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Study Genetic Distances Amonge Nine Okra (Abelmoschus esculentus) genotypes UsingTen ISSR markers

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Abstract:

Ten from molecular marker ISSRs (Inter - Simple Sequence Repeats) were used to find genetic diversity, genetic relationship, and DNA fingerprint of nine Okra (Abelmoschus esculentus) genotypes. Primers varied among them in giving unique DNA fingerprints, primers (UBC-809, HB12, and HBS10) gave a unique fingerprint for one genotype of okra, while primers (844A, UBC808) give unique fingerprint for four genotypes. High genetic distance was (0.722) between Egypt and Hasnawia while low genetic distance was (0.074) between Hasnawia and Lahluba. Cluster analysis (Phylogenetic tree) grouped studied genotypes in to two main cluster, the first large one included six genotype(Mosulia , Houseagrl, Khnisiraa ,Hasnawia ,Lahluba and Batra) and other small one includes three genotype (Soutl,Egypt and Zasco seed).ISSR marker could reveal genetic relationship in studied genotypes according to their origin, thus it gave an excellent tool to breeding programs help breeders .

***Keywords**: Okra, ISSR markers, Genetic diversity, Dendrogram, cluster analysis*

Introduction:

A kind of an annually herb, Okra (Abelmoschus esculentus) is a Dicotyledonae, owned by the Malvaceae family. (Islam, 2019). It is a diploid with somatic chromosome numbers 2n widely used for many purposes having many industrial, domestic uses, and also exhibit medicinal properties. Okra is eaten as a food vegetable. It is a healthy vegetable with lots of fibers and folate (Bello et al., 2015 and Fufa, 2019).

The definition of genetic diversity is any variability of a population that could be measure quantitatively, that be inverted the balance between lack genetic variation and mutation (Carvalho et al., 2019).

There are important to project germplasm genetic composition to modern-day crops and compare them with their related species and ancestors. This will provide information's about their phylogenetic relationship and produce a chance to find a new useful gene, because accessions that possess distinct DNA profiles are always satisfying novel alleles with a huge number (Messmer et al., 1992).

Investigation about Genetic diversity help to more check a pedigree of biological development and acclimation power. It participates in the protection and employment of the resources of biology. Moreover, information about genes, singles, type, and communities supply an ever major comprehension of biodiversity so the most adequate strategies for environmental keeping could be development (Bertoni et al., 2010).

A fragment of DNA knows as DNA markers are used to indicating (mutations/variations), help to find out polymorphism between alleles of a gene for a private series of DNA or various genotypes. Such fragments are detected by using

particular molecular technology when it linked with a critical site within the genome (Lateef, 2015).

Farther and farther markers in recent years are used to determined genetic diversity such as Amplified Fragment Length Polymorphism (AFLP) , Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), and DNA amplification finger (DAF), (Ying et al., 2015).

By Zietkiewicz (1994) ISSR marker technique was proposed. Studying the genetic diversity of captive in this manner is widely used. It has utility, such as high polymorphism, simple operation good constancy, and no time-lost. (Yuna et al., 2014 and Huang et al., 2017).

Research on the genetic diversity of plants has widely used this mechanism, for instance, cotton (Wu et al., 2001), ryegrass (Ghariani et al., 2003), and ipomoea (Huang and Sun, 2000). The extent of the SSR sequence 3' or 5' termini is considered fundamental to utilize Microsatellite DNA as a primer, and 2 to 4 indiscriminate nucleotides (Shuldiner et al., 1990).

The information based on DNA marker and their consequence variation would be of huge pleasure in okra cultivation programs facts to limit the genetically identical and morphology analogous plant variety or not (Duzyaman, 2005).

II.Materials and methods

DNA extraction and PCR amplification: nine Okra genotypes with diverse characterization and pedigree were used for DNA extraction **(table.1)**.

Powder dry leaves were used for DNA extraction using I-genomic plant (DNA extraction Mini Kit). Intron Biotechnology/ Korea.

PCR reaction mixture was prepared as follows: 5µl template DNA and5 µl of primer (10 pmole/µl), 5 µl Sterilized deionized distilled water was added to AccuPower® TLA PCR PreMix tubes to the final volume of (20 µl)., ten ISSR markers were examined for fingerprinting genotypes as listed in **table .2**. Amplification was performed in thermocycler programmed according to annealing temperatures produced in **table .3**.

The amplified DNA product was separated by electrophoresis on 1.2 % agarose gels stained with ethidium bromide (3 µl of ethidium bromide solution) electrophoresis runs for 1.5 hr at 70V and then visualized under UV light and photographs.

The presence of a product was identified as (1) and absence was identified as (0). Data were scored for all genotypes, their amplification reproduction, and primers. The result then scrod into NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), Version 1.8 (Applied Biostatistics) program [19].

A Dendrogram design was based on genetic distance: genetic distance (GD) =1-genetic similarity (GS) using the Unweighted Pair-Group Method with Arithmetical Average (UPGMA).

Table (1) Genotypes characteristic as proposed by the Institute of seed examination and certification

No.	Name	Pedigree
1	Batra	Iraq
2	Lahluba	Iraq
3	Hasnauia	Iraq
4	Musiliaa	Iraq
5	Khnisiraa	Iraq
6	Houseagrl seed Okra hybrid F1	Iraq
7	Soutl	Turkish
8	Zasco seeds	Holand
9	Egypt	Egypt

Table (2) Primers name and their sequences which have been used as ISSRs markers.

No.	ISSRs Primers names	Sequence 5' ⟶ 3'
1	844A	5' CTC TCT CTC TCT CTC TGC 3'
2	HB12	5' CAG CAG CAG GC 3'
3	844B	5' CTC TCT CTC TCT CTA 3'
4	17899A	5' CAC ACA CAC ACA AG 3'
5	17889A	5' CAC ACA CAC ACA AC 3'
6	HBS10	5' GAG AGA GAG AGA CC 3'
7	UBC-808	AGA GAG AGA GAG AGA GC
8	UBC-809	AGA GAG AGA GAG AGA GG
9	UBC-811	5'GAGAGAGAGAGAGAGAAC3'
10	UBC814	5'CTCTCTCTCTCTCTCTCTA3'

Table (3): ISSRs primers with their PCR amplification programmes.

ISSRs Primer	Step	Temperature	Time
UBC811 (52 C°) UBC-808 (52 C°) UBC809 (52 C°) 844B (37 C°) UBC-814 (53 C°)	Initial denaturation	94C°	4 min
	No. of Cycles = 40Cycles		
	Denaturation	94C°	1min
	Annealing	variable	1min
	Extension	72C°	1min
	Final extension	72C°	7min
Muhammad *et al*.,2017, Sofalian *et al* .,2008			
ISSRs Primer	**Step**	**Temperature**	**Time**
HB12 844A 17889A 17899A HBS10	Initial denaturation	94C°	5 min
	No. of Cycles = 30Cycles		
	Denaturation	94C°	1min
	Annealing	48C°	1min
	Extension	72C°	1min
	Final extension	72C°	7min
Sofalian *et al*., 2008 and Abou Deif *et al* ., 2013 with slight modification			

III.Results and Discussion

Using Bio drop apparatus the concentration of isolated DNA was 75.92µg/ml with purity 1.9, ISSRs profile results showed variation among studied genotypes through the presence of monomorphic, polymorphic, and unique bands. Primers (844A and UBC808) give a unique fingerprint for four genotypes while Primers HBS10, HB12, and UBC809 gave a unique fingerprint for one genotype, primer UBC811 failed to give a unique fingerprint **Table 4**. The presence of unique bands increased the chance for a primer to give a unique fingerprint (AL.Tamimi, 2014).

Results show that the primer (UBC811) gave the higher value for polymorphism was (75) and primers (HBS10, UBC809) gives the lower value of polymorphism (12.5). Polymorphism increased with an increasing number of polymorphic bands in response to the main band. (Hunter and Gaston, 1988 and Graham and McNichol, 1995). High polymorphic bands of any primer can be used to detect polymorphic markers which will supply hopeful data for consistency and genetic lucidity experiment of crops (Pal and Singh, 2013).

Product of PCR amplification fragment shown in figure (2). Results in the **table (5)** show that the higher molecular size was3902 bp in primer UBC808 and the lower molecular size was 149 in primer UBC809.

Size of amplified fragments related to primer sequence annealed with the DNA template. (Mahpara *et al*., 2012). Any change at a volume of the amplified result could be occurred by Insertions and deletions (Powell *et al*., 1996 and Fadoul *et al*.,2013).

Higher calculate of main bands was 10 in primer UBC808 while the lower was 4 bands in primer UBC811. The higher number of amplified bans was 71 bands in primer UBC809 while the lower number of amplified bans was 21 bands in primer UBC811**.**

Variation in several main and amplified bands are mainly due to primer structure and a large numeral of annealing site for primers determined which is farther benefit rather primers have the lowest numeral of annealing sites. In this status the enumerate of amplified bands will be larger thus awarding the best opportunity for determining DNA polymorphisms through the population (Williams *et al*., 1990 and Tahir, 2014).

Most of the chosen primers in this study were di-nucleotide motif primers, primers of dinucleotide repeat markers revealed maximum amplification and especially 3'anchored di-nucleotide motif primers which gave clear amplified products (Pujor et al, 1999).

Monomorphic bands were seven in primer (UBC809) while in primers (844A, UBC811) were only one band. AlJudy (2004) reported that the genome contains constant identical sequences commonly referred to as a conserved sequence. Monomorphic bands are a type of these sequences, which reveal that genotypes that belong to one species share some genome sequences and differ in others (Russel, et al., 1997; Al-Judy, 2004; AL-Badeiry, 2013 and AL-Tamimi, 2014).

The higher polymorphic bands were six in (17889A) while primers (HBS10, 844B, and UBC809) gave only one polymorphic band. Studies reported that primers with di-nucleotides GA sequence give low polymorphism (Akkaya *et al*.,1992; Singh and Jaiswal,2016), this was correct for primer HBS10 while primer 844B and UBC809 gave low polymorphism, this may result from nucleotide sequence changes by deletion or insertions that may change the level of polymorphism by changing primer annealing sites (Powell *et al*., 1996 and Fadoul *et al*.,2013).

The greater measure of unique bands was two at both primers (HB12, UBC808, and 844A) but primers (844B, 17889A, and HBS10) gave just alone band. The rest (UBC811, 17899A, and UBC809) failed to give an alone band. The existence of like bands refer to that primer familiar in the genome a unique annealing site, so excess possibility of produce a unique plant variety fingerprint (Grewal et al.,2007 ; Vishwanath et al., 2010; Fadoul et al.,2013 and AL-Tamimi ,2014).

Primer (UBC809) gave the lowest value for efficiency (0.0140) in contrast to primer844A which gave a higher value (0.133). Discriminatory value in primers 17889A was the highest (26.086) while primers (HBS10, UBC809, and 844B) gave the lowest value (4.347). Both efficiency and discriminatory value of primer concerned with its ability to give a unique fingerprint. (Newton and Graham , 1997; Arif et al., 2010; AL -Badeiry , 2013 and AL-Tamimi., 2014).

The results in **table (6)** exhibit that the greatest genetic distance was watched at Hasnawia (originate from Iraq) and Egypt (originate from Egypt) genotypes (0.722) while the lowest genetic distance was between Hasnawia (Iraq) and Lahluba (Iraq) genotypes (0.074).

The genealogy of the cultivars may be causing the variation in genetic similarity because some of them have a general ancestry or not, scholars assurance that there is the ability to form collection could be joint to the participation of hereditary materials from one separate universal predecessors; it would elucidate the genetic resemblance, the presence of universal predecessors might affect the resemblance through the crops in the present study. (Morale et al., 2011).

According to the dendrogram produced in **figure (1),** there were two major groups, the first collection contain (Soutl, Egypt and Zasco) while the other collection included six individuals (Mosulia, Houseagrl, Khnisiraa, Hasnawia Lahluba, and Batra).varieties in clusters group according to their origin. Plant verities originating from similar or nearby geographic origins would give a higher level of homogeneously between them so agrees with the thesis of autochthonal origin (Belaj et al., 2001).

AL-Badeiry (2013) agrees with this study that the presence of genetic similarity between certain varieties present in the same location.

Table (4) Okra genotypes fingerprinting (DNA profile) using ten ISSRs primers and their sequences.

No.	Primers	Sequence (5'-3')	Varieties finger printing	No. of varieties fingerprint
1	**844A**	5' CTC TCT CTC TCT CTC TGC 3'	4,7,8,9	4
2	**17889A**	5' CAC ACA CAC ACA AC 3'	3,4,9	3
3	**HB12**	5' CAG CAG CAG GC 3'	9	1
4	**844B**	CTCTCTCTCTCTCTCTCTA	3,4	2
5	**UBC-811**	5'GAGAGAGAGAGAGAGAAC3'	-	0
6	**UBC-808**	AGA GAG AGA GAG AGA GC	1,2,8,9	4
7	**UBC-809**	AGA GAG AGA GAG AGA GG	1	1
8	**HBS10**	5' GAG AGA GAG AGA CC 3'	1	1
9	**17899A**	5' CAC ACA CAC ACA AG 3'	1,2,3	3
10	**UBC814**	5'CTCTCTCTCTCTCTCTCTA3'	No amplification product	

Table (5) Summarized results of ISSRs amplification product include :Amplified bands molecular size range in bp ; No. of : main amplified ,monomorphic , polymorphic and unique bands ; primer polymorphism (%) , efficiency and discriminatory value (%) .

No.	Primers	Amplified bands molecular size in bp	Number of Main bands	Number of Amplified bands	Number of Monomorphic band	Number of Polymorphic band	Number of Unique bands	Primer Polymorphism (%)	Primer Efficiency	Primer Discriminatory Value (%)
1	844A	194- 1139	7	30	1	4	2	57.14	0.133	17.391
2	HB12	169-1862	7	42	3	2	2	28.57	0.0476	8.695
3	844B	232-890	6	39	4	1	1	16.66	0.0256	4.347
4	17899A	175-1221	8	62	5	3	-	37.5	0.0483	13.043
5	17889A	157- 1189	9	46	2	6	1	66.66	0.130	26.086
6	HBS10	167-1985	8	59	6	1	1	12.5	0.0169	4.347
7	UBC808	207-3902	10	69	6	2	2	20	0.0289	8.695
8	UBC809	149-1059	8	71	7	1	-	12.5	0.0140	4.347
9	UBC811	261-772	4	21	1	3	-	75	0.0471	13.043
10										
Total no. of bands			67	439	35	23	9	-	-	-
Average bands per primer			7.44	48.77	3.88	2.55	1	-	-	-

Table (6) The genetic distance

G.	Egypt	Zasco	Soutl	Houseagrl	Khnisiraa	Mosulia	Hasnawia	Lahluba	Batra
Egypt	0.000								
Zasco	0.352	0.000							
Soutl	0.537	0.519	0.000						
Houseagrl	0.704	0.574	0.463	0.000					
Khnisiraa	0.620	0.491	0.491	0.083	0.000				
Mosulia	0.583	0.454	0.454	0.194	0.111	0.000			
Hasnawia	0.722	0.481	0.481	0.315	0.287	0.324	0.000		
Lahluba	0.648	0.407	0.556	0.389	0.361	0.398	0.074	0.000	
Batra	0.602	0.361	0.620	0.398	0.315	0.352	0.194	0.120	0.000

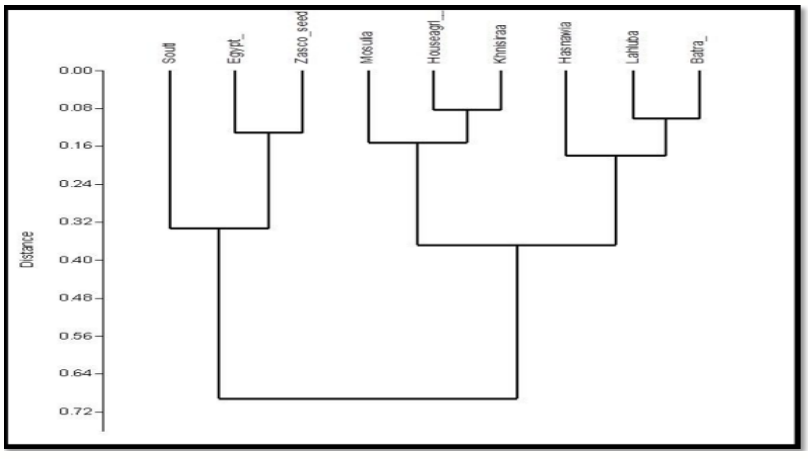
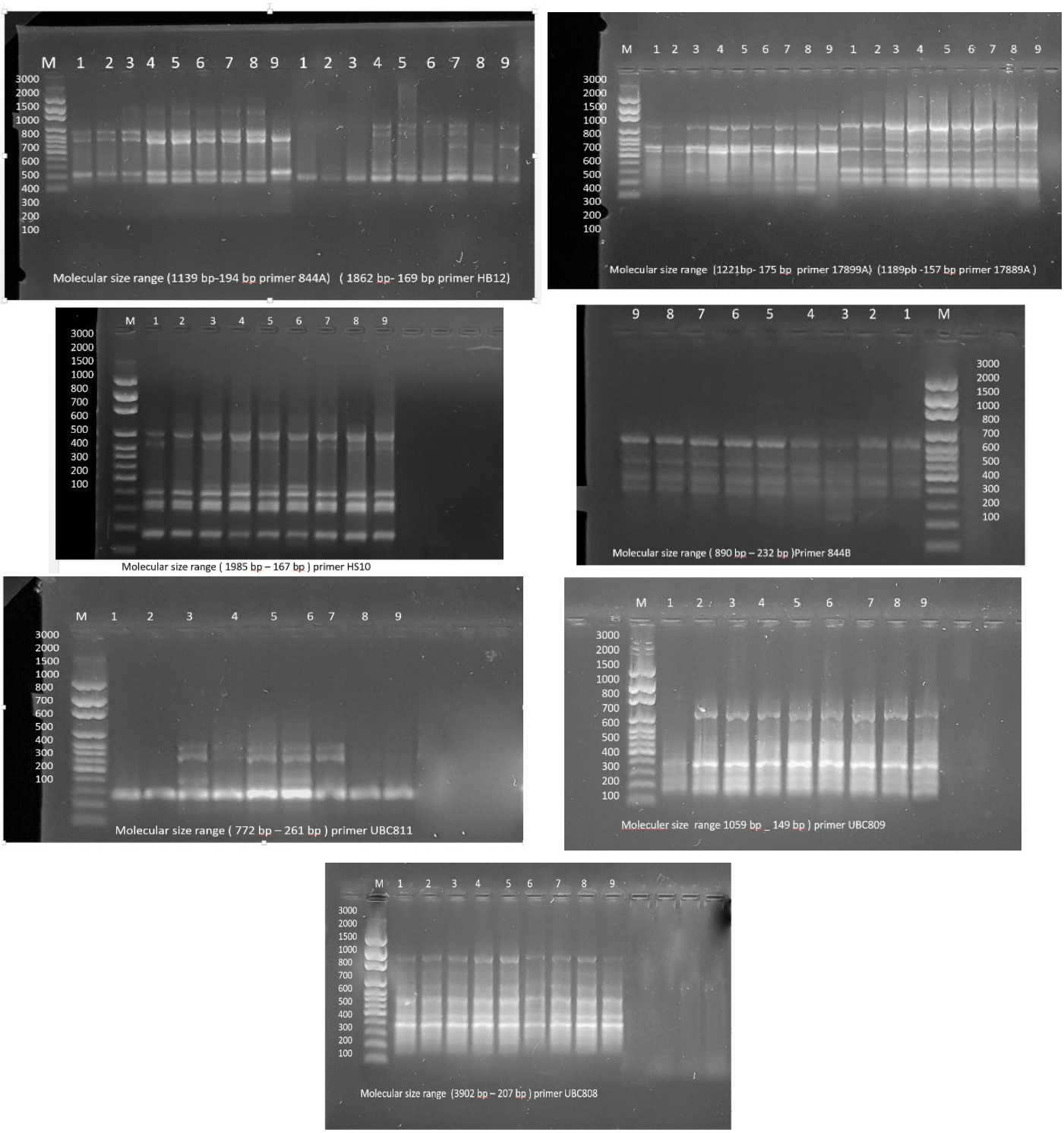


Figure (1) UPGMA dendrogram



Okra genotypes

Figure (2) The electrophoresis profile obtained by ISSR primers, lane M: DNA ladder and lanes (1-9)

References

[1]. Abou-Deif, M. H.; Rashed, M. A.; Sallam, M. A. A.; Mostafa, E. A. H. and Ramadan,W.A.(2013).Characterization of Twenty Wheat Varieties by ISSR Markers. Middle-East Journal of Scientific Research 15 (2): 168-175.

[2]. Akkaya, M.S.; Bhagwat, A.A. and Cregan, P.B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. Genetics, 132: 1131–9.

[3]. Al–Badeiry, Nidhal Abdul Hussein Massan 2013. Molecular and Cytological Studies on Some Zea mays Varieties in Iraq.Ph.D. thesis, University of Kufa, Faculty of Science, Department of Biology, Iraq.

[4]. Al-Judy, N.J. (2004).Detecting of DNA Fingerprints and Genetic Relationship Analysis in Local and Improved Rice (Oryza sativa L.)Varieties in Iraq Using RAPD Markers.Ph.D thesis, College of Science, Baghdad University, pp 166.

[5]. AL-Tamimi, Attyaf, Jameel. Thamir 2014 . Genetic Diversity of Some Tomato Genotypes Using RAPD and SSR markers in Iraq.Ph.D. thesis.Faculty of science.The University of Kufa. p 183.

[6]. Arif, I. A.; Bakir, M. A.; Khan, H. A.; Al-Farhan, A. H.; Al-Homaidan, A. A.; Bahkali, A. H.; AlSadoon, M. and Shobrak, M. (2010).Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment.Genet. Mol. Res., 9 (4): 2191-2198.

[7]. Belaj, A.; Trujillo, R. and Rallo, L. (2001). Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an Olive germplasm bank. J. Amer. Soc. Hort. Sci., 126 (1): 64-71.

[8]. Bertoni B.W., M.P. de C Telles, M.G. Malosso, S.C. Torres, J.O. Pereira, M.V. Lourenço, S. de C França, A.M. Pereira. 2010. Genetic diversity in natural populations of Jacaranda decurrens Cham determined using RAPD and AFLP markers. Genet Mol. Biol. 33(3): 532–538.

[9]. Bello, H.S.; Mustapha, A.; Isa, M.A., and Rahila, T.C. (2015). The in vitro antibacterial activity of okra (Abelmoschus esculentus) against some selected bacteria from Maiduguri, North-Eastern Nigeria. Int J Biosci Nanosci, 2(4):84-88.

[10]. Carvalho, Y.G.S.; Vitorino, L.C.; de Souza, U.J. B., and Bessa, L.A. (2019).Recent Trends in Research on the Genetic Diversity of Plants: Implications for Conservation. Diversity, 11- 62.

[11]. Fadoul, H. E.; El Siddig, M. A.; and El Hussein, A. A. (2013).Assessment of genetic diversity among Sudanese wheat cultivars using RAPD markers. INT J CURR SCI. 6: E 51-57.

[12]. Fufa, N. (2019). Propagation Methods of Okra (Abelmoschus esculentus L.) and its Application Used in Vitro Plant Regeneration. Acta Scientific Agriculture, 3 (10):125-130.

[13]. Ghariani, S.; Trifi-Farah, N.; Chakroun, M.; Marghalin, S. and Arrakchi, M. (2003). Genetic diversity in Tunisian perennial ryegrass revealed by ISSR markers. Genetic Gen Resour Crop Evol 50: 809-815.

[14]. Graham, J. and McNicol, R. J. (1995). An examination of the ability of RAPD markers to determine the relationships within and between Rubus spp. Theo. Appl. Gene., 90: 1128-1132.

[15]. Ghariani, S.; Trifi-Farah, N.; Chakroun, M.; Marghalin, S. and Marrakchi, M. (2003). Genetic diversity in Tunisian perennial ryegrass revealed by ISSR markers.

Genetic Gen Resour Crop Evol 50: 809-815.

[16] Grewal, A.; Kharb, P.; Malik, R.; Jain, S.and. Jain, R. K. (2007).Assessment of genetic diversity among some Indian wheat cultivars using random amplified polymorphic DNA (RAPD) markers .indian Journal of Biotechnology, 6, pp 18-23.
Huang, J.; Liu1, S.; Guo, X.; Zhao, Y.; Li, M.; Zhang, C.; Zhu, J. and Ye, J. (2017). Genetic Diversity and Evolutionary Analysis of Okra (Abelmoschus esculentus (L.) Moench) Germplasm Resources based on ISSR Markers Genetics and Molecular Biology Research. 1 (1):2.
[17]
.
[18] Huang, J.C.and Sun. M. (2000). Genetic diversity and relationships of sweet potato and its wild relatives in Jpomoea series Batatas (Convolvulaceae) as revealed by ISSR and restriction analysis of chloroplast DNA.Theor Appl Genet 100: 1050-1060.

[19] Hunter, P. R. and Gaston, M. A. (1988). Numerical index of the discriminatory ability of Simpson's index of diversity. J. Clin. Mic., 26:2465-2466.
[20] Islam, M.T. (2019). Phytochemical information and pharmacological activities of Okra (Abelmoschus esculentus): A literature-based review. Phytotherapy Research, 33(1(:72-80.
[21] Lateef, D.D. (2015). DNA marker technologies in plants and applications for crop improvements. Journal of Biosciences and Medicines, 3(05) p.7.

[22] Mahpara, S. Farooq, J. Ali, Z.; Petrescu-Mag, I. V.andHussain, F. (2012).Assessment of genetic distance among wheat genotypes through RAPD markers.Advances in Agriculture &Botanics International Journal of the Bioflux Society. Volume 4 (1):31-35.

[23] Messmer, M.M.; Melchinger,A.E.; Boppenmaier,J.; Herrmann,R.G. and Brunklaus-Jung,E. (1992). RFLP analysis of early-maturing Eurpeonmaizgermplsm.Theor.Appl.Genet. 83: 1003-1112.

[24] Muhammad, R.W.; Qayyum, A.; Ahmad1, M.Q.; Hamza, A.; Yousaf, M.; Ahmad, B.; Younas, M.; Malik, W.; Liaqat, S. and Noor, E. (2017). Characterization of maize genotypes for genetic diversity based on inter simple sequence repeats. Genetics and Molecular Research 16 (1).

[25] Newton, C. R. and Graham, A. (1997).Polymerase Chain Reaction.2nd ed. Bios.Scientific Publishers Ltd., Oxford, U.K.
[26] Pal, D. and Singh, M. (2013).Molecular Profiling and RAPD analysis of Commercial Hybrid Parental Lines in Tomato and Chili. IJIRSET, 2(9): 4288- 4292.

Powell, W.; Morgante, M.; Andre C.; Hanafey, M.; Vogel, J.; Tingey, S. and Rafalski, A. (1996).The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers
[27] for germplasm analysis. Mol Breed 2: 225-238.
.
[28] Pujor, S.; Tamhankar, S.A.; Rao, V.S.; Gupta, V.S.; Naik, S. and Ranjekar, P.K. (1999). Arbitrarily primed-PCR based diversity assessment reflects hierarchical groupings of

Indian tetraploid wheat genotypes. Theor. Appl. Genet., 99: 868-876.

[29]. Russell, J.R.; Fuller, J.D.; Macaulay, M.; Hatz, B.; Jahoor, A.; Powell, W. and Waugh, R. (1997).Direct comparison of levels of genetic variation among barley accessions detected by RFLP, AFLPs, SSRs and RAPDs.Theor. Appl. Genet. 95: 714-722.

[30]. Sofalian, O.; Chaparzadeh, N. and Dolati, M.(2008). Genetic diversity in spring wheat landraces from northwest of Iran assessed by ISSR markers. Notul. Bot. Hort. Agric., Cluj-Napoca, 37: 252-256.

[31]. Tahir, N. A. (2014).Genetic Variability Evaluation Among Iraqi Rice (Oryza sativa L) Varieties using RAPD Markers and Protein Profiling. Jordan Journal of Biological Sciences, 7(1): 13 – 18.

[32]. Vishwanath, K.; Prasanna, K. P. R., Pallavi, H. M.; Rajendra P.; Ramegowda, S. and Devaraju, P. J. (2010). Identification of Tomato (Lycopersiconesculentum) Varieties through Total Soluble Seed Proteins Research Journal of Agricultural Sciences, 2(1): 08-12.

[33]. Williams, J. G.; Kubelik, A. R.; Livak, K. J.; Rafaski, J. A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18 (22):6531-6535.

[34]. Wu, Y.T.; Zhang, T.Z. and Yin, J.M. (2001). Genetic diversity of upland Cotton accessions detected by a molecular marker and morphological traits. J Genet Genomics 28:1040-1050.

[35]. Yuan C.Y.; Wang P.; Chen P.; Xiao W. J.; Zhang1 C.; Hu S.; Zhou P.; Chang H.P.; He Z.; Hu R.; Lu X. T.; Ye J.Z. and Guo X. H. (2015). Genetic diversity revealed by morphological traits and ISSR markers in 48 Okras (Abelmoschus escullentus L.) Physiol Mol Biol Plants. 21(3):359–364

[36]. Zietkiewicz, E.; Rafalski, A. and Labuda, D. (1994).Genome fingerprinting by Simple Sequence Repeat (SSR) - anchored polymerase chain reaction amplification. Genomics 20: 176-183.