1	1	1	0	1	1	1

Table 5. Molecular markers generated by eight RAPD primers for eleven cereal aphid species in Egypt.

	A.corni	*T.africana*	*R.maidis*	*R.padi*	*S.graminum*	*S. rotundiventrusi*	*S.minuta*	*S.avenae*	*M.dirhediom*	*H. proni*	*S. scirpus*
OPC-01	850 603	574 111	333	698 457	903 786 394	671 298	220	740 494 400	764	529	678 174
OPC-13	1221	1064	881	610 516	849	1126 800 689	1160 900 565	731	598 317	988 272 198 105	1308
OPD-07	886		639		795				935		
OPE-03	807 500	1053 319	1104	251			622	996 889		401	694 550 465
OPE-06	1022 800			368		698	856 628	224		749	1132 287
OPI-17	587	513 392	302		1343 1091 761 537 162	294	728	1184	231	561	
OPL-20	1144	1089	949	315		305		1010			
OPQ-15	477	1018	229	378	653 550	299	845			309	1162
Total	**11**	**9**	**7**	**8**	**12**	**9**	**9**	**9**	**5**	**9**	**9**

Table 6. Molecular markers generated by five ISSRs primers for eleven cereal aphid species in Egypt.

	A.corni	*T.africana*	*R.maidis*	*R.padi*	*S.graminum*	*S. rotundiventrus*	*S.minuta*	*S.avenae*	*M.dirhediom*	*H. proni*	*S. scirpus*
HP-09	1027 882 508	738 385	699 412	213	1109	780 627	274	310		465	117
HP-11	1273 124	1140	854	716	1301 269	1198 203	574	605	524	191	344
HP-12	506		398	685	842 127	254		460 271 95	285 140	586 208	
HP-13	581 123	1016 725	499 197	322	858 507	428	904	993	286	214	792
HP-14	963 283	757	560	600	49	400 304	672	500 223	72		
Total	**10**	**6**	**7**	**5**	**8**	**8**	**4**	**8**	**5**	**5**	**3**

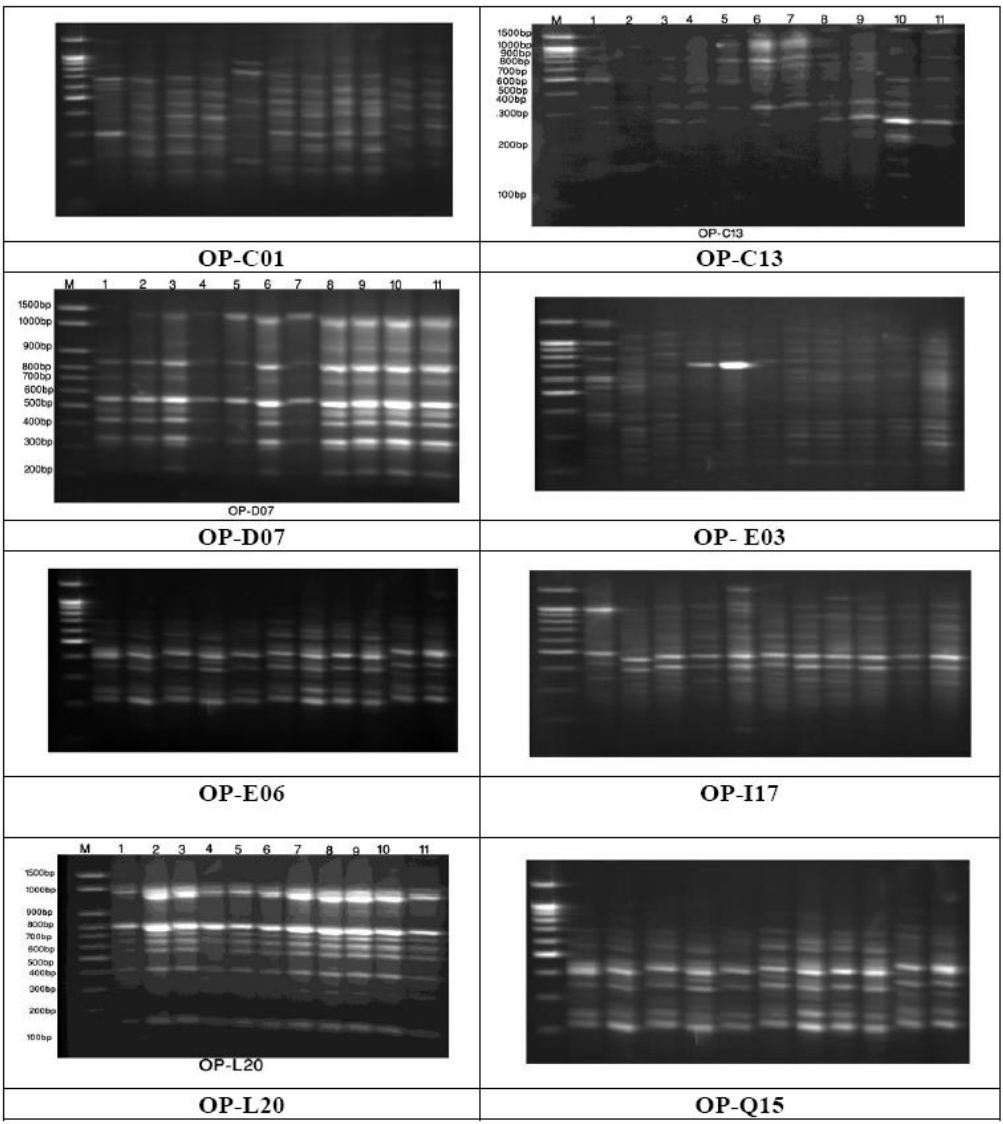

Figure 1. RAPD banding patterns of eleven cereal aphid species generated by eight random primers. M, 1500 bp marker; 1. *Anoecia corni*, 2. *Tetrenura africana*; 3. *Rhopalosiphum maidis*, 4. *Rhopalosiphum padi*, 5. *Schizaphis graminum*, 6. *Schizaphis rotundiventrus*, 7. *Schizaphis minuta*, 8. *Sitobion avaenae*, 9. *Metopolophum dirhodum*, 10. *Hyalopterous pruni*, 11. *Saltusaphis scirpus*.

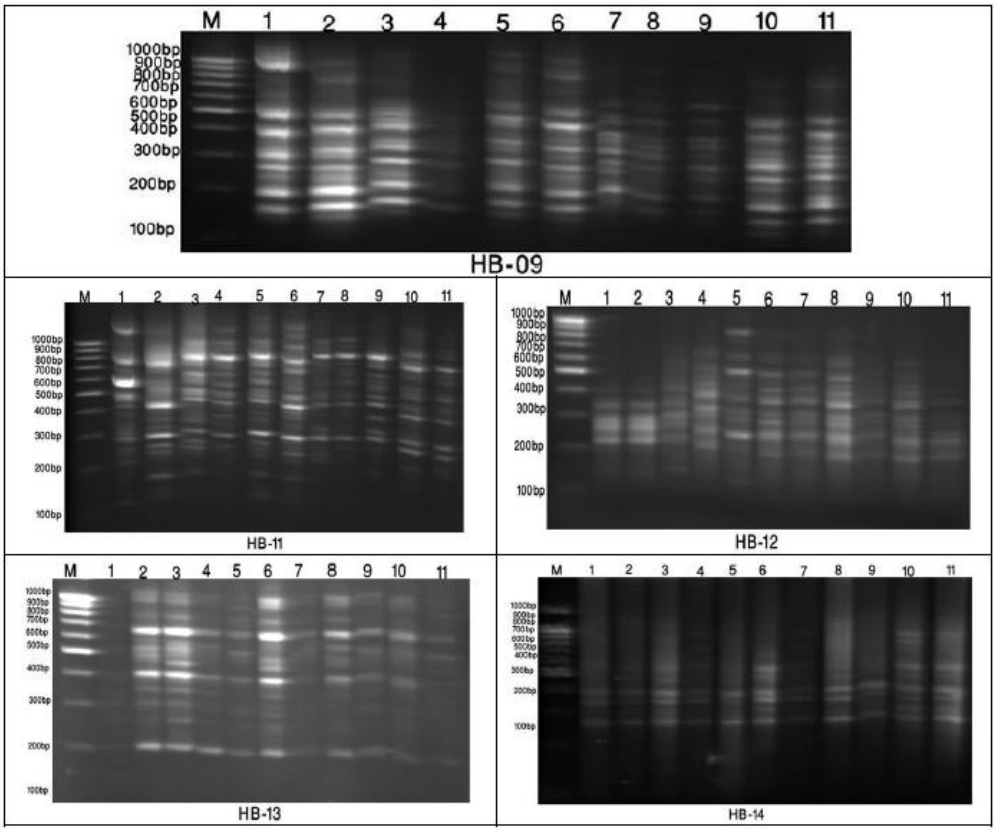

Figure 2. ISSR banding patterns of eleven cereal aphid species generated by five primers. M, 1000 bp marker; 1. *Anoecia corni*, 2. *Tetrenura africana*; 3. *Rhopalosiphum maidis*, 4. *Rhopalosiphum padi*, 5. *Schizaphis graminum*, 6. *Schizaphis rotundiventrus*, 7. *Schizaphis minuta*, 8. *Sitobion avaenae*, 9. *Metopolophum dirhodum*, 10. *Hyalopterous pruni*, 11. *Saltusaphis scirpus*.

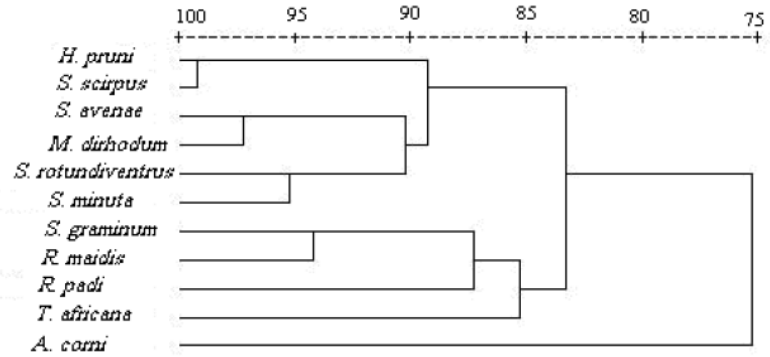

Figure 3. Phylogenetic dendrogram among eleven cereal aphid species based on three criteria; RAPD markers, ISSRs markers and certain diagnostic morphological characters.