IDENTIFICATION OF GENETIC DIVERSITY AMONG BASIL (OCIMUM SP.) ACCESSIONS COLLECTED FROM AFRICA, EUROPE, ASIA, AND USA

YASMIN A. A. ABURIGAL[1*], NADA B. HAMZA [2], AWATIF M. SIRIBLE[3], ELFADL Y. ELMOGTABA[4], ISMAIL H. HUSSEIN[5]

[1]Faculty of Agricultural Sciences, University of Gezira, P. O. Box 20 Wad Medani, Sudan.
[2]Commission for Biotechnology and Genetic Engineering, National Centre for Research, Khartoum, Sudan.
[3]Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan.
[4,5] National Oilseeds Processing Research Institute (NOPRI), University of Gezira,WadMedani, Sudan.

*****Corresponding author: aburigalyasmin@gmail.com*

ABSTRACT: Basil (*Ocimum* sp.), belonging to family Lamiaceae is very important for their therapeutic potentials and medicinal properties. In the present study, 62 basil accessions collected from different regions of the world were analyzed for the assessment of genetic diversity using inter simple sequence repeat (ISSR). Four primers generated a total of 36 amplified fragments with an average of nine polymorphic bands per primer. The maximum number of fragments was produced by primers 807 and 872 with 100% polymorphism, while the minimum number of fragments was produced by primers 841 and 858 with 100% polymorphism. The highest similarity index was 0.59 and the least similarly index was 0.04. The unweighted pair- group method was done using UPGMA clustering, classified the studied accessions in two major groups. It was concluded that high genetic variations were detected among the 62 Basil accessions. Based on the findings of this study, the ISSR markers are very useful for analyzing the genetic variations for exploitation of basil in industrial applications.

KEY WORDS: *Basil, Genetic diversity, ISSR markers.*

1. INTRODUCTION

Sweet Basil (*Ocimum basilicum*), locally known as Rehyan, family Lamiaceae**,** is one of the most important aromatic and medicinal plants for health benefits**.** The genus *Ocimum* is native to the tropical and subtropical regions of Africa [1], Asia, Central and South America [2]. The taxonomy of *Ocimum* is complex due to inter-specific hybridization and polyploidy of the species in the genus. Pushpangadan, [3] recognized more than 150 species; however Paton,[4] proposed that *Ocimum* had only 65 species, and that other attributions should be considered as synonyms.In order to improve the further breeding or collection of basil, understanding its genetic diversity is important. In recent years, a variety of DNA-based techniques have been employed to study genetic variation in *Ocimum spp* such as Random Amplified Polymorphic DNA (RAPD) [5,6&7], Amplified Fragment Length Polymorphic (AFLPs) [8,9], Inter Simple Sequence Repeat (ISSR) [10] and Sequence-Related Amplified Polymorphism (SRAP) [11]. For the initial assessment of genetic diversity in plant species, The ISSR marker is the most

powerful DNA-marker due to its simplicity, ease and speed, and the wide availability of universal primers. These can be used to estimate the genetic diversity among plants and genetic materials. When used in concert with other morphological, physiological, and chemical properties, ISSR markers provide reliable criteria that can be used for selection of the best varieties. The objective of the present work is to investigate the relationships among 62 basil accessions collected from different regions of the world using ISSR markers,as a key step in the basil breeding program.

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2. METHODOLOGY

2.1. Seed sources

Basil seeds used in these studies were obtained from the Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben (Germany) (Table 1).Seeds of each accession were grown in 30 cm diameter earthen pots in a greenhouse for four weeks.

Table 1: The *Ocimum* acessions used in this study

No.	Accession No.	Latin name	Observation	Country
1	OCI 353	*Ocimum basilicum* L. var. *purpurascensBenth*		Yemen
2	OCI 108	*Ocimumbasilicum* L. var.*basilicum*		Iraq
3	OCI 30	*Ocimumbasilicum* L. var.*basilicum*		Argentine
4	OCI 334	*Ocimumbasilicum L.* var. *purpurascensBenth*		Egypt
5	OCI 237	*Ocimumbasilicum L.* var.*purpurascensBenth*		RuBland
6	OCI 29	*Ocimum×citriodorum Vis. ×O.basilicum ssp. Basilicum Var. purpurascensBenth*		Armenia
7	OCI 86	*Ocimumbasilicum L.* var.*basilicum*		Soviet union
8	OCI 354	*Ocimumbasilicum L.* var. *purpurascensBenth*		Yemen
9	OCI 217	*Ocimumbasilicum L.* var.*purpurascensBenth*		Armenia
10	OCI 32	*Ocimumbasilicum L.* var.*purpurascensBenth*		Armenia
11	OCI 181	*Ocimumbasilicum L.* var.*purpurascensBenth*	Persian Anise-scented Basil	Unknown
12	OCI 194	*Ocimumbasilicum L.* var.*purpurascensBenth*	Hebaq	Algeria
13	OCI 158	*Ocimumbasilicum L.* var.*purpurascensBenth*	Kartulirechani(=georgi schesBasilikum)	Georgia
14	OCI 238	*Ocimumbasilicum L.* var.*basilicum*		RuBland
15	OCI 61	*Ocimumbasilicum L.* var.*purpurascensBenth*		Italy
16	OCI 338	*Ocimumbasilicum L.* var.*purpurascensBenth*		Armenia
17	OCI 150	*Ocimumbasilicum L.* var.*basilicum*		Maldives
18	OCI 370	*Ocimumbasilicum L.* var.*basilicum*	Albahaca Graude Verde	Spain
19	OCI 56 A	*Ocimumbasilicum L.* var.*basilicum*	Lactucaefolium	Unknown
20	OCI 343	*Ocimumbasilicum L.* var.*purpurascensBenth*		Kazakhstan
21	OCI 102	*Ocimumbasilicum L.* var.*basilicum*		Rumania
22	OCI 237	*Ocimumbasilicum L.* var.*purpurascensBenth*		RuBland
23	OCI 228	*Ocimumbasilicum L.* var.*basilicum*		USA
24	OCI 225	*Ocimumbasilicum L.* var.*basilicum*		USA
25	OCI 1	*Ocimumbasilicum L.* var.*basilicum*		Germany

26	OCI 23	*Ocimumbasilicum L.* var.*purpurascensBenth*	Dark Opal	Unknown
27	OCI 49	*Ocimumbasilicum L.* var.*basilicum*	Napoletano	Italy
28	OCI 65	*Ocimum×citriodorum Vis. ×O.basilicum ssp. Basilicum* Var. *purpurascensBenth*	Sasklavi	Georgia
29	OCI 67A	*Ocimumbasilicum L.* var.*purpurascensBenth*		Unknown
30	OCI 67 B	*Ocimumbasilicum L.* var.*basilicum*		Soviet Union
31	OCI 73	*Ocimumbasilicum L.* var.*basilicum*	Comune aFogliaPiccola	Italy
32	OCI 74 A	*Ocimumbasilicum L.* var.*basilicum*		Georgia
33	OCI 75 A	*Ocimumbasilicum L.* var.*basilicum*	Sasklavi	Georgia
34	OCI 76 B	*Ocimumbasilicum L.* var. *differmeBenth*		Italy
35	OCI 80	*Ocimumbasilicum L.* var.*basilicum*	BasilicogiganteGenovese	Italy
36	OCI 106	*Ocimumbasilicum L.* var.*purpurascensBenth*		Iraq
37	OCI 102	*Ocimumbasilicum L.* var.*basilicum*		Rumania
38	OCI 114	*Ocimumbasilicum L.* var.*basilicum*		Italy
39	OCI 118	*Ocimumbasilicum L.* var.*basilicum*		Togo
40	OCI-Sulait	*Ocimum basilicum L.* var.*basilicum*		Sudan
41	OCI-Butana	*Ocimum basilicum L.* var.*basilicum*		Sudan
42	OCI 141	*Ocimum basilicum L.* var.*basilicum*		Maldives
43	OCI 160	*Ocimum basilicum L.* var.*basilicum*	Albahacalimon	Cuba
44	OCI 165	*Ocimum basilicum L.* var.*basilicum*	BasilicoFino Verde	Italy
45	OCI 180	*Ocimum basilicum L.* var.*basilicum*	Lettuce-leaved Basil	Unknown
46	OCI 185	*Ocimum basilicum L.* var.*basilicum*	Lettuce-leaved Basil	Unknown
47	OCI 186	*Ocimum basilicum L.* var. *purpurascens Benth*		China
48	OCI 194	*Ocimum basilicum L.* var. *purpurascens Benth*	Hebaq	Algeria
49	OCI 197	*Ocimum basilicum L.* var. *purpurascens Benth*		Maldives
50	OCI 281	*Ocimum basilicum L.* var. *thyriflourm (L.)Benth*	Siam Queen	Thailand
51	OCI 308	*Ocimum basilicum L.* var. *purpurascens Benth*		Reunion (REU)
52	OCI 333	*Ocimum basilicum L.* var.*basilicum*		Madagascar
53	OCI 341	*Ocimum basilicum L.* var.*basilicum*		Azerbaijan
54	OCI 372	*Ocimum basilicum L.* var.*basilicum*	BASILICO COMMEODOROSO	Italy
55	OCI 340	*Ocimum basilicum L.* var. *purpurascens Benth*		Kirgizstan
56	OCI-Gezira	*Ocimum basilicum L.* var.*basilicum*		Sudan
57	OCI 43	*Ocimum basilicum L.* var.*basilicum*	Ohre	CSFR(CSK)
58	OCI 174	*Ocimum basilicum L.* var.*basilicum*	Genovese Basil	Unknown
59	OCI 186	*Ocimum basilicum L.* var. *purpurascensBenth*		China (CHN)
60	OCI 284	*Ocimum basilicum L.* var.*basilicum*		Japan (JPN)
61	OCI 343	*Ocimum basilicum L.* var. *purpurascensBenth*		Kazakhstan
62	OCI 225	*Ocimum basilicum L.* var.*basilicum*		USA

2.2. DNA Extraction

Genomic DNA was extracted from fresh leaf tissues for each genotype using the modified CTAB method [12] as follows:

Two grams of fresh leaf tissue were ground using dry ice in a Blender. The fine powder was then transferred into 13 ml Falcon tube and 6 ml of preheated CTAB buffer (60ºC) were added. The solution was incubated in a shaking water bath at 60ºC with gentley shaking for 30 min

and left to cool at room temperature for 5 min. Three ml of chloroform\isoamyl alcohol (24:1) were added, and the solution was mixed gently but thoroughly for 10 min and then centrifuged at 4000 rpm for 15 min. The aqueous phase was transferred with a pipette into a new sterile tube and the precipitate was discarded. The previous steps were repeated, and the same volume of chloroform\isoamyl alcohol (24:1) was added again. Three ml of cold 2- propanol was added to cover and mixed gently to precipitate in freezer for 30 min. The solution was centrifuged at 4000 rpm for 5 min. The supernatant was discarded and three ml of 70% ethanol was added. The previous step was repeated again with repeated washing. The solution was centrifuged again at 4000 rpm for 5 min. The supernatant was discarded and the pellet was allowed to dry for 30 min at room temperature. Finally, the DNA pellet was dissolved in 100ul of TE buffer and kept overnight at 4ºC. DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis.

2.2.1. Primer selection and PCR Protocol for ISSR techniques

Four primers that produced strongly amplified polymorphic bands with these test templates were selected for ISSR-PCR analysis (Table 2). Each reaction contained 25 ul total volume of reaction. For each of the primers, a master mix was done separately for the 62 basil samples.The PCR reactions were carried out in 25 µl volume containing 15 µl sterile distilled water, 2.5 µl 10Xbuffer, 2.5 µl (2 mM/µl) DNTPs, 1.5 µl (50 mM) MgCl2, 2 µl (10 pmol/µl) primer, 0.1 µl BSA, 0.5 µl (5u/ µl) Taq DNA polymerase and 1 µl (10 to30 ng/µl) template DNA, for each sample.

2.2.2. Inter-Simple Sequence Repeat Techniques (ISSR)

The PCR amplification protocol was programmed for 5 min at 94°C for initial denaturation, followed by 40 cycles of 1min at 94°C, 1 min at (42°C for ISSR) and 1 min at 72°C, final extension was programmed for 7 min at 72°C followed by hold time at 4°C until samples were collected.

2.3.Data Scoring

For each primer, the number of polymorphic and monomorphic bands was determined. Data was scored as (0) for absence of a fragment and (1) for presence of a fragment. Coefficient of similarity trees were produced by clustering the similarity data with the un-weighted pair group method using statistical software package **STATISTICA** ver. 9.

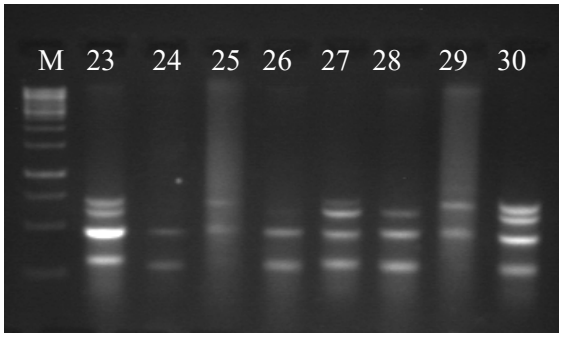
3. RESULTS AND DISCUSSION

In the present study, 62 basil accessions collected from different regions of the world were analyzed for the assessment of genetic diversity with ISSR markers. Four primers generated a total of 36 amplified fragments with an average 9 polymorphic bands per primer. The maximum number of fragment bands was produced by the primers 807 and 872 with 100% polymorphism, while the minimum number of fragments was produced by the primers 841 and 858 with 100% polymorphism (Table 3).

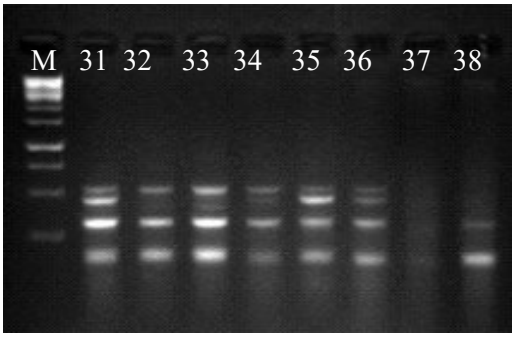
Table 3: Total number of bands, number of Polymorphic and Monomorphic bands for ISSR primers

Primer	Sequence (5'- 3')	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage of polymorphic bands(%)
807	(AG)8T	10	10	0	100
841	(GA)8YC	8	8	0	100
858	(TG)8RT	8	8	0	100
872	(GATA)4	10	10	0	100
Total		36	36		
Average		9	9		

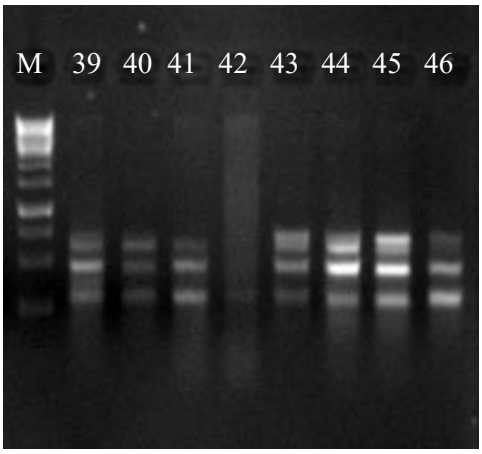
The high level of polymorphism found suggests that the ISSR technique can be useful for studying diversity in *Ocimum spp.* These results are in agreement with [10] who indicated that ISSR is useful to evaluate genetic diversity among basil accessions. Also, Lal et al. 2012 [13] using RAPD, ISSR and SSR Markers for detecting genetic diversity within six different species of *Ocimum*, found that the ISSR gave a satisfactory amplification. Pattern of ISSR fragments produced by the primer 841 and 872 is shown in Fig. 1 and 2.



(A)



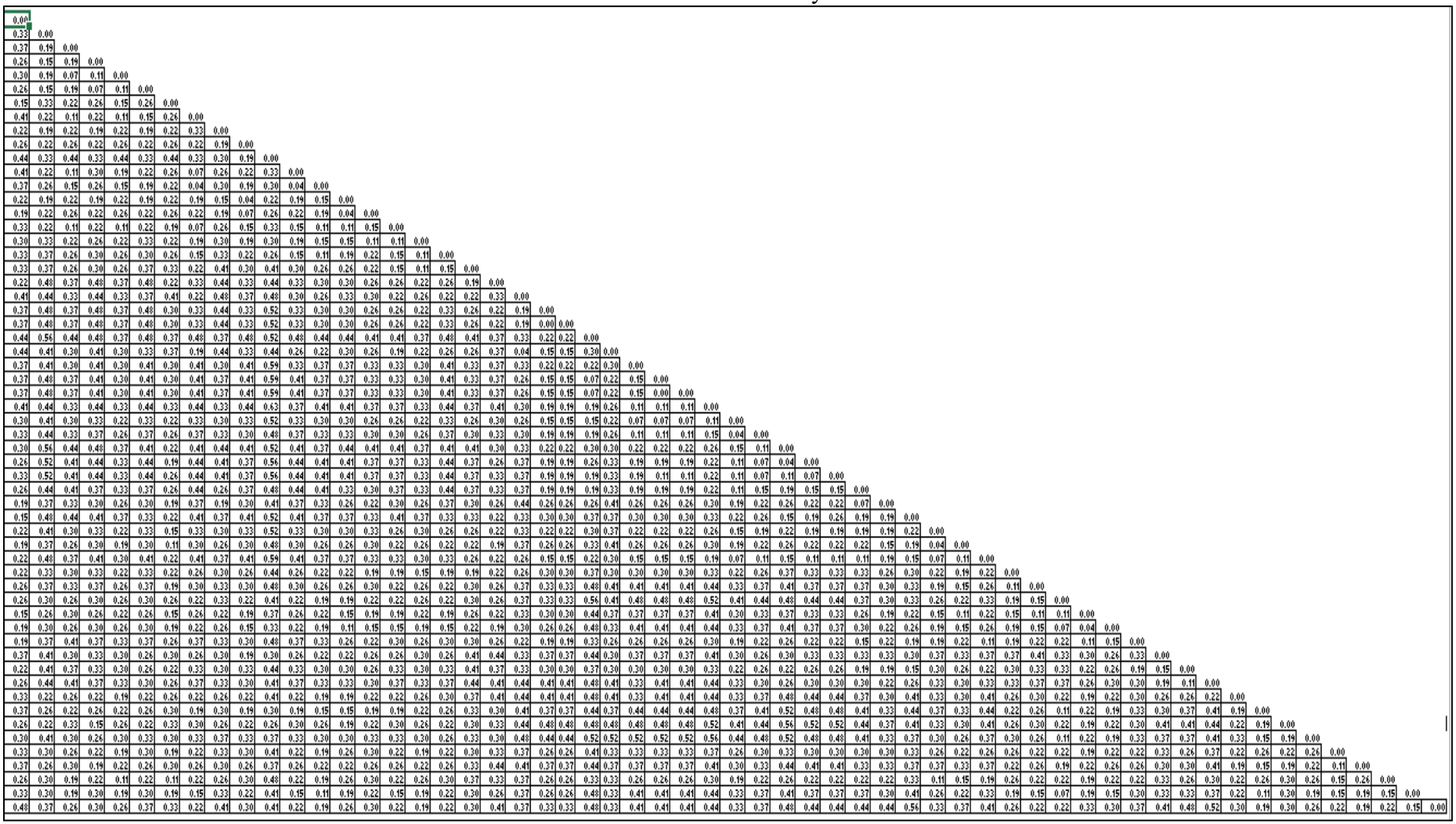
(B)



(C)

Fig.1.(A,B,C): ISSR DNA fragment pattern using primer 841 with some Basil accessions

Table 4:Genetic distance matrix for sixty-two basil accessions



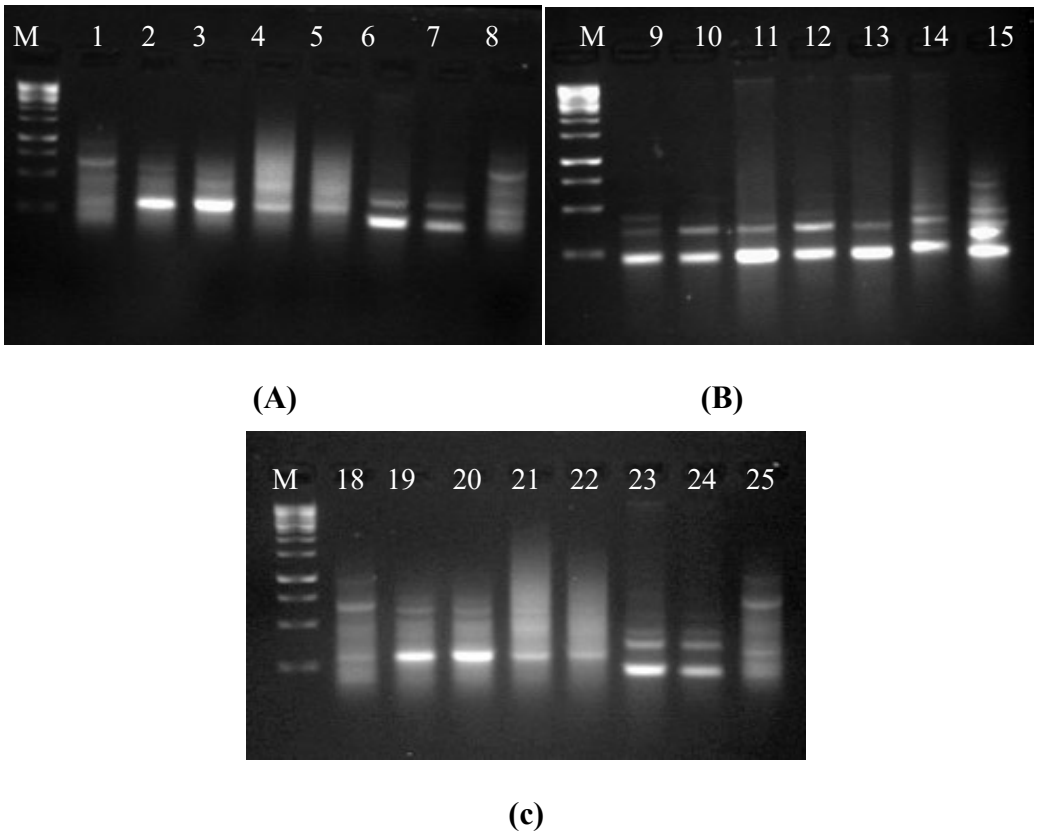


Fig. 2 (A,B,C): ISSR DNA fragment pattern using primer 872 with some Basil accessions

A genetic distance matrix for 62 basil accessions gave values from 0.59 to 0.04. The highest similarity index (0.59) was observed between accessions from Soviet Union and Italy as well as between accessions from Georgia and Italy, while the least similarity index was 0.04, as indicated in Table 4.

In order to obtain the relationships among the 62 basil accessions, a dendogram was constructed using UPGMA clustering algorithm based on ISSR data as shown in Fig. 3. The dendogram showed two main groups. The first group was divided into two clusters, A and B. Cluster A was subdivided into two sub-clusters; sub-cluster one contained accessions (Yemen, Iraq, Rumania, RuBlnd, USA, Soviet Union, Armenia, Kazakhstan, Algeria and Maldives), while sub-cluster two contained accessions (Armenia, Yemen, Sudan-Kennana, Georgia, RuBland, Spain, unknown and Italy). Cluster B contained accessions (China, Kazakhstan, Kirgizstan, CSFR, Sudan- South Darfur, China and USA). Accession no.15 (from Italy) was genetically different from all accessions in group one as it appeared out of the group. The second group clustered the basil accessions into two clusters C and D. Cluster C is divided into two sub-clusters; sub-cluster 1 and sub-cluster 2. Sub-cluster 1 contained accessions (Argintine, USA, Egypt, RuBland and Sudan- Abuharaz),whereas sub-cluster 2 contained basil accessions (Unknown, Japan, Maldives, Cuba, Italy, Togo, Sudan- Silate, Algeria and Thailand). Cluster D is divided into two sub-clusters; sub-cluster 1 contained accessions (Germany, unknown and Italy), whereas sub-cluster 2 contained accessions (Georgia, Italy, Soviet Union, Iraq and Rumania).In Group 2, basil accessions Reunion, Madagascar, and Azerbaijan are shown to be genetically different from all basil accessions within the group. The results showed high genetic

variations among the 62 Basil accessions. Aghaei, et al. 2012 [10] identified the genetic relationships of 50 Iranian basil accessions using ISSR markers and they found relatively acceptable genetic diversity within available basil accessions.

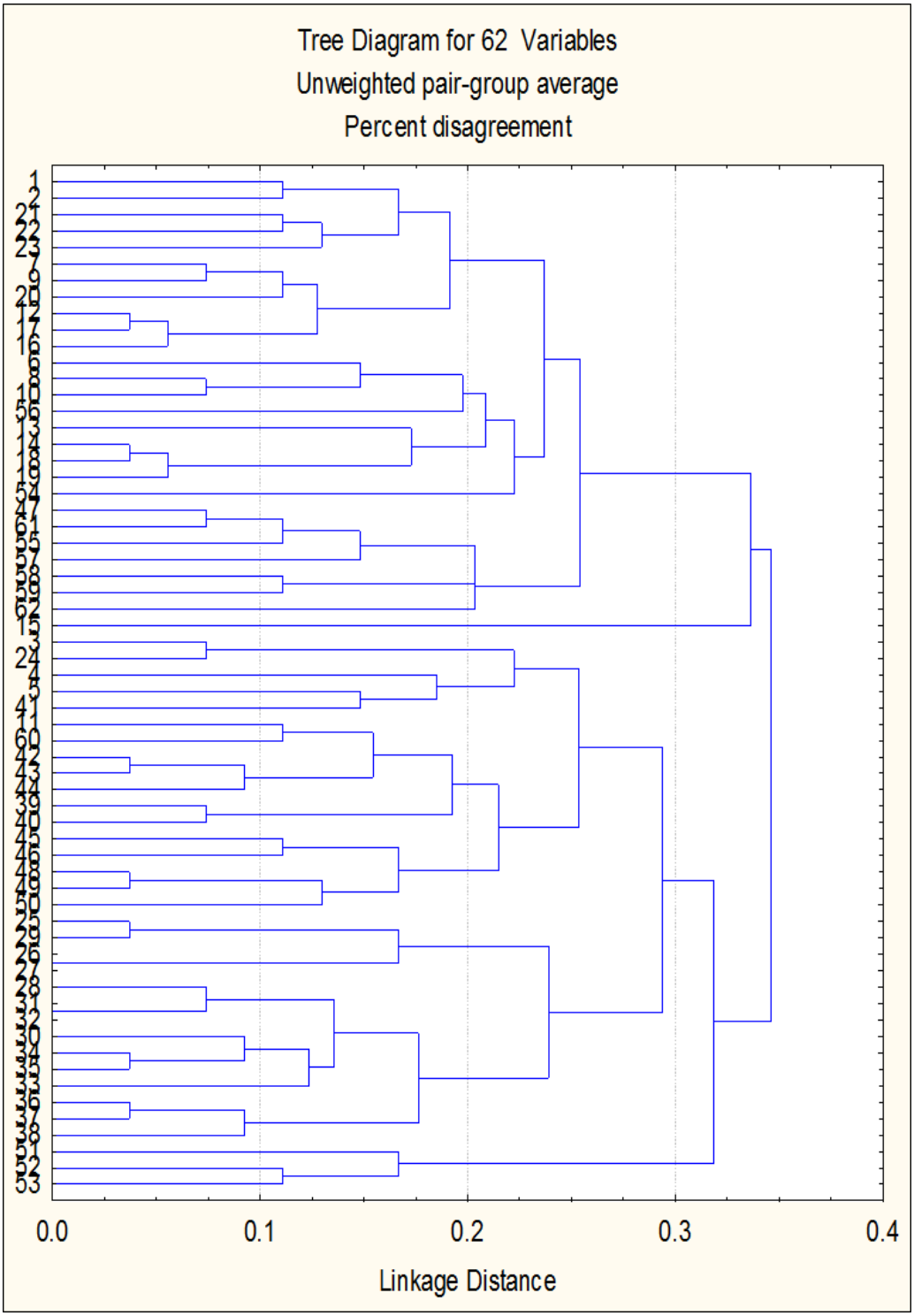


Fig.3. UPGMA- Dendogram results from analysis of four ISSR primers showing the relationship among 62 *Ocimum basilicum*

4. CONCLUSION

ISSR markers are very useful for the study of the genetic diversity among *Ocimum sp,* and this high level of polymorphism can be useful for the maintenance of germplasm banks and the efficient selection of parents for breeding.

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