Molecular identification of some alfalfa (*Medicago sativa* L.) cultivars grown in Egypt and Saudi Arabia using RAPD and ISSR

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Mona H. Hussein* and Rania A. A. Younis**
* Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt
** Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

ABSTRACT

The level of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) variation present in seven alfalfa (Medicago sativa L.) cultivars from Egyptian and Saudi Arabian germplasm sources were evaluated. Both molecular approaches revealed a low degree of intra-cultivar polymorphism (less than 10% across both analyses), while high degree of inter-cultivar genetic polymorphism (over 90% across both analyses) and enabled each cultivar to be uniquely fingerprinted. The genetic relationships among cultivars were analysed by calculating the pairwise similarity matrix between genotypes according to Nei and Li's genetic similarity coefficient (NLc) and applying the Unweighted Pair Group Method using Arithmetic Average (UPGMA) to plot dendrograms. Both analyses were able to separate the Saudi Arabian from the Egyptian cultivars. It was concluded that RAPD and ISSR could be useful and powerful tools for assessing genetic variation and genetic relationships as well as improving tetraploid alfalfa via marker-assisted selection.

Key words: *Molecular identification, alfalfa, RAPD, ISSR.*

INTRODUCTION

Alfalfa (Lucerne), *Medicago sativa* L., is the most important forage crop grown in the temperate regions over 21 million hectares cultivated worldwide (Michaud *et al.*, 1988). It is a model organism because of the ready manipulation of its ploidy level. Cultivated alfalfa is autotetraploid (2n = 4x = 32), allogamous and seed-propagated (Barnes *et al.*, 1988). Tetrasomic inheritance and pronounced inbreeding depression (McCoy and Bingham, 1988) have delayed the genetic characterization of alfalfa compared with other major crops. Alfalfa is often called "Queen of the forages" as it has the highest feeding value of all commonly grown hay crops for farm animals. It produces more protein per hectare than grain or oil seed crops. It has a high mineral content with at least 10 different vitamins, especially vitamin A. These characteristics make its use as hay meal or silage a desirable component for most farm animals. Alfalfa, in combination with the bacterium *Rhizobium meliloti*, is a highly effective symbiosis for biological nitrogen fixation.

Information about germplasm diversity in cultivated plant species is relevant for plant breeding (Hallauer and Miranda, 1988). In particular, it is often necessary to distinguish between germplasm sources of different

origin, especially when they are individually registered by a Seed Certification Board. The knowledge of the extent of genomic variability and relationship in alfalfa cultivars and ecotypes bearing desirable agronomic traits is an area that could benefit from detailed molecular genetic analyses.

Several approaches to variety identification are based upon the use of polymerase chain reaction (PCR with primers specific to various protein genes. Random amplified polymorphic DNA (RAPD) analysis involves the amplification of small sequences of target DNA using random primer. Until recently, the discrimination power of restriction fragment length polymorphism (RFLP) has been extensively studied in alfalfa (Businelli *et al.*, 1993, Kidwell *et al.*, 1994, Brummer *et al.*, 1995, Pupitli *et al.*, 1996, Vitale *et al.*, 1996), nevertheless, the use of this method for a wide range studies is limited by the large amount of relatively pure DNA needed as well as the time-consuming procedure. Conversely, RAPD analysis is a quick and inexpensive non-radioactive method and has also been widely used in plant population studies (Powell *et al.*, 1995). In alfalfa, RAPDs have been evaluated for segregation analyses (Echt *et al.*, 1992, Barcaccia *et al.*, 1995), for assessing germplasm introgression (McCoy and Echt, 1993), constructing genetic maps (Echt *et al.*, 1992, Kiss *et al.*, 1993, Yu and Pauls, 1993b), as well as assessing intra- and inter-species variation among annual medics (Brummer *et al.*, 1995) and estimating relationships among alfalfa populations (Yu and Pauls, 1993a, Crochemore *et al.*, 1996).

The genome of alfalfa (comprising 80% repetitive DNA), like those of other eukaryotes, contains a class of sequences termed microsatellites or simple sequence repeats (SSRs). They are short stretches of DNA that are "hypervariable" within population and among different species. They are characterized by mono-, di- or trinucleotide repeats that have 4-10 repeat units side by side. A marker system called inter-simple sequence repeats (ISSRs) has only recently been developed as an anonymous, RAPDs-like approach that accesses variation in the numerous microsatellite regions dispersed throughout the various genomes (particularly the nuclear genome) and circumvents the challenge of characterizing individual loci that other molecular approaches require. In the current study, we report the use of RAPD and ISSR analyses for assessing the inter- and intra-cultivar relationships among a number of cultivated alfalfa of Egyptian as well as Saudi Arabian origin.

MATERIALS AND METHODS

Plant material

Seven alfalfa cultivars, obtained from different sources, were used. Names, sources and major characteristics of these cultivars are as the following:

Serial no.	Cultivar name	Source	Major chracteristic
1	Saudi	Saudi Arabian	Salt tolerance
2	Wezara 1	MALR*, Egypt	Mass production and it has a high number of root nodules
3	Wezara 2	MALR*, Egypt	Mass production
4	Ismailia 1	MALR*, Egypt	Disease tolerance specially for roots
5	Ismailia 94	MALR*, Egypt	Disease tolerance specially for roots
6	Siwa Algama	Dept. Field Crops, Fac. Agric., Cairo Univ.	Salt tolerance
7	Siwa 1	MALR*, Egypt	Salt tolerance

**MALR= Ministry of Agriculture and Land Reclamation.*

DNA preparation

Seeds were allowed to germinate and seedlings were grown in greenhouse up to the appearance of leaf 3. Three leaves were collected from each plant and three plants per cultivar were subjected to molecular analysis. Leaves (200 mg) were ground to a powder using liquid nitrogen in microfuge tubes and DNAs were isolated using plant Genomic DNA Mini-Prep Kit (V-gene Biotechnology, China, cat. no. 110420-25) according to manufacturer manual. DNA samples of each cultivar were analysed individually to detect intra-cultivar variations and bulked to detect inter-cultivar variations.

PCR conditions and electrophoresis

An initial screening of 30 RAPD decamer and 20 ISSR (successfully utilized in other plant species, Nagaoka and Ogihara, 1997, Awasthi *et al.*, 2004) primers was performed in order to test readability and amplification profiles for polymorphism. Ismailia 1 and Ismailia 94 cultivars were used for this step. After this screening procedure, eight RAPD and twelve ISSR primers were chosen for analysis. Names and sequences of the eight RAPD and twelve ISSR primers are shown below. To detect polymorphism within each cultivar (intra-cultivar), PCR with one RAPD (eg. O18) and one ISSR (HB12) primers was performed on individual DNA samples of each cultivar.

RAPD Primers

Name	Sequence
B01	GTTTCGCTCC
B06	TGCTCTGCCC
B07	GGTGACGCAG
B10	CTGCTGGGAC
B04	GGACTGGAGT
O06	CCACGGGAAG
O07	CAGCACTGAC
O18	CTCGCTATCC

ISSR Primers

Name	Sequence
814	$(CT)_8TG$
844A	$(CT)_8AC$
844B	$(CT)_8GC$
T7898A	$(CA)_6AC$
17898B	$(CA)_6GT$
17899A	$(CA)_6AG$
17899B	$(CA)_6GG$
HB8	$(GA)_6GG$
HB9	$(GT)_6GG$
HB10	$(GA)_6CC$
HB11	$(GT)_6CC$
HB12	$(CCA)_3GC$

PCR for both analysis was performed in 25 µl volume containing 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 20 µM primer, 50 ng genomic DNA and 1 U Taq DNA polymerase (Bioron, Germany). All reactions were performed in a Perkin Elmer 2400 thermal cycler. RAPD program was performed as 1 cycle of 94°C for 4 min and 40 cycles of 94°C for 1 min, 35°C for 1 min and 72°C for 2 min. Then, a final extension step 72°C for 8 min was done. The ISSR program was performed as 1 cycle of 94°C for 4 min and 35 cycles of 94°C for 1 min, 44°C for 45 sec, 72°C for 1.5 min. Then, a final extension step of 72°C for 8 min was done. To visualize the PCR products, 15 µl of each reaction was loaded on 1.8% agarose gel. The gel was run at 90 V for about 1 hr and visualized with UV transilluminator and photographed using UVP gel documentation system. For each amplification, a negative control reaction without DNA template was included. PCR reactions, that generated high level of polymorphism across both types of analyses, were repeated twice in order to verify the reproducibility of polymorphic bands scored. This procedure allowed only

those bands present in all replicated experiments to be scored as markers. Amplicon sizes were estimated using both 100-bp and 1-kb DNA standards (Bioron, Germany).

Data analysis

Reproducible bands visualized on the gels were scored using a binary code (1/0) for their presence or absence for both RAPD and ISSR based on the UVP gel documentation system (GelWorks 1D advanced software, UVP). The data were used to calculate the pairwise similarity matrix between genotypes according to Nei and Li's genetic similarity coefficient (NLc): $2N_{ij}/(2N_{ij} + N_i + N_j)$, where N_{ij} is the number of bands common to samples i and j, while N_i and N_j are the number of bands unique to sample i and j, respectively (Nei and Li, 1979). The genetic coefficient of Nei and Li represents the fraction of shared DNA fragments between two samples. It ranges from 0 to 1, the complete genetic identity corresponding to 1. Relationships among the tested cultivars were calculated by the Unweighted Pair Group Method using Arithmetic Average (UPGMA) and visualized through a genetic relatedness dendrogram.

RESULTS AND DISCUSSION

In this work, the utility of RAPD and ISSR markers in the analysis of alfalfa germplasm was studied. To our knowledge, this is the first report of an analysis of genetic variability in cultivated tetraploid alfalfa using both RAPD and ISSR analyses. In order to identify primers useful in distinguishing genotypes, Ismailia 1 and Ismailia 94 cultivars were examined. The optimal number of primers, required to discriminate among genomic DNA of two cultivars, depends on the level of polymorphism generated by type of molecular analysis (eg., RAPD, ISSR, etc.). Suggestions about the amount of genetic distance required to classify two correlated plant accessions as distinct cultivars have been reported by several authors (Cabrita *et al.*, 2001, Papadopoulou *et al.*, 2002). For our purpose, 20 primers (8 RAPD and 12 ISSR) out of 50 (40%) with informative patterns were selected on the basis of the number of amplification products and the stability of the patterns. Then, analysis was extended with the chosen primers to characterize the seven unidentified cultivars.

RAPD and ISSR fingerprints obtained using O18 and HB12 primers, respectively, generated little concerns with the sample identity and/or heterogeneity within the two exotic cultivars Wezara 1 and 2 and the Saudi cultivar. Less than 10% intra-cultivar polymorphism was found for the three cultivars across the two types of analyses (data provided upon request). As being dominant markers, however, bulking (pooling) strategy in RAPD and ISSR analyses is ideal to saturate such a level of intra-cultivar polymorphism with almost no effects on the accuracy of the obtained results. Mengoni *et al.* (2000) indicated that this level of intra-cultivar polymorphism in alfalfa, following the procedure of analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992), is statistically insignificant. On the other hand, results of intra-cultivar polymorphism for the other cultivars demonstrated homogeneity of as high as 100%.

Identification of RAPD markers

A high level of polymorphism was generated utilizing the eight RAPD primers (Figure 1 and Table 1). A total number of 97 RAPD bands, separated by electrophoresis on agarose gel across all cultivars, was obtained. Of these, 92 bands were polymorphic (95%) and only five were monomorphic (5%).

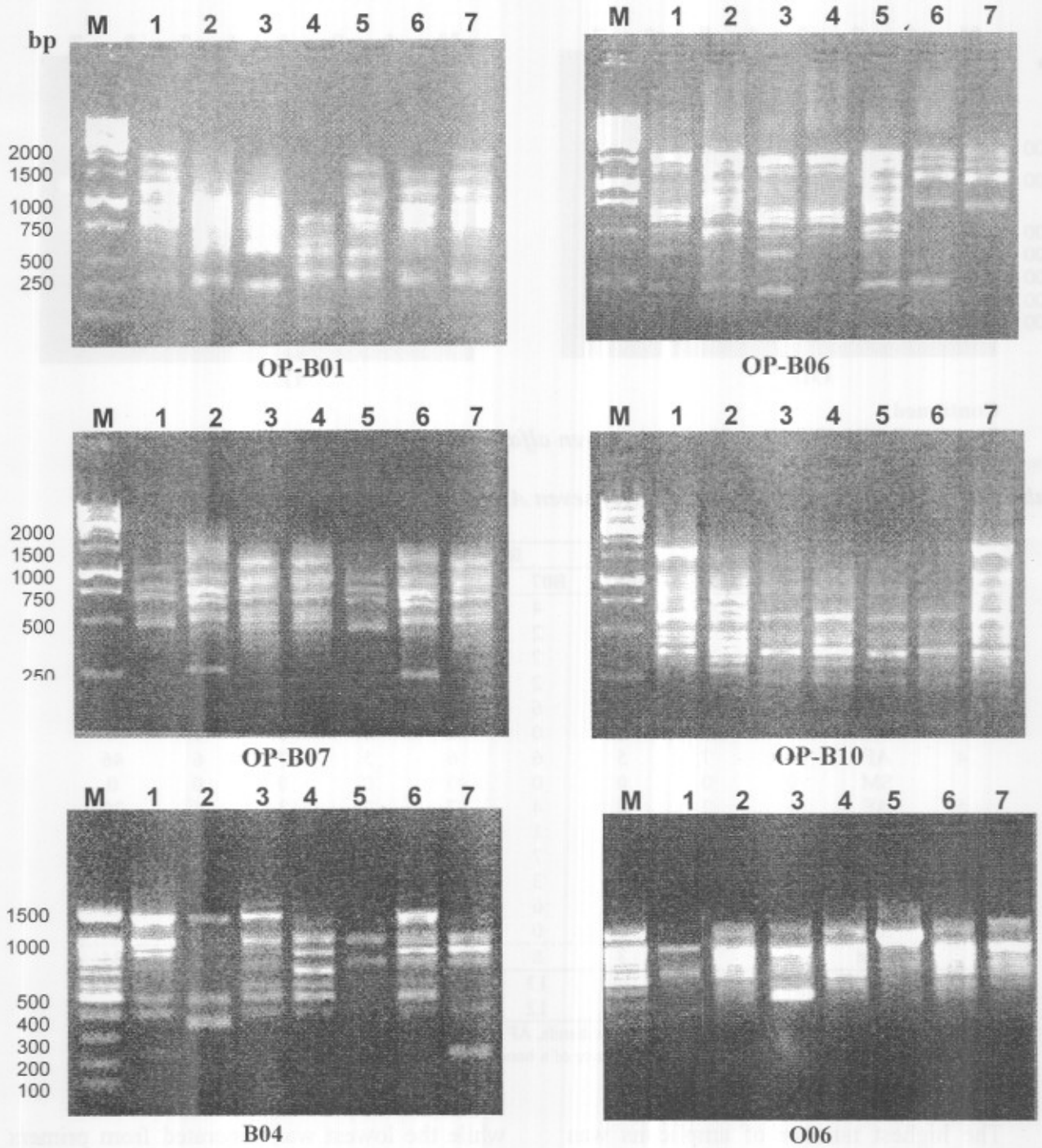

Fig. (1): RAPD PCR profiles of the seven alfalfa cultivers with different primers.

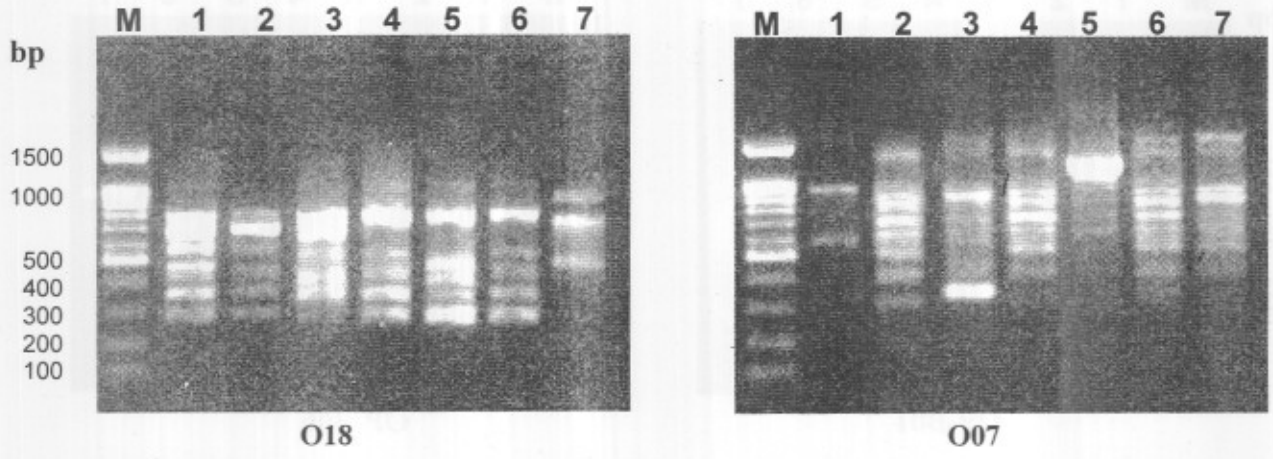

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Fig. (1): RAPD PCR profiles of the seven alfalfa cultivers with different primers.

Table (1): Number of amplified markers of seven Alfalfa cultivars based on RAPD – PCR. analysis.

Alfalfa cultivars		RAPD Primers								
		B01	B04	B06	B07	B10	O06	O07	O18	Total
1	AF	6	6	7	4	7	2	2	7	41
	SM	0	1	0	2	1	1	1	1	7
2	AF	6	3	9	7	10	6	8	6	55
	SM	0	1	1	2	2	0	2	0	8
3	AF	6	5	8	6	7	4	5	5	46
	SM	0	1	1	0	0	0	1	1	4
4	AF	4	7	5	6	6	5	7	6	46
	SM	0	0	0	0	0	0	0	0	0
5	AF	7	2	8	4	7	2	3	6	39
	SM	1	0	1	1	0	1	1	0	5
6	AF	5	5	5	7	4	5	9	5	45
	SM	0	0	0	3	1	0	1	0	5
7	AF	6	4	4	6	8	5	7	3	43
	SM	0	2	0	0	0	0	0	3	5
	TSM	1	5	3	8	4	2	6	5	34
	TAF	12	13	14	13	11	10	14	10	97
	PB	11	13	14	12	9	10	14	9	92

TAF = Total amplified fragment, PB = Polymorphic bands, AF = Amplified fragment,
SM = marker, including either the presence or absence of a band in cultivars,
TSM = Total no. of markers across cultivars.

The highest number of amplicons was generated from Wezara 1 cultivar (55 amplicons), while Ismailia 94 cultivar generated the lowest (39 amplicons). The highest number of amplicons was generated from primers B06 and O07 (14 amplicons), while the lowest was generated from primers O06 and O18 (10 amplicons). Five primers, out of eight, generated no monomorphic amplicons. They are primers B06, B07, B04, O06 and O07. A number of 34 amplicons were useful cultivar-specific markers in which 25 of

them were scored for the presence of a unique band for a given cultivars (positive marker), while nine were scored for the absence of a common band (negative marker). The highest number of cultivar-specific markers (eight) was scored for Wezara 1 cultivar, while the analysis failed to detect markers specific for Ismailia 1 cultivar. Primers B07 and O07 generated the highest number of cultivar-specific markers (eight), while primer B01 generated the lowest (one). In conclusion, all RAPD primers used in the present study allowed for enough distinction among the seven Alfalfa cultivars. Overall comparison among cultivars across the eight primers revealed the power of RAPD in distinguishing among Alfalfa cultivars grown in the same location (ex., Ismailia and Siwa). These markers can be used in subsequent experiments to detect molecular markers for genes with economic importance in Alfalfa cultivars. Recent works demonstrated that a low number of RAPD amplicons per primer was sufficient to produce useful fingerprints for alfalfa cultivar discrimination (Mengoni *et al*., 2000).

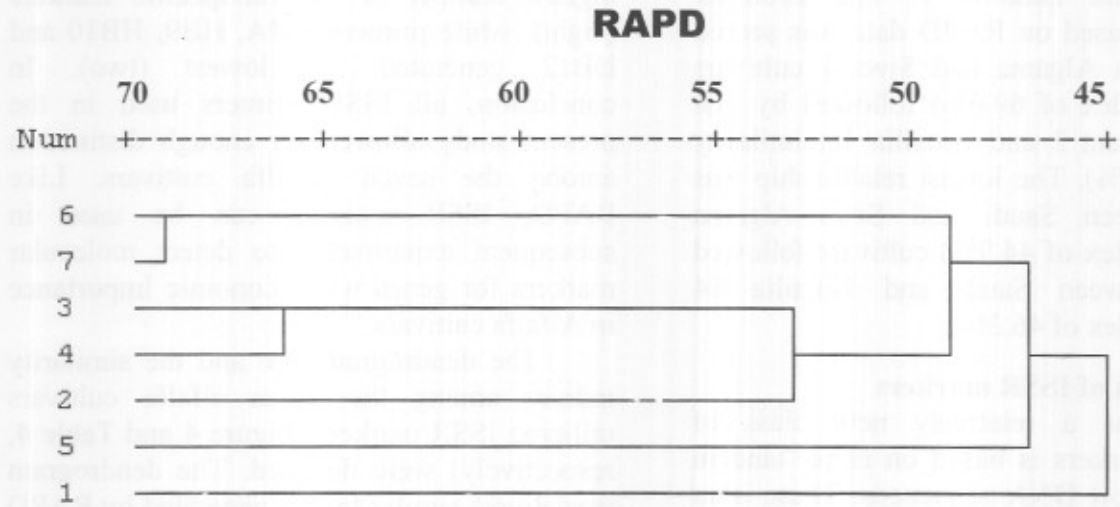

Fig. (2): Dendrogram indicating the relationships among the seven alfalfa cultivars based on RAPD analysis.

Table (2): Similarity matrix among the seven alfalfa cultivars based on RAPD analysis.

	1	2	3	4	5	6
1						
2	57.1					
3	56.2	62.1				
4	51.7	64.1	68.1			
5	46.3	56.3	52.9	55.2		
6	44.9	60.2	55.3	66.0	50.6	
7	50.6	59.4	50.0	56.5	47.1	69.6

The dendrogram tree and the similarity indices among the seven alfalfa cultivars utilizing RAPD markers (Figure 2 and Table 2, respectively) were detected. The dendrogram divided the cultivars into two clusters, the first contained Saudi cultivar and the second contained the rest. The second cluster was divided into two sub-clusters, the first contained Ismailia 94 and the second contained the other five cultivars. The second sub-cluster was divided into two groups, the first contained two cultivars (Siwa Algama and Siwa 1), while the second contained Wezara 1, Wezara 2 and Ismailia 1. The strongest relationship based on RAPD data was scored between Siwa Algama and Siwa 1 cultivars (similarity index of 69.6%) followed by that between Wezara 2 and Ismailia 1 (similarity index of 68.1%). The lowest relationship was scored between Saudi and Siwa Algama (similarity index of 44.9%) cultivars followed by that between Saudi and Ismailia 94 (similarity index of 46.3%).

Identification of ISSR markers

ISSR as a relatively new class of molecular markers is based on in ter tandem repeats of short DNA sequences. These inter repeats are highly polymorphic, even among closely related genotypes, due to the lack of functional constraints in these non-functioning regions. Similarly, a high level of polymorphism was generated utilizing the 12 ISSR primers (Figure 3 and Table 3). A total number of 100 ISSR bands was obtained. Of these, 87 bands were polymorphic (87%) and only 13 were monomorphic (13%). The highest number of amplicons was generated from Wezara 1 cultivar (57 amplicons), while Siwa 1 cultivar generated the lowest (44 amplicons). The highest number of amplicons was generated from primer HB11 (13 amplicons), while the lowest was generated from primer 814 (5 amplicons). Three primers, out of 12, generated no monomorphic amplicons. They are primers 844A, 844B and HB12. A number of 44 amplicons were useful cultivar-specific markers in which 31 of them were scored for the presence of a unique band for a given cultivars (positive marker), while 13 were scored for the absence of a common band (negative marker). The highest number of cultivar-specific markers (seven) was scored for Ismailia 1, Ismailia 94 and Siwa 1 cultivars, while the lowest number of cultivar-specific markers was, unexpectedly, scored for Saudi cultivar. Primer 17899B generated the highest number of cultivar-specific markers (eight), while primers 844A, HB9, HB10 and HB12 generated the lowest (two). In conclusion, all ISSR primers used in the present study allowed for enough distinction among the seven Alfalfa cultivars. Like RAPD, ISSR markers can be used in subsequent experiments to detect molecular markers for genes with economic importance in Alfalfa cultivars.

The dendrogram tree and the similarity indices among the seven alfalfa cultivars utilizing ISSR markers (Figure 4 and Table 4, respectively) were detected. The dendrogram was almost similar to that generated by RAPD data. It divided the cultivars into the same clusters, sub-clusters and groups generated by RAPD data. It was expected that data generated by markers with similar type of inheritance are almost similar. The strongest relationship based on ISSR data was scored between Siwa Algama and Siwa 1 cultivars (similarity index of 76.6%) followed by those between Wezara 2 and Siwa algama (similarity index of 72.3%) and between Wezara 2 and Ismailia 1 (similarity index of 71.0%). The lowest relationship was scored between Saudi and Ismailia 1 cultivars (similarity index of 51.5%) followed by that between Saudi and Siwa Algama (similarity index of 52.9%).

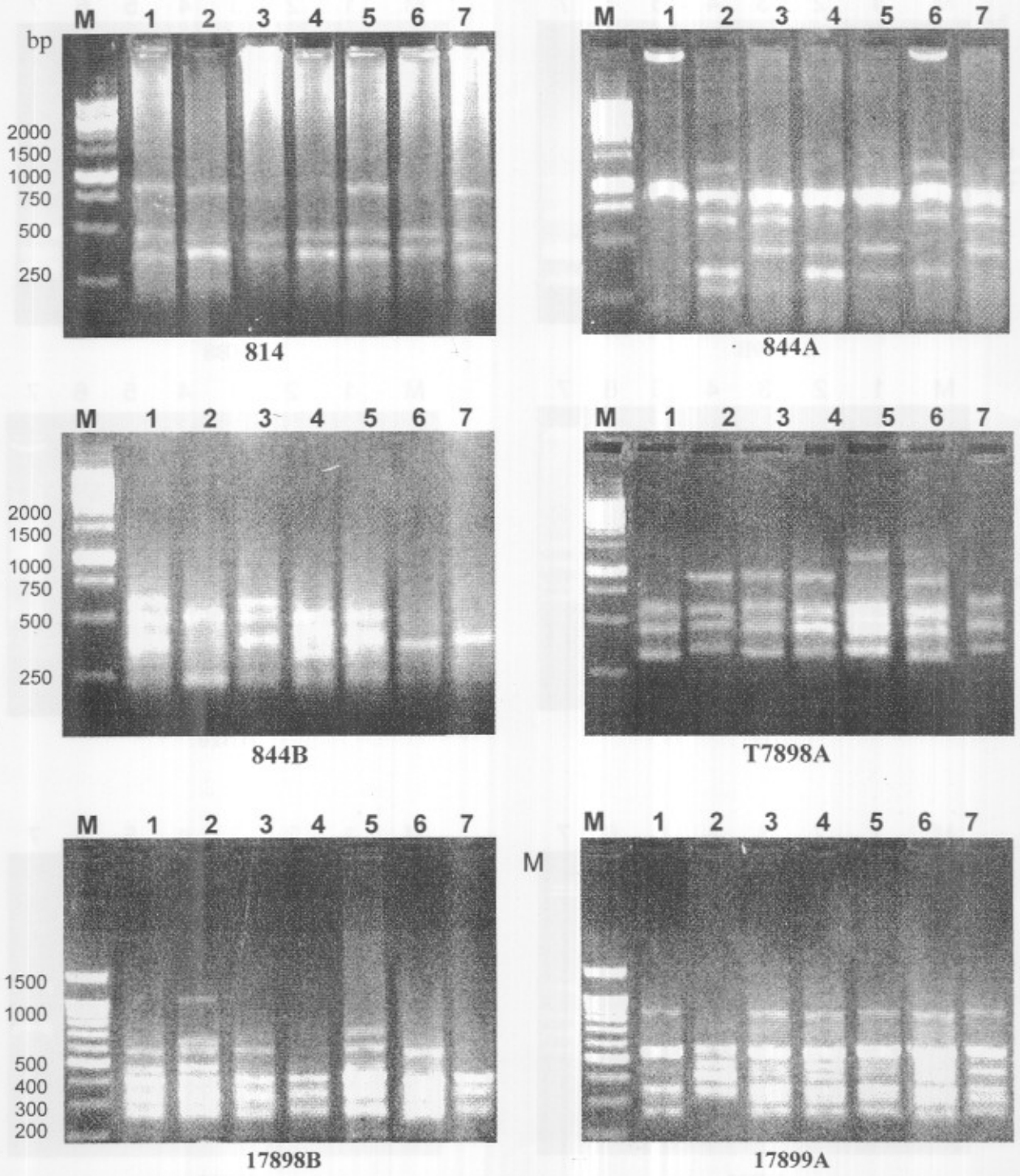

Fig. (3): ISSR-PCR profiles of the seven alfalfa cultivers with different primers.

Fig, (3) Cont.

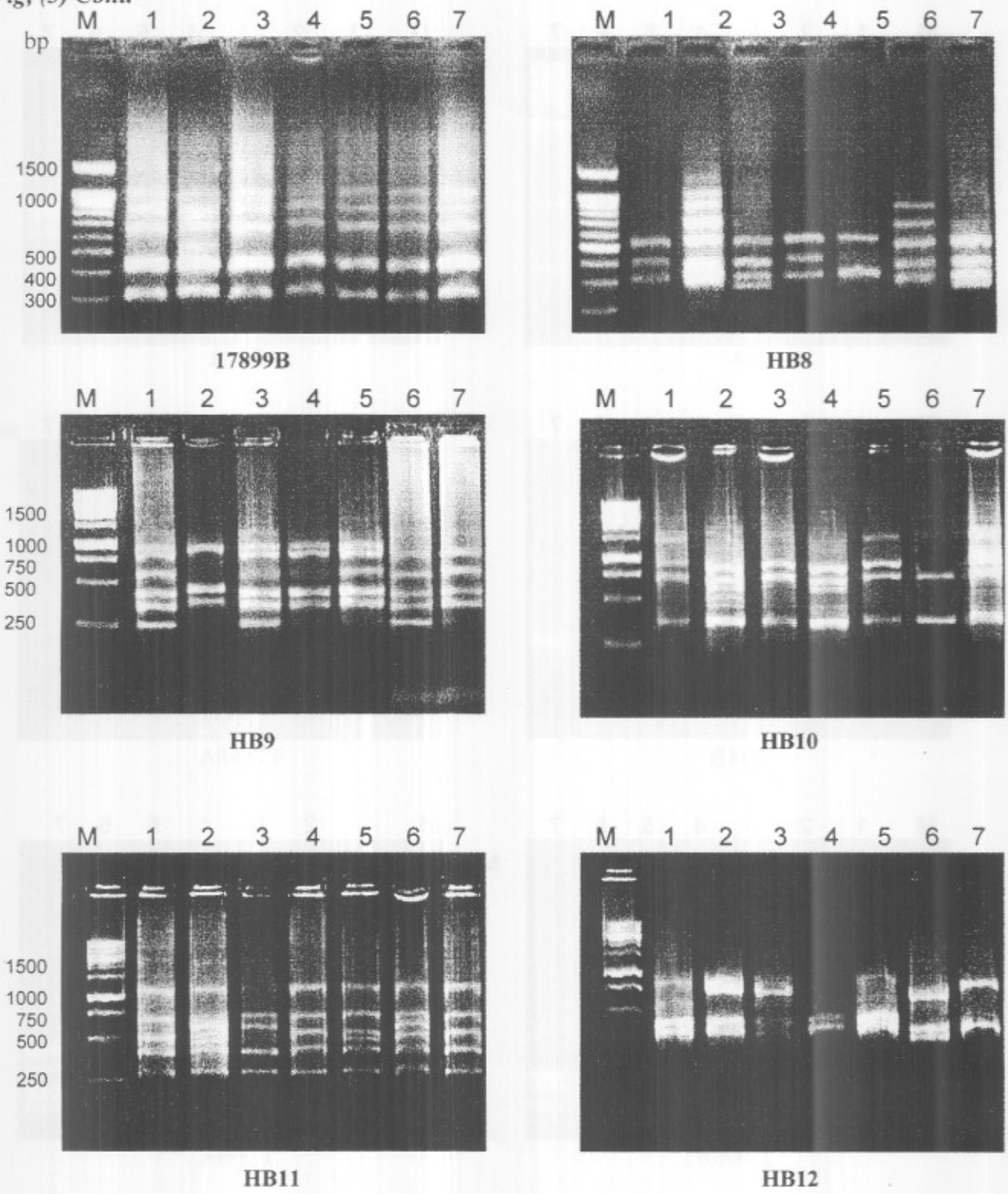

Fig. (3): ISSR-PCR profiles of the seven alfalfa cultivers with different primers.

Table (3): Number of amplified fragments markers of seven Alfalfa cultivars based on ISSR analysis.

		ISSR Primers												
Alfalfa cultivars		814	844A	844B	17898A	17898B	17899A	17899B	HB8	HB9	HB10	HB11	HB12	Total
1	AF	3	1	3	3	5	4	5	3	6	4	5	3	45
	SM	1	1	0	0	0	0	0	0	0	0	1	0	3
2	AF	3	5	2	4	7	4	6	7	3	6	6	4	57
	SM	0	1	0	0	2	2	2	2	2	2	0	0	13
3	AF	2	3	4	4	4	7	5	4	6	3	5	2	49
	SM	0	0	0	0	0	0	0	0	0	0	2	1	3
4	AF	2	4	4	4	3	7	4	3	5	4	5	2	47
	SM	0	0	2	0	0	0	4	1	0	0	0	0	7
5	AF	3	4	3	4	5	5	5	2	5	4	6	3	49
	SM	0	0	0	2	1	1	2	0	0	0	0	0	6
6	AF	2	4	1	5	4	6	5	5	5	2	6	3	48
	SM	0	0	0	1	0	0	0	0	0	0	3	1	5
7	AF	3	3	2	3	3	6	5	4	4	4	5	2	44
	SM	2	0	1	1	2	1	0	0	0	0	0	0	7
	TSM	3	2	3	4	5	4	8	3	2	2	6	2	44
	TAF	5	7	7	9	9	9	10	9	6	9	13	7	100
	PB	4	7	7	8	7	7	9	7	4	8	12	7	87

TAF = Total amplified fragment, PB = Polymorphic bands, AF =Amplified fragment,
SM = marker, including either the presence or absence of a band in cultivar,
TSM = Total no. of markers across cultivars.

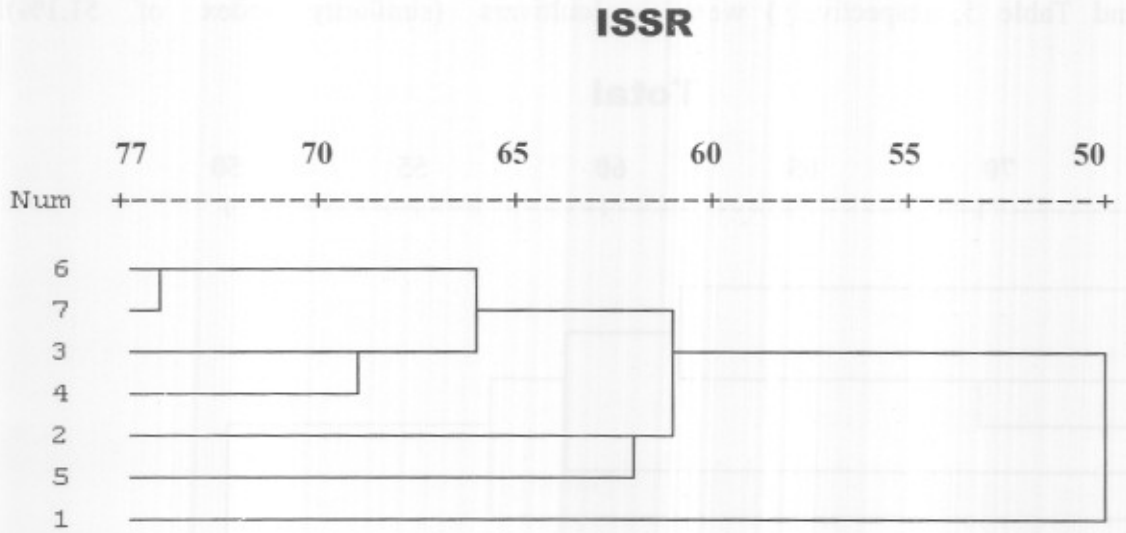

Fig. (4): Dendrogram indicating the relationships among the seven alfalfa cultivars based on ISSR analysis.

Table (4): Similarity matrix % among the seven alfalfa cultivars based on ISSR analysis.

	1	2	3	4	5	6
1						
2	62.9					
3	58.5	61.9				
4	51.5	63.0	71.0			
5	65.4	65.3	68.8	61.5		
6	52.9	66.7	72.3	65.2	56.5	
7	58.5	68.0	69.4	68.8	68.8	76.6

Comparative analysis of genetic variation between RAPD and ISSR markers

As for the rate of polymorphism and structure of data, it can be concluded that both types of analyses were in harmony regardless of the difference in primer number and strategy of amplification. They basically share the type of marker inheritance (dominance) that reflects the feasibility of utilizing bulking strategy in the present work. The dendrogram tree and the similarity indices among the seven alfalfa cultivars across both types of markers (Figure 5 and Table 5, respectively) were detected. The dendrogram from the combined data was almost similar to that generated by either one. It divided the cultivars into the same clusters, sub-clusters and groups. The strongest relationship based on combined data was scored between Siwa Algama and Siwa 1 cultivars (similarity index of 72.5%) followed by that between Wezara 2 and Ismailia 1 (similarity index of 69.2%). The lowest relationship was scored between Saudi and Siwa Algama (similarity index of 48.9%) followed by that between Saudi and Ismailia 1cultivars (similarity index of 51.1%).

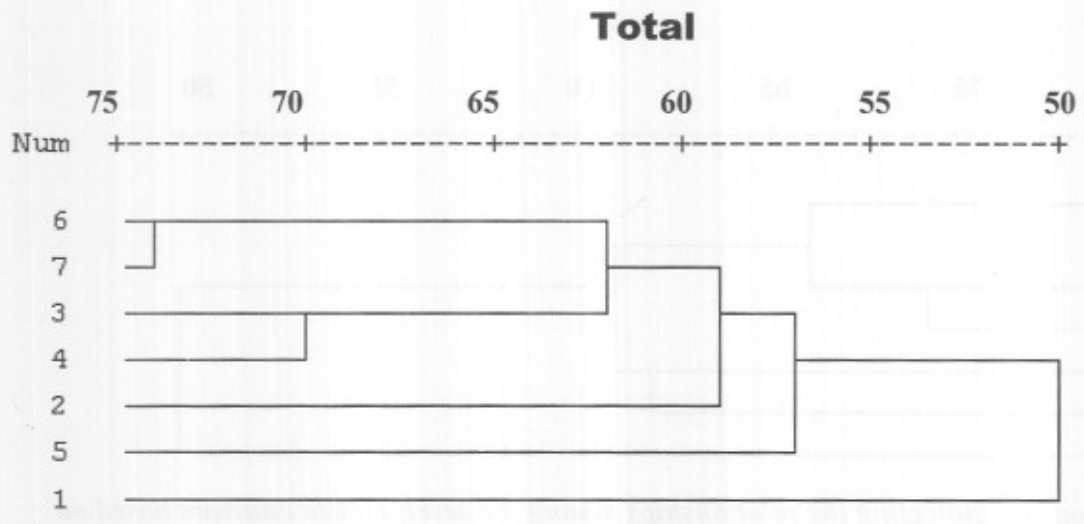

Fig. (5): Dendrogram indicating the relationships among the seven alfalfa cultivars based on RAPD and ISSR analysis.

Table (5): Similarity matrix % among the seven alfalfa cultivars based on RAPD and ISSR analysis.

	1	2	3	4	5	6
1						
2	60.0					
3	57.0	61.9				
4	51.1	63.5	69.2			
5	56.5	60.6	60.8	58.0		
6	48.9	62.5	63.8	65.6	53.4	
7	54.7	62.9	59.9	62.6	58.4	72.5

In molecular analysis, intra- or inter-cultivars polymorphism is a direct consequence of the differences existing at genomic DNA level. Molecular analyses utilized in the present work provided a reliable, specific and highly sensitive method for DNA typing and genetic characterization of a multiplicity of alfalfa genotypes. These PCR-based procedures represent an improvement of our understanding of genetic variability within or across cultivars. Since RAPD and ISSR markers are transmitted by inheritance, they constitute a better typifying system than those based on phenotypic determination. The polymorphism observed in the present work (around 97%) was sufficient to differentiate among all the samples analyzed and provided an innovative method to identify cultivars similar from a phenotypic point of view in agreement with similar earlier studies on alfalfa cultivars (Mengoni *et al*., 2000). Such molecular analyses can be an invaluable tool for safeguarding and improving alfalfa species. Selection assisted by these molecular markers may also be helpful to produce new cultivars with improved productivity, abiotic tolerance and pest resistance. Furthermore, the molecular marker-assisted selection may become important in the assessment of the genotypes used in the *Medicago* L. improvement programs in Egypt.

REFERENCES

Awasthi, A.k., Nagaraja, G.M., Naik, G.V., Kanginakudru, S., Thangavelu, K. and Nagaraju J. (2004). Genetic diversity and relationships in mulberry (genus Morus) as revealed by RAPD and ISSR marker assays. BMC Genet. 5(1):1.

Barcaccia, G., Tosti, N., Falistocco, E. and Veronesi, F. (1995). Cytological, morphological and molecular analyses of controlled progenies from meiotic mutants of alfalfa producing unreduced gametes. Theor. Appl. Genet. 91, 1008-1015.

Barnes, D. K., Goplen. B. P. and Baylor, J. E. (1988). Highlights in the USA and Canada. In: A. A. Hanson, D. K. Barnes, and R. R. Hill (eds), Alfalfa and Alfalfa Improvement. Monogr. 29, 1-24. J. Soc. Agron. Madison.

Brummer, E.C., Bouton J.H. and Kochert G. (1995). Analysis of annual Medicago species using RAPD markers. Genome. Apr,38(2):362-7.

Businelli, S., Pupilli, F., Damiani, F. and Arcioni, S. (1993). Selection of homologous probes for the characterization of Medicago genotypes through RFLP analysis. 3. Genet. Breed. 47, 333-340.

Cabrita L.F, Aksoy, U., Hepaksoy, S. and Leitio, J.M. (2001). Suitability of isozyme, RAPD and AFLP markers to assess genetic

differences and relatedness among fig (*Ficus canca* L) clones. SciHortic (Amsterdam) 87:261-273

Crochemore, M. L., Huyghe, C., Kerlan, M. C. and Julier, B. (1996). Partitioning and distribution of RAPD variation in a set of populations of *Medicago sativa* complex. Agronomie 16, 421-432.

Echt, C.S., Erdahl, L.A. and McCoy, T.J. (1992). Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome, Feb. 35(1):84-7.

Excoffier, L., Smouse, P. E. and Quattro, M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479-491.

Hallauer, A. R. and Miranda, J. B. (1988). Quantitative Genetics in Maize Breeding, 2nd ed., Iowa State Univ. Press, Ames.

Kidwell, K. K., Austin, D. F. and Osborn, T. C. (1994). RFLP evaluation of nine Medicago populations representing the original germplasm sources for north American alfalfa cultivars. Crop Sci. 34, 230-236.

Kiss, G. B., Csanadi, G., Kalman, K., Kalo, P. and Okresz, L. (1993). Construction of a basic genetic map for alfalfa using RFLP, RAPD, isozyme and morphological markers. Mol. Gen. Genet. 238, 129-137.

McCoy, T. J., and Bingham, E. I. (1988). Cytology and cytogenetics of alfalfa. In: A. A. Hanson, D. K. Barnes, and R. R. Hill (edt), Alfalfa and Alfalfa Improvement, Monogr. 29, 737-776. Soc. Agron., Madison.

McCoy, T. J. and Echt, C. S. (1993). Potential of trispecies bridge crosses and random amplified polymorphic DNA markers for introgression of *Medicago hestanica* and *M. pironae* germplasm into alfalfa (M. sat wa). Genome 36, 594-601.

Mengoni A., Gori A. and Bazzicalupo, M. (2000). Use of RAPD and microsatellite (SSR) variation to assess genetics relationships among population of tetraploid alfalfa, *Medicago sativa*. Plant Breeding 119, 311-317.

Michaud, R., Lehman, W. F. and Runbaugh, M. D. (1988). World distribution and historical development. In: A. A. Hanson, D. K. Barnes, and R. R. Hill (edt), Alfalfa and Alfalfa Improvement, Monogr. 29. Soc. Agron., Madison.

Nagaoka, T. and Ogihara Y. (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94:597-602.

Nei, M. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl .Acad. Sci., USA 76:5269-5273

Papadopoulou,. K., Ehaliotis, C., Tourna, M., Kastanis, P., Karydis, I. and Zervakis, G. (2002). Genetic relatedness among dioecious *Ficus carica* L cultivars by random amplified polymorphic DNA analysis, and evaluation of agronomic and morphological characters. Genetica 114:183-194

Powell, W., Orazco-Castillo, C., Chaluers, K. 1. , Provan, J. and Waugh, R. (1995). Polymerase chain reaction-based assays for the characterization of plant genetic resources. Electrophoresis 16, 1 726-1730.

Pupitli, F., Businelli, S., Paolocci, F., Scotti, C., Damiani, F. and S. Arcioni, (1996). Extent of RFLP variability in tetraploid populations of alfalfa, *Medicago sativa*. Plant Breeding 115, 106-112.

Vitale, M., Pupilli, F., Scotti, C. and Arcjoni, S. (1996). Extent of RFLP and RAPD variability in tetraploid populations of alfalfa. Proc. XVI EGF Meeting, Italy, 305-308.

Yu, P. and Pauls, K. P. (1993a). Rapid estimation of genetic relatedness among heterogeneous populations of alfalfa by random amplification of bulked genomic DNA samples. Theor. Appl. Genet. 86, 788-794.

Yu, P. and Pauls, K. P. (1993b). Segregation of random amplified polymorpbic DNA markers and strategies for molecular mapping in tetraploid alfalfa. Genome 36, 844-851.

الملخص العربي

التوصيف الجزيئى لبعض أصناف البرسيم الحجازى المزروعة فى مصر والمملكة العربية السعودية باستخدام تقنيات الـ RAPD والـ ISSR

منى هاشم حسين * – رانيا أحمد يونس**

* قسم الوراثة – كلية الزراعة – جامعة القاهرة-جمهورية مصر العربية.

** قسم الوراثة – كلية الزراعة – جامعة عين شمس-جمهورية مصر العربية.

لقد تم تقدير مستوى التباين العشوائى لل د ن أ المجسم (RAPD) ، وأيضا التباين فيما بين التتابعات البسيطة المكررة (ISSR) فى جينومات سبعة أصناف من البرسيم الحجازى المزروعة فى مصر والمملكة العربية السعودية. أوضحت نتائج كلتا التقنيتين وجود درجة منخفضة من التباين polymorphism الوراثى داخل كل صنف (أقل من 10% فى كل من التحليلين)، بينما ظهرت درجة عالية من التباين الوراثى genetic polymorphism (أكثر من 90% فى كل من التحليلين) بين الاصناف – مما مكن التبصيم المتفرد uniquely fingerprinted لجينوم كل صنف. كما أمكن تحليل القرابة الوراثية بين الاصناف السبعة من البرسيم الحجازى – التى شملتها الدراسة – بحساب مصفوفة التماثل الزوجية pairwaise similarity matrix بين التراكيب الوراثية طبقا لمعامل Nei & Li بتطبيق طريقة UPGMA لرسم شجرة القرابة. وبينت النتائج ان كلا التحليلين كان قادراً على فصل جينوم الصنف السعودى عن جينومات الاصناف المصرية بدرجة واضحة ولقد استنتج من هذه الدراسة ان تقنيتى الـ RAPD والـ ISSR – واللتان أعطيتا نتائج متشابهة تقريبا – كانتا وسائل مفيدة وفعالة فى تحديد التباين الوراثى وعلاقة القرابة الوراثية، وامكانية تحسين البرسيم الحجازى من خلال الانتخاب بمساعدة الواسمات الجزيئية للـ د ن أ Marker-assisted selection (MAS).