BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

Molecular characterization of four *Eruca sativa L*. Cultivars using ISSR markers

Russul Abd Al mahde Faisal Al kufee[1] and Attyaf Jameel Thamir[2]
University of Kufa, Faculty of Science,Department of Biology, Iraq

ABSTRACT
The current study was conducted at University of Kufa ,Faculty of Science,Biology Department for molecular identification of four *Eruca sativa L*. cultivars (1-Turish 2-Syrian 3-Turkish 4-Eygyptian) using tenInter Simple Sequence Repeat (ISSR).Results indicates thatprimers 844A , HBS10 , UBC811 , UBC812 , UBC852 and HB12 success in giving all treatments a unique fingerprint.Highest value for molecular size 3238bp in primer HB12,while lowest value for molecular size was 155bp in primer UBC811. Highest polymorphism was 81.81% in primer UBC817. Higher values for main bands,polymorphic bands,primer effiecency and discriminatory value were 19 band, 15 band ,0.14 and 18.75 respectively in primer UBC812. Primer UBC852 produced higest value for both monomorphic bands (10 bands) and amplified bands (54 band) .Monomorphic bands in primers 844A and UBC817 and polymorphism in primer 844A. Highest genetic distance was 0.49886 between1-Turkish and 2-Syrian cultivars,while lowest genetic distance was 0.34435 between 2-Syrian and 4-Egyptian cultivars .Genetic relationship among cultivars illustrates that *Eruca sativa* cultivars arranged among two major custers ,the first small one included only 1-Turkish cultivar,the other large one included 2-Syrian 3- Turkish 4- Egyptian cultivars . These three cultivars ditributed among two subcluster ,the first small one included only 3- Turkish cultivar , while the other large subcluster included 2-Syrian and 4- Egyptian cultivars .In general looke for results,ISSR markers produce an excellent tool in studying genetic diversity and germ plasm identification in E.sativa
Keywords: *E.sativa* **,**ISSR ,phyllogenetic tree,fingerprint

INTRODUCTION

Eruca sativa is commonly known as rocket plant. The local Iraqi name is jarjeer , it is a member of mustard (Brassicaceae) family originated in the Mediterranean region coast, also grown in the Middle-East, South Asia, and all over the world (Jaafar and Jaafar .,2019).The airy tender fresh parts of plant was used as a medicinal remedy for various diseases (Kishore et al.,2017). Jarjeer seeds known to have high oil, protein, and glucosinolate content (El Nagar and Mekawi .,2014).Study of genetic diversity(variation in genes and genotypes) using molecular markers offer numerous advantages overconventional phenotype based alternativesas they are stable and detectable in alltissues regardless of growth, differentiation, development, or defense status of the cell.(Rao and Hodgkin .,2002; Dhutmal et al.,2018) ISSR(inter simple sequence repeat) (Garg and Sharma.,2015) and RAPDS (Randomly amplified polymorphic DNA (Al-Qurainy et al.,2010) are both used to evaluate genetic diversity in *E. sativa* germplasm.Both are simple,inexpensive, need noknowledge of the target sequence, and are easy to apply and in data analysis (Bahadur et al. 2015).Antioxidant activity ,antibacterial and antifungal activity are all related to plant constitutent of bioactive compounds , high performans liquid chromatography (HPLC) ,is a versatile, robust, and widely used technique for the isolation of natural products , it is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify , quantify and purify the individual components of the mixture (Boligon and Athayde., 2014). The selection of genotypes with a high genetic distance in terms of the molecular marker, along with desirable agronomic traits, can be effective in future breeding programs to produce new superior hybrids (Zafar-Pashanezhad et al.,2020) , it's a critical step in plant breeding programes for determining superior hybrid , thus this study aimed to evaluate geneticdiversity among *E. sativa* cultivars, examining their antibacterial,antifungal and antioxidant activity and finally determination of seed oil

constitnuents. ISSRs are DNA fragments of about 100–3000 bp located between adjacent, oppositely oriented microsatellite regions (Kumar *et al*., 2009). ISSR regions lying within the range of microsatellite repeats are highly effective in revealing inter and intra-specific polymorphisms. ISSR markers are effective in evaluating genetic variability of individuals with close relationship (Surendran and Udayan, 2020). ISSR is a simple, fast, and efficient technique that produces amplified products of 200–2000 bp in length. The technique is highly reproducible due to the use of longer primers, which allow for high annealing temperatures (Reddy *et al*., 2002).

MATERIALS AND METHODS

Seeds of four *Eruca sative* Linn L. cultivars (1-Turkish 2-Syrian 3- Turkish 4- Egyptian) were provided from local market ,seeds sowing was conducted at the orchid of agriculture division at the University of Kufa using plastic pots filled with beatmoss to get fresh leaves for DNA extraction and molecular markers application of RAPD markers .Seeds and leaves illustrates in figure (1).

Figure (1) Leaves and seeds of *Eruca sative* cultivars (1-Turkish 2-Syrian 3- Turkish 4- Egyptian

DNA extraction

Fresh seedling leaves were used to take apical fresh leaves for genomic DNA extraction using Genomic DNA Mini Kit provided from Geneaid Biotech.

Primers

The Primers were provided by Bioneer Corporation in lyophilized form, dissolved in TE buffer to obtain 100 pmol/µl as a final concentration (stock solutions). Working solutions 10 pmole/µl were prepared from stock solutions, ten primers were used in application of ISSR markers (Sofalian *et al* .,2008 , Abou-Deif *et al* ., 2013; Singh and Sengar, 2015 and Muhammad *et al*.,2017) in tables (1) with their nucleotide sequences and names of each primer.

Table (1)Primers used as ISSRs markers.

Primer name	Sequence 5' → 3'	Temperature
844A	CTC TCT CTC TCT CTC TGC	48 C°
UBC820	GTGTGTGTGTGTGTGTC	45 C°

UBC816	CAC ACA CAC ACA CAC AT	52 C°
HBS10	GAG AGA GAG AGA CC	48 C°
UBC811	GAGAGAGAGAGAGAGAAC	52 C°
UBC817	CAC ACA CAC ACA CAC AA	52 C°
UBC812	GAG AGA GAG AGA GAG AA	52 C°
UBC852	AGATAGACAGACA	49 C°
17889A	CAC ACA CAC ACA AC	48 C°
HB12	CAG CAG CAG GC	48 C°

PCR content and amplification programe

PCR Pre Mix master mix. Bioneer Corporation USA, (0.2ml) thin-wall 8-strip tubes with attached cup / 96 tubes were used,(*Top* DNA polymerase(1U), (dATP,dCTP,dGTP,dTTP)(Each 250 µM), Reaction Buffer with 1.5 mM Mgcl2(1X) and Stabilizer and tracking dye, 100 bp DNA ladder used.

According to the Experimental Protocol of AccuPower® TLA PCR PreMix(at volume of 5 µl), the PCR reaction mixture was prepared as follows: 5µl template DNA and 5 µl of primer (10 pmole/µl), were added to each AccuPower® TLA PCR Pre Mix tube. Sterilized deionized distilled water was added to AccuPower® TLA PCR PreMix tubes to the final volume of 20 µl.Performing PCR of samples: the amplified of each primer were done according to annealing temperatures and following programe of initial temperature at 94C˚ for 3 min, 40 Cycles of (denaturation at 94C˚ for 1min , annealing :variable , extension at 72 C˚ for 1min and final extension at 72 C˚ for 5min .

Agarose gel electrophoresis

The gel electrophoresis methods were done according to Sambrook and Russel (2001) using 1.2% agarose at 70volt for two hours .

Statistical analysis

The photographs resulted from agarose gel electrophoresis was used to score data, presence of a product was identified as (1) and absence was identified as (0), data then entered into PAST statistic vital program, Version 62.1 (Hammer *et al.,* 2001) and analyzed using SIMQUAL (Similarity for Qualitative Data) routine to generate genetic similarity index (Nei and Li, 1979): GS =2Nij (Ni+Nj). Nij is the number of bands in common between genotypes I and j, and Ni and Nj are the total number of bands observed for genotypes I and j, a dendrogram was constructed based on genetic distance (GD=1-GS) using the Unweighted Pair- Group Method with Arithmetical Average (UPGMA). Polymorphism, primer efficiency, and discriminatory value were calculated for each primer using the following three equations as described by Hunter and Gaston (1988) and by Graham and McNicol (1995).

RESULTS

Determination of concentration of isolated DNA was performed by using Bio drop apparatus. The concentration was 79.81µg/ml with purity 1.9, This accompanied by the locations of bands near wells and their intensity which shows their good quality and high molecular size. The result of electrophoresis of DNA samples using 0.9% agarose gel was shown in Figure (2). (Sambrook and Russell, 2001).

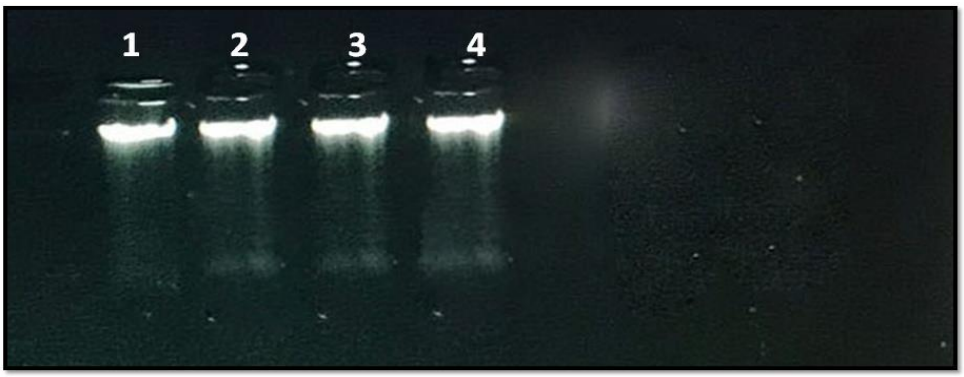


Figure (2) Genomic DNA agarose gel electrophoresis for *Eruca sativa* cultivars 1- Turkish 2-Syrian 3- Turkish 4- Egyptian

No.	Primer	Treatments	No. of fingerprint
1	844A	1,2,3,4	4
2	UBC820	2,3	2
3	UBC816	1,3	2
4	HBS10	1,2,3,4	4
5	UBC811	1,2,3,4	4
6	UBC817	3	1
7	UBC812	1,2,3,4	4
8	UBC852	1,2,3,4	4
9	17889A	3,4	2
10	HB12	1,2,3,4	4

DNA fingerprint detected by ISSR markers

Results in table (2)show thatprimers 844A , HBS10 , UBC811 , UBC812 , UBC852 and HB12 success in giving all treatments a unique fingerprint , while primer UBC817 gave only 3- Turkish cultivar a unique fingerprint .

Total ISSR marker analysis

In table (3), highest value for molecular size 3238bp in primer HB12,while lowest value for molecular size was 155bp in primer UBC811. Highest polymorphism was 81.81% in primer UBC817.Higher values for main bands,polymorphic bands,primer effiecency and discriminatory value were 19 band, 15 band ,0.14 and 18.75 respectively in primer UBC812.

Primer UBC852 produced higest value for both monomorphic bands (10 bands) and amplified bands (54 band) .Other lowest value for studied criteria were main ,amplified and polymorphic bands , efficiency and discriminatory value in primers UBC816 and 17889A.

Table(3) 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 (%)

Monomorphic bands in primers 844A and UBC817 and polymorphism in primer 844A.

Genetic relationships

Results in table (4) showed that highest genetic distance was 0.49886 between1-Turkish and 2-Syrian cultivars ,while lowest genetic distance was 0.34435 between 2-Syrian and 4- Egyptian cultivars .

Cultivars	1-Turkish	2-Syrian	3- Turkish	4- Egyptian
1-Turkish	0			
2-Syrian	0.49886	0		
3- Turkish	0.40745	0.40726	0	
4- Egyptian	0.36095	0.34435	0.36095	0

Primers	Molecular size	Main bands	Amplified bands	Monomorphic band	Polymorphic band	Polymorphism (%)	Efficiency	Discriminatory Value (%)
844A	1241-177	12	28	2	10	33.33	0.35	12.5
UBC820	1219-352	7	19	3	4	57.14	0.21	5
UBC816	1170-344	5	16	3	2	40	0.12	2.5
HBS10	1319-243	10	22	3	7	70	0.31	8.75
UBC811	1640-155	15	38	6	9	60	0.23	11.25
UBC817	1268-259	11	27	2	9	81.81	0.33	11.25
UBC812	1776-179	19	36	4	15	78.94	0.41	18.75
UBC852	2685-226	18	54	10	8	44.44	0.14	10
17889A	800-221	5	16	3	2	40	0.12	2.5
HB12	3238-178	18	40	4	14	77.77	0.35	17.5
Total					80			

Table (4) The genetic distance values among *Eruca sativa* cultivars 1-Turkish 2-Syrian 3-Turkish 4- Egyptian using ISSR markers.

Phylogenetic tree

Genetic relationship among cultivars in figure (3) illustrates that *Eruca sativa* cultivars arranged among two major custers ,the first small one included only 1-Turkish cultivar,the other large one included 2-Syrian 3- Turkish 4- Egyptian cultivars . These three cultivars ditributed among two subcluster ,the first small one included only 3- Turkish cultivar , while the other large subcluster included 2-Syrian and 4- Egyptian cultivars .

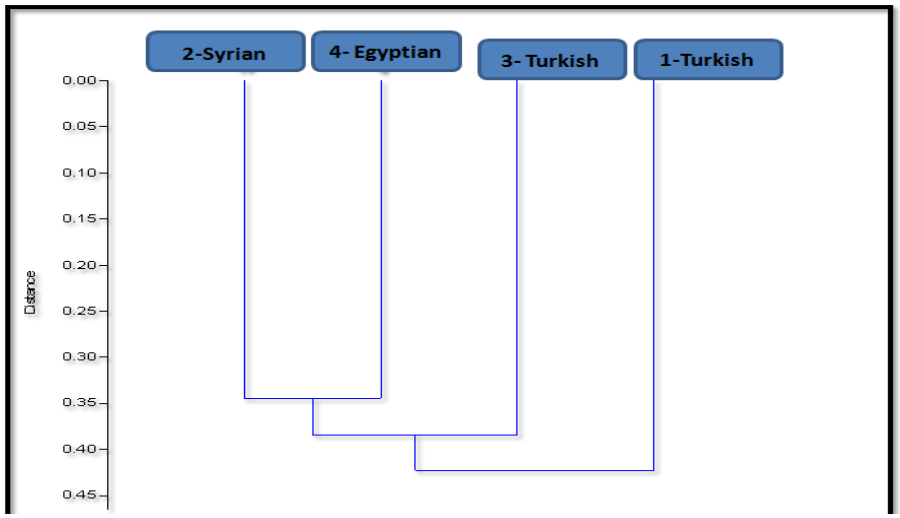


Figure (3) UPGMA dendrogram illustrating the trees of genetic relationship between *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian using ISSR markers.

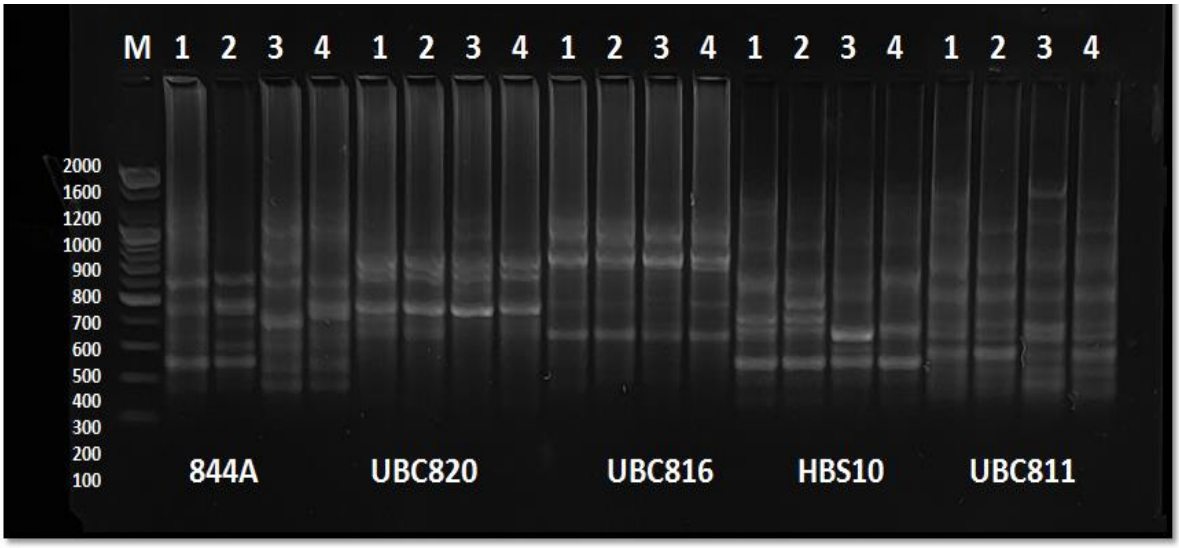
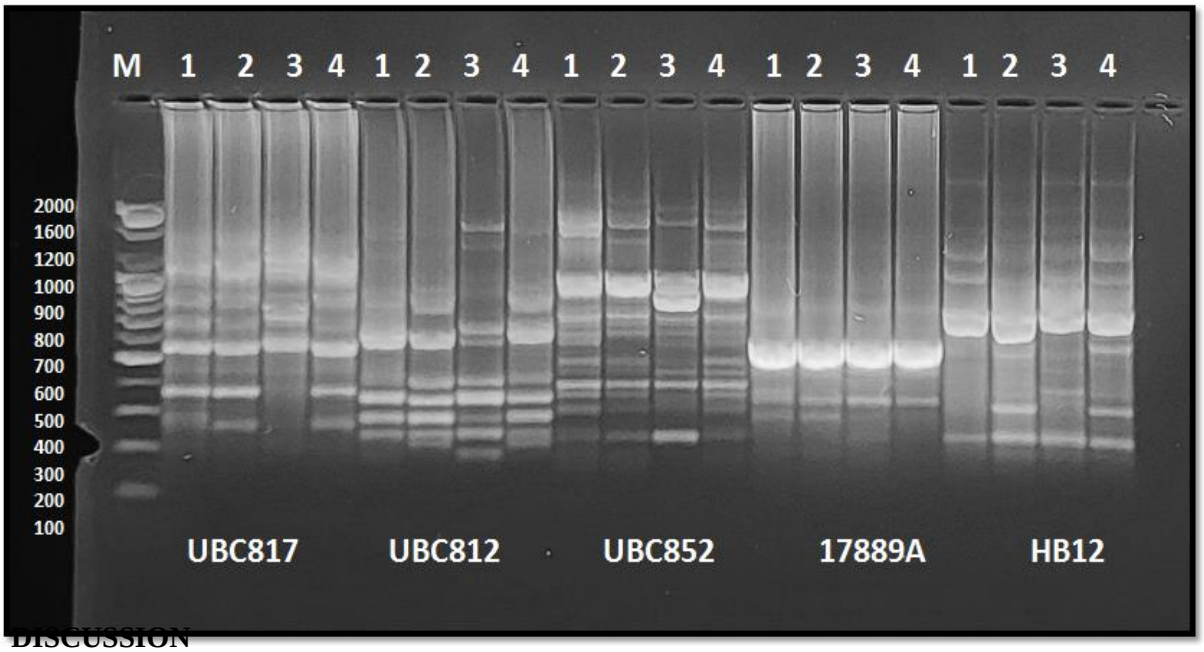


Figure (4) Amplification product of primers 844A ,UBC820, UBC816, HBS10 and UBC811 , M: DNA ladder , *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian



DISCUSSION

Figure (5) Amplification product of primers UBC817 ,UBC812 ,UBC852 , 17889A and HB12 , M: DNA ladder , *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian

Polymorphism increased with increasing number of polymorphic bands (Hunter and Gaston, 1988 ;Graham and McNichol, 1995), and increases chance of producing unique fingerprint. Primer which produces high polymorphic bands can be further used as polymorphic marker which will prove promising in identification and genetic purity testing of crops (Pal and Singh, 2013).Studies confirmed that GA sequence showed stable amplification and rich polymorphism (Liu *et al*., 2010;Yousefi*et al*.,2015)as in primers HDS10,UBC811 and UBC812. Polymorphism level increased by using tri- HB12 nucleotide sequence as confirmed by Pujor *et al*, (1999).The higher molecular size and lower molecular size of primers related to primer sequence annealed with DNA template. (Mahpara*et al*., 2012).Insertions and deletions could change the size of the amplified product by changing distance between annealing sites of primers (Powell *et al*., 1996;Fadoul*et al*.,2013). The higher number of main bands and amplified are mainly due to primer structure and that some primers recognize a high number of annealing site, which is more useful than primers recognizing lower number of annealing sites. In this case the number of amplified bands will be higher, thus giving a better chance for detecting DNA polymorphisms among individuals (Williams *et al*., 1990;Tahir, 2014).Appearance of unique unique fingerprint refer to the presence unique bands which related to primer ability to recognized a unique annealing site in genome , (Grewal*et al*.,2007;Vishwanath *et al*., 2010; Fadoul*et al*.,2013;AL-Tamimi,2014). Monomorphic bands are 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 and AL-Badeiry, 2013; AL-Tamimi, 2014).Both efficiency and discriminatory value of primer concerned with its ability to give uniquefingerprint .(Arif *et al*., 2010 ;AL-Tamimi,2014 and –Al Ghufaili and Al-Tamimi,2017),this was clearly observed in primers HB12,UBC811,844A,HBS10,UBC812 and UBC852. Variation in genetic similarity may be attributed to the genealogy of the cultivars, because some of them have a common parent or not, authors

emphasized that there is possibility of forming groups could be linked to the sharing of genetic material from one distant common ancestor; it would explain the genetic similarity, the presence of common ancestors might have influenced the similarity among the cultivars in the present study. (Morale *et al*., 2011).The evolution leading to adaptation to different agroecological conditions maymutate the SSR sequences ,which lead to differences in the ISSR amplification pattern result in diverse phyllogenetic relationships Regardless of different geographical locations of the place of release of varieties and different ploidy levels.(Singh and Jaiswal.,2016).

REFERENCES

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.

Al–Badeiry , N. A.M.(2013).Molecular and Cytological Studies on Some Zea mays Varieties in Iraq .Phd thesis, University of Kufa ,Faculty of Science ,Department of Biology , Iraq.

AL- Ghufaili,M.H.; Al-Tamimi,A.J.T.(2017). Genetic Relationship amongSome Wheat Genotypes Using Ten ISSR Markers, IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN:2278-3008, p-ISSN:2319-7676, 12(3): 30-36.

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.

AL-Tamimi, A. J.T. (2014). Genetic Diversity of Some Tomato Genotypes Using RAPD and SSR markers in Iraq .PhD thesis .Faculity of science.University of kufa. p 183.

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.

Al-Qurainy, F.; Alameri , A.A. and Khan, S..(2010).RAPD profile for the assessment of genotoxicity on a medicinal plant; Eruca sativa . journal of Medicinal Plants Research Vol. 4(7), pp. 579-586, 4 April, 2010

Bahadur . B; Rajam . M. V; Sahijram . L and Krishnamurthy . K.V .(2015) . Plant Biology and Biotechnology . Volume II: Plant Genomics and Biotechnology. https://www.springer.com/gp/book

Boligon , A. A. and Athayde, M. L. .(2014).Importance of HPLC in Analysis of Plants Extracts,Austin Chromatography- 1 (3): 1-2

Dhutmal, R. R.; Mundhe, A. G. and Mor, A. W..(2018)..Molecular Marker Techniques: A Review,nt.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 816-82

El -Nagar ,M.M and Mekawi, E.M.(2014). Comparison of different genotypes of rocket (Eruca sativa) in terms of chemical compounds extracted from seeds and in the callus induced from tissue culture. Int J Appl Res Rev 2014;3:1074-82

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.

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. J , 90: 1128-1132.

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.

Garg , G. and Sharma, V ..(2015).Assessment of fatty acid content and genetic diversity in Eruca sativa (L.) (Taramira) using ISSR markers., Biomass and Bioenergy,67, 118-129

Hunter, P. R. and Gaston, M. A. (1988). Numerical index of discriminatory ability of simpson's index of diversity. J. Clin. Mic., 26:2465-2466.

Hammer, O.; Harper,D.A.T and Ryan,P.D.(2001). PAST: Palaeontological Statistics software package for education and data analysis. Paleontologia Eletronica 4(1):1-9.

Jaafar, N.S . and Jaafar, I. S. (2019). Eruca sativa linn.: pharmacognostical and pharmacological properties and pharmaceutical preparations.Asian J Pharm Clin Res, Vol 12, Issue 3, 2019, 39-45.

Kumar, P.; Gupta, V.K.; Misra, A.K.; Modi, D.R. and Pandey, B.K. (2009). Potential of molecular markers in plant biotechnology, Plant omics, 2(4):141.
Kishore, L. ; Kaur , N. ; Kajal , A. and Singh, R. (2017). Extraction,characterization and evaluation of Eruca sativa against Streptozotocin-induced diabetic nephropathy in rat. Bangladesh J Pharm ;12:216-27.
Liu, D.; X.He; G.Liu and B.Huang (2010). Genetic diversity and phylogenetic relationship of Tadehagi in southeast China evaluated by intersimple sequence repeat (ISSR). Genet.Resour. Crop Evol. 58: 679-687.
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 on the basis of inter simple sequence repeats. Genetics and Molecular Research J. 16 ,(1): gmr16019438.
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.
Morale,G.F.;Resende, J. T.V.; Faria, M.V.; Andrade, M. C. ; Resende, L.V.; Delatorre, C. A. and Da Silva, P.R..(2011).Genetic similarity among strawberry cultivars assessed by RAPD and ISSR markers. Sci. Agric., 68(6):665-670.
Nei, M. and Li, W. H. (1979). Mathematical modern for studying genetic variation in terms of restriction endonuclease. Pro. Nat. Acad. Sci., 74: 5269-5273.
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.
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.
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 for germplasm analysis. Mol Breed 2: 225-238Reddy, M.P.; Sarla, N. and Siddiq, E.A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Kluwer Academic Publishers, Printed in the Netherlands. Euphytica, 128:9–17.
Rao, V. R. and Hodgkin, T.(2002).Genetic diversity and conservation and utilization of plant genetic resources, Plant Cell, Tissue and Organ Culture 68: 1–19, 2002.
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
Sambrook, J. and Russell, D. W. (2001). In vitro application of DNA by the polymerase chain Reaction, in molecular cloning. A laboratory manual. 3rd ed., Cold Spring Harbor Laboratory Press, New York. Chapter 8: 691-733.
Singh, A. and Sengar, R.S. (2015). DNA Fingerprinting Based Decoding of Indica Rice (*Oryza sativa* L) Via Molecular Marker (SSR, ISSR, & RAPD) in Aerobic Condition. Adv. Crop. Sci. Tech., 3(2): 1-8.
Singh, P. and Jaiswal J.P. (2016).Assessing genetic diversity in bread wheat using inter simple sequence repeat (ISSR) markers. Asian Journal ofn bio science.11 (1):141-14
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.
Surendran, S. and Udayan, P.S. (2020). Evaluation of genetic variability between yellow vein mosaic disease resistant gamma-irradiated okra mutant and parent, Current Botany,11: 38-42
Tahir, N. A. (2014).Genetic Variability Evaluation A mong Iraqi Rice (Oryza sativa L) Varieties using RAPD Markers and Protein Profiling. Jordan Journal of Biological Sciences, 7(1): 13 – 18

Vishwanath, K.; Prasanna, K. P. R., Pallvi, 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.

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.

Yousefi, V.; Najaphy, A.; Zebarjadi, A. and Safari, H. (2015).Molecular characterization of Thymus species using ISSR markers. Journal of Animal & Plant Sciences, 25(4): 1087-1094

Zafar-Pashanezhad , M.; Shahbazi, E.; Golkar, P. and Shiran, B..(2020).Genetic variation of *Eruca sativa* L. genotypes revealed by agro-morphological traits and ISSR molecular markers , industrial crop and product 145, 111992.