RESEARCH ARTICLE
Published 31th Desember 2023
https://doi.org/10.21070/nabatia.v11i2.1630

Molecular Characteristics Study of The Plants of Two Species Form The Genus Eruca Mill of The Brassicaceae Family, Which are Growing in Iraq

Ghassan Radam Idan Al.Taie
Department of Biology, College of Education for Pure Science, University of Diyala, Diyala-Iraq

Abstract. *The Internal Transcribed Spacer ITS method was used to diagnose the two species (Eruca sativa Mill. And Eruca vesicaria L.) These plants belong to the Eruca Mill genus from the Brassicaceae family. This study was conducted during the spring season of the year (2022-2023), as all samples were taken from different regions in Iraq, after being classified from the Iraqi flora. (E. sativa Mill and E. vesicaria L) of the Brassicaceae family to find out the degree of relatedness between the two species. of wildly growing plants in iraq, as the Deoxyribonucleic acid DNA bundles were extracted from agarose gel and the process of finding the sequence of nitrogenous bases was performed. Eruca two species. E. sativa Mill. And E. vesicaria L. which belong to the Brassicaceae family. With a percentage of 100% and 99.83% concordance. This method is considered one of the accurate methods in the diagnosis of genus at the level of the one species and between two species in addition to its ease to compare and and differentiate between the two species.*

Keywords: *Eruca Sativa Mill and Eruca Vesicaria L., Brassicaceae Family, Sequence Of Nitrogenous Bases, Molecular (gen ITS).*

I. INTRODUCTION

Eruca sativa with other species types such as *Eruca vesicaria* are an herb vegetables related to a family of the Brassicaceae as in [1]. This is due to the environment of Mediterranean region, but also widely grown all over the world. Both types considered as a medicinal plant [2]. *Eruca sativa* , which is also called in the second name of watercress "salad," is leafy salad type which that an eatable Brassicaceae family. Glucosinolates in addition to other important phytochemicals such as flavanols, vitamins and minerals which demonstrated to be beneficial for human health [3]. Through examination and chemical analysis, the ethanolic extract from the leaves of the *Eruca sativa* plant has the potential to treat hyperuricemia and its associated diseases [4]. Watercress is an important industrial crop with the ability to grow in difficult climatic conditions and on lands with poor fertility [5].

Light-emitting diode lamps provide a calibration among the conditions of lighting versus it's quality/quantity for the in artificial growth environments. Hence, photoperiod extension facilitating accurate resource management and crop performance *Eruca vesicaria* [6]. Numerous scientific studies demonstrate how plants react differently to different light levels, even when different species are exposed to the same light conditions [7].
Oilseed crop Taramira *Eruca sativa* L. has a wide range of industrial and medicinal uses [8]**.** Details about the functional characteristics, phytochemical composition, and antioxidant activity of underutilized plants found in their leaves, including figl *Raphanus sativus* L. and girgir *Eruca sativa* L. . It is important as a good source of phytochemicals [9].

Against the tested strains, the antimicrobial activities of all the tested oils varied, *Eruca* oils were the most active oils [10]. Antibiotic-resistant Gram-positive (Staphylococcus aureus and Bacillus subtilis) as well as Gram-negative (*Escherichia coli*, *Pseudomoms aeruginosa*, and *Shigella flexneri*) bacteria are shown to be susceptible to the antimicrobial effects of *Eruca sativa* seed oil. These seed oils are full of nutrients and phytochemicals, but because of the possible health risks linked to erucic acid, their high content may make it unsuitable for use in food applications. [11].

The study's findings showed that *E. sativa* essential oil is more resistant to self-oxidation, of higher quality, has a longer shelf life, and may be used as cooking or salad oil. and has antimicrobial properties against seven different kinds of harmful bacteria to humans. It is also edible. demonstrated antibiotic resistance [12].

study indicated that marinating meat with fresh watercress leaves and ethanolic extract [13]. The relationship between genetic diversity, population structure, and the use of morphological traits is related to changing some quantitative traits [14]**.**

This crop is of great economic importance due to the oil content of its seeds, as well as some other parts of the plant, such as the edible leaves, which fodder is one of its utilizations. Humans have extracted oil due to the privilege of its seeds, which is named locally as Taramira. Cruciferae family plants mostly depend on insect pollination, the number and quantity of seeds can be increased through pollination [15] Honey bees have a role in pollinating Brassicaceae plants.

It has been observed that *E. sativa* attracts a large number of insect pollinators, and this may be attributed to the difference in the amount of nectar and its chemical properties, as well as the number of flowers and pollen grains [16]. In the future, possible antifungal, cancer, and insect resistance medicines may be developed using EsNap of the *E. sativa* species [17].

The chloroplast genome has been considered an "ultra-barcode" for species recognition [18]. the chloroplast genome is regarded as a "ultra-barcode" for species identification. For evaluating genome evolution and the phylogenetic relationships of complex angiosperm families, the chloroplast (cp) genome is a perfect model [19]. Plasmodimes are ideal molecular markers for species identification and phylogenetic research because they are haploid, maternally inherited, have a highly conserved structure, low mutation rates, and a slow evolutionary pace [20].

The great change that took place in the science of classification began as a result of the development that occurred

in the fields of molecular biology, and when specialists in the science of molecular classification provided very important information through their analysis of the data of the Deoxyribonucleic acid DNA sequence [21]. I currently use molecular data to draw genetic tree for organisms of all kinds and without specific groups that can be easily distinguished [22].

The study of genetic and molecular variation can benefit from the use of DNA or molecular markers [23]. Genetic relatedness and genetic distance between the distributions of *E. sativa* cultivars are unrelated to their geographical origin. RAPD markers are an effective tool in studying *E. sativa* [24]. stated that molecular DNA indicators help to draw family tree, understand genetic structure and evolution, as well as infer genetic variations, no matter how slight, between species genera one, especially DNA indicators based on the polymerase chain reaction (PCR) enzyme, such as DNA barcoding. It is a rapidly developing newer method that helps to diagnose species using fixed DNA sequences [25].

The DNA barcoding method relies on the amplification of primers, as small standard DNA sequences are amplified that are used to identify species by repossession of small DNA sequences, which A barcode is called from a standard part of the genome of samples to be studied [26]. The barcode gene method is specialized in terms of its application to plants and animals, as the mitochondrial gene Cytochrome oxidase and ITS are used on animals, and the mitochondrial genes rbcL and matk are used on plants. Depending on the primer used, different Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD PCR) reactions produced different results [27]. The genetic diversity of E. sativa was determined by combining sequence-related amplified polymorphism (SRAP) and start codon targeted polymorphism (SCoT), which may be helpful in enhancing population genetics and producing superior hybrids in E. sativa. However, no discernible similarities were found between the genotypes' geographic origin and the patterns related regarding the molecular markers [28].

E. sativa is a valuable resource for enhancing other Brassica species since, according to phylogenetic studies, it is phylogenetically similar to the significant Brassica species [29]. enhanced knowledge of the genetic variety within the Eruca plant gene pool, which will aid in identifying the genetic basis for plant and leaf features in future genomes, identifying sources of variation, and selecting the finest cultivars for cultivated species breeding programs [30]. For each crop development initiative, genetic diversity is essential for identifying elite genotypes [31]. confirmed that the common bands indicate similarity in the genetic material of that region of the genome for studied genotype, It may be represent similarity in genetic characteristics, anatomical traits, or similarity in environmental requirements. The used primer identified the genetic variations required for comparison between the studied species, which can be used as molecular genetic indicators.

II. METHODS

Specimens Collection: This study was conducted at the Molecular Genetics Labssoratory of the College of Education for Pure Sciences / University of Diyala – Iraq. plant specimens were collected from different areas in Iraq, with a total of 40 specimens, 10 samples for each species. The in Soft samples were placed in nylon bags and kept in the refrigerator and carried over to the lab so that DNA could be extracted.

DNA Extraction: The DNA Promiga kit, produced by the Korean company Macrogen, was used to extract DNA from fresh plant leaf samples, which were then stored at 4°C. Spectrophotometry was then used to estimate the amount and quality of extracted DNA. The precise estimation of DNA content is achieved by the application of the spectrophotometric approach. Given that DNA has a maximum UV absorption spectrum at 260 nm, the concentration of DNA may be found by measuring the optical density (OD) in a spectrophotometer at this wavelength.

ITS gene amplification: The primer used amplification for ITS gene was ITS -f (5'- TCCGTAGGTGAACCTGCGG-3') and ITS R (5'- TCCTCCGCTTATTGATATGC -3') [32]. The PCR mixture contains 5 µL of overall master mix, 10 µL of the forward and revers primers, 1.5 µL of DNA, and 16.5 µL of deionized water, totaling 25 µL. The thermopolymer programming consists of five minutes of initial denaturation at 95°C, thirty seconds of denaturation at 35 cycles, thirty seconds of annealing at 55°C, forty seconds of extension at 72°C, and seven minutes of final extension at 72°C. The PCR results were examined using a UV lamp in an LG2020 Gel Documentation System, electrophoresed, examined on a 1% agarose gel, and stained with 0.5µl ethidium bromide.

For direct sequencing, ten PCR product samples of each kind were shipped to the Macrogene firm in South Korea.

Sequencing of ITS gene: Using BioEditing Software version 7, the ITS gene's nucleotide sequence was compared to samples from the two species Eruca sativa and Eruca vesicaria. Sequences that were similar were combined into a single haplotype and translated through sequencing.

III. RESULT AND DISCUSSIONS

The resulting data were analyzed from the DNA segments, and all of them contained single bundles, and there were no cases of genetic polymorphism, the bundles that appeared in the gel, and the information obtained from the amplification of the DNA segments was recorded by PCR technology for two species *E. sativa* and *E. vesicaria* used with ITS.

3.1. Analysis of genetic relationships and measurement of genetic distances.

Genetic relationships were analyzed and genetic distances measured by calculating the data obtained from the analysis of the locations of DNA segments, which were projected onto specific sites of the gel after electrophoresis. Genetic distance (GD) was estimated. Each species is shown based on the data matrix using Genius PC software by Macrogen Corporatio via email https: www.geneious.com for the purpose of performing cluster analysis to draw a tree diagram of the two species of the genus *Eruca* using the ITS gene.

The species *E. sativa and E. vesicaria* belonging to the genus *Eruca* under study showed one bundle at 500bp base pair and all bundles were of the single type (Figure 1) .

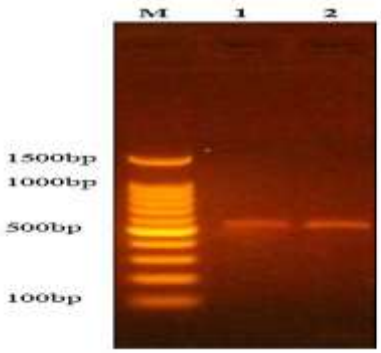

Figure 1. Electrophoresis of DNA amplification results extracted from Eruca L. using primers especially ITS. M represents the molecular size index (bp100), meaning the ladder marker. (1.5 acarose) and 500 represents the PCR product.

3.2. Cluster Analysis

Dendrogram genetic analysis tree was designed according to the method of [33] and showed the genetic distance.

The genetic analysis tree of the species *E. sativa* contained two main groups, the first group branched into two sub-groups, and the species falls within the first sub-group Figure 2.

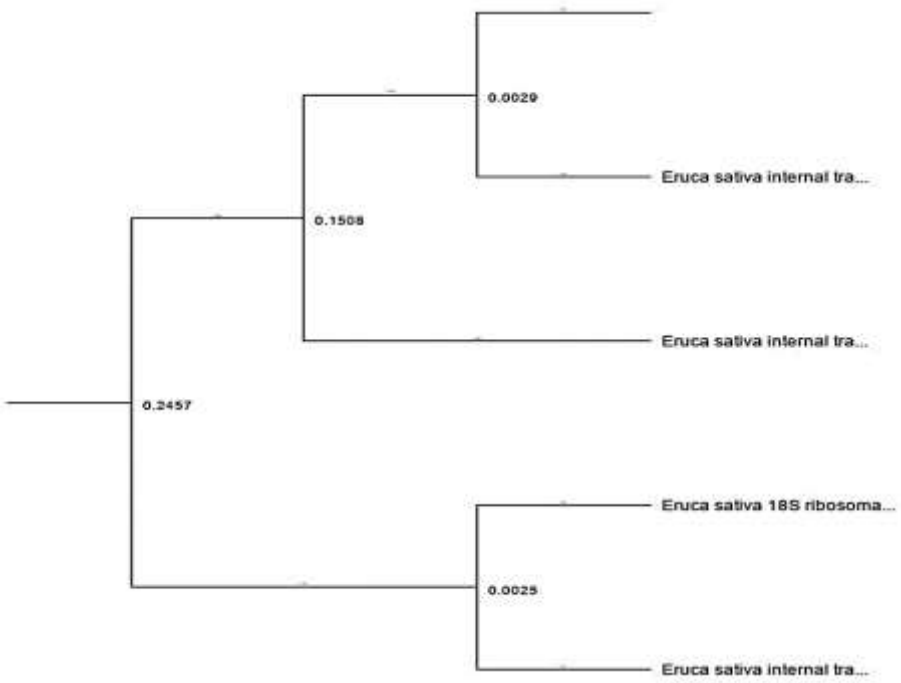

Figure 2. Cluster analysis chart (kinship tree) for *E. sativa*

The percentage of match was this species *E. sativa* with the samples in the Gen Bank, as the similarity rate was about 100%, and the ID code AY254536 in the NCBI existing sample *E. sativa*, as the length of the gene was 670 subunits of ribosomes (S5.8), the classification code in the Gen Bank 29727 [22], Figure 3.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Eruca sativa 18S ribosomal RNA, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete s	1083	1083	100%	0.0	100.00%	AY254536.1
Eruca vesicaria subsp. sativa genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: YB018	1059	1059	100%	0.0	99.15%	LC090305.1
Eruca sativa internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1035	1035	100%	0.0	98.30%	DQ249821.1
Eruca vesicaria subsp. sativa voucher EDNA15-0042413 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence;	520	520	48%	1e-148	99.65%	KX282139.1
Eruca vesicaria subsp. sativa voucher EDNA15-0042404 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence;	512	512	48%	2e-146	98.94%	KX282137.1
Eruca vesicaria subsp. sativa voucher EDNA15-0043142 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence;	496	496	46%	2e-141	99.26%	KX282136.1
Eruca vesicaria subsp. sativa voucher 6540281206060032 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence;	484	484	44%	5e-138	100.00%	KF454470.1
Eruca vesicaria subsp. sativa voucher 65230276 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S	484	484	44%	5e-138	100.00%	KF454469.1
Eruca sativa internal transcribed spacer 1, complete sequence	431	431	42%	6e-122	97.98%	AF039896.1
Eruca vesicaria subsp. sativa voucher CCDB-24915-F07 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence;	416	416	38%	2e-117	100.00%	MG234605.1
Eruca vesicaria subsp. sativa voucher EDNA15-0042410 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence;	344	344	40%	8e-96	89.08%	KX282138.1
Eruca sativa internal transcribed spacer 2, complete sequence	311	311	29%	8e-86	99.42%	AF040237.1

The genetic analysis tree of the species *E. vesicaria* contained a main group, the first group branched into two sub-groups, and the species falls within the first sub-group, Figure 4 .

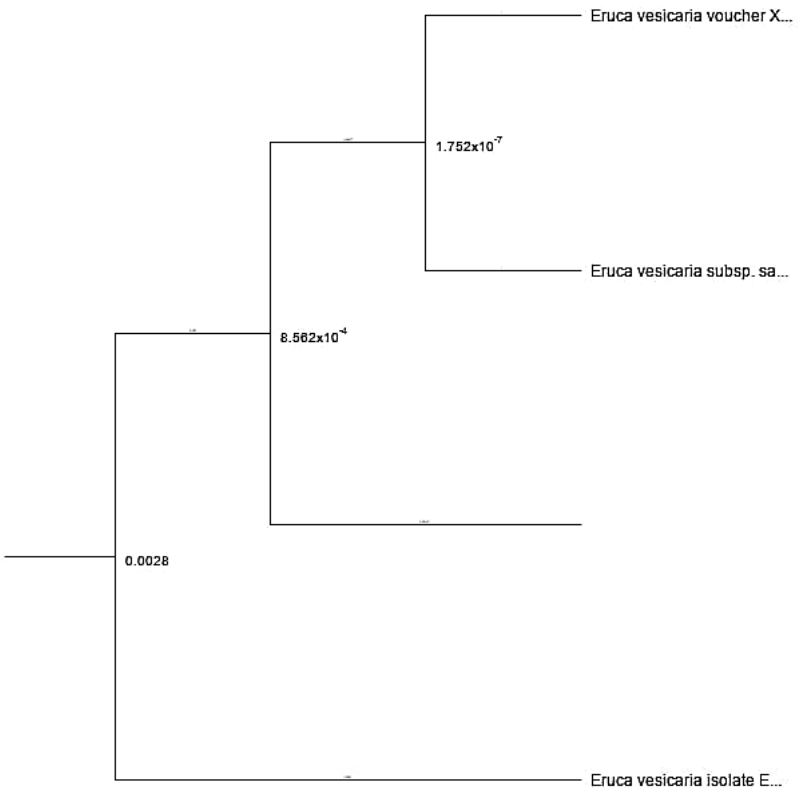

Figure 4. Cluster analysis chart (kinship tree) for *E. vesicaria*.

The percentage of congruence in this type E. vesicaria with the samples in the Gen Bank was 99.83%. The ID KX824487 in the Gen Bank was for a sample between the type E. vesicaria, as its length was 586 and ribosome units S5.8, classification code 180536 in the Gen Bank [34],Figure 5.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Eruca vesicaria voucher XFMa-Bra35 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribe	1077	1077	100%	0.0	99.83%	KX824487.1
Eruca vesicaria subsp. sativa genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: YB018	1070	1070	100%	0.0	99.49%	LC090095.1
Eruca vesicaria isolate Eruves01_15_01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr	1061	1061	100%	0.0	99.32%	MF192769.1
Eruca sativa 18S ribosomal RNA, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete seq	1055	1055	100%	0.0	99.15%	AY254536.1
Eruca pinnatifida isolate Erupinn01_15_01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and interna	1050	1050	100%	0.0	98.98%	MF192765.1
Moricandia foleyi voucher UPM-GERM-5549 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1046	1046	100%	0.0	98.81%	EF601903.1
Eruca foleyi isolate Mfy01_15_01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri	1038	1038	100%	0.0	98.63%	MF192769.1
Eruca sativa internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1029	1029	100%	0.0	98.13%	DQ249821.1
Eruca vesicaria isolate SW78 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed space	1013	1013	95%	0.0	99.46%	AY722459.1
Diplotaxis acris voucher KSUFS397 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transc	966	966	100%	0.0	98.42%	KF850565.1
Diplotaxis tenuifolia genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: YB019	933	933	100%	0.0	95.40%	LC090096.1
Diplotaxis tenuifolia isolate DT small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete s	933	933	100%	0.0	95.40%	MW606071.1
Diplotaxis tenuifolia 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), clone Dte-ITS-1	933	933	100%	0.0	95.40%	AM905721.1
Diplotaxis tenuifolia voucher UPM-GERM-6666 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequen	933	933	100%	0.0	95.40%	EF601913.1
Brassica repanda subsp. tenuisiliqua clone 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and interna	929	929	100%	0.0	95.23%	JQ042821.1

Figure 5. represents the species similarity with the species in the *E. vesicaria* gene bank.

We notice a difference in the nitrogenous bases, one mutation occurred for the sample *E. vesicaria* at site 46, as the nitrogenous base A was replaced with the two universes G, as it was observed there is a difference in nitrogenous bases [35], we note that the reason for the occurrence of mutations is the result of chemical changes and factors environment as shown in, Figure 6.

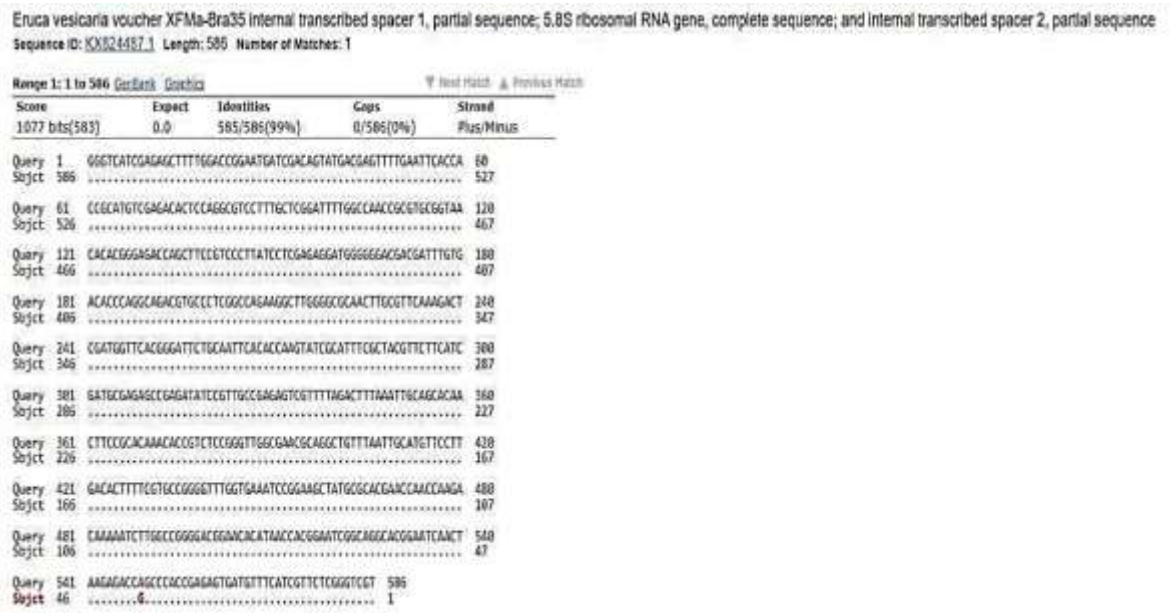

Figure 6. shows the data obtained from the NCBI database and the genetic mutations of *E. vesicaria.*

The current study has contributed to giving important results in diagnosing the genus *E. sativa*, according to what was shown in the data bases. The percentage of conformity of this type, *E. sativa*, with the samples in the Gen Bank was 100%, as ID AY254536 was in the Gen Bank, as the length of the gene was 670 rRNA units equal to S5.8, and no difference was observed in the nitrogenous bases. As for the species *E. vesicaria*, information was entered to identify the species *E. vesicaria* in the database. The result showed that the sequence of nitrogenous bases belonged to the species *E. vesicaria* by knowing the sequence of nitrogenous bases in the NCBI site, and the result was as shown in Figure 6.

IV. CONCLUSION

The result showed that the sequence of nitrogenous bases belongs to the type *E. vesicaria*, and with a percentage of 99.83, we note the occurrence of a genetic mutation in this type.
Therefore, molecular techniques that allow comparison of the nitrogenous base sequences of the genus *Eruca*. Unknown with known genera within a database is one of the most accurate and easy methods than the traditional methods, as this method is based on the amplification of the ITS region within the DNA to diagnose species at the genus level.

REFERENCE

[1] M. Sharifi-Rad *et al.*, "Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases," *Frontiers in Physiology*, vol. 11. 2020, doi: 10.3389/fphys.2020.00694.
[2] A. U. Khan *et al.*, "Production of organic fertilizers from rocket seed (Eruca sativa l.), chicken peat and moringa oleifera leaves for growing linseed under water deficit stress," *Sustain.*, vol. 13, no. 1, 2021, doi: 10.3390/su13010059.
[3] M. Puranik, "Towards the development of an Eruca sativa crop with improved nutritional and flavour qualities by investigating the relationship between phytochemical and sensory attributes of breeding lines

cultivated in different environments and identification of molecular markers for sugars ," University of Reading, UK, 2022.

[4] A. F. Teixeira, J. de Souza, D. D. Dophine, J. D. de Souza Filho, and D. A. Saúde-Guimarães, "Chemical Analysis of Eruca sativa Ethanolic Extract and Its Effects on Hyperuricaemia," *Molecules*, vol. 27, no. 5, 2022, doi: 10.3390/molecules27051506.

[5] E. Bakhshandeh, H. Pirdashti, F. Vahabinia, and M. Gholamhossieni, "Quantification of the Effect of Environmental Factors on Seed Germination and Seedling Growth of Eruca (Eruca sativa) Using Mathematical Models," *J. Plant Growth Regul.*, vol. 39, no. 1, 2020, doi: 10.1007/s00344-019-09974-1.

[6] S. Proietti, S. Moscatello, F. Riccio, P. Downey, and A. Battistelli, "Continuous Lighting Promotes Plant Growth, Light Conversion Efficiency, and Nutritional Quality of Eruca vesicaria (L.) Cav. in Controlled Environment With Minor Effects Due to Light Quality," *Front. Plant Sci.*, vol. 12, 2021, doi: 10.3389/fpls.2021.730119.

[7] R. Paradiso and S. Proietti, "Light-Quality Manipulation to Control Plant Growth and Photomorphogenesis in Greenhouse Horticulture: The State of the Art and the Opportunities of Modern LED Systems," *Journal of Plant Growth Regulation*, vol. 41, no. 2. 2022, doi: 10.1007/s00344-021-10337-y.

[8] M. Zafar-Pashanezhad, E. Shahbazi, P. Golkar, and B. Shiran, "Genetic variation of Eruca sativa L. genotypes revealed by agro-morphological traits and ISSR molecular markers," *Ind. Crops Prod.*, vol. 145, 2020, doi: 10.1016/j.indcrop.2019.111992.

[9] E. O. Keyata, Y. B. Tola, G. Bultosa, and S. F. Forsido, "Phytochemical contents, antioxidant activity and functional properties of Raphanus sativus L, Eruca sativa L. and Hibiscus sabdariffa L. growing in Ethiopia," *Heliyon*, vol. 7, no. 1, 2021, doi: 10.1016/j.heliyon.2021.e05939.

[10] R. H. Bassyouni, Z. Kamel, A. A. Algameel, G. Ismail, and S. N. Gaber, "In-vitro determination of antimicrobial activities of Eruca sativa seed oil against antibiotic-resistant gram-negative clinical isolates from neonates: a future prospect," *BMC Complement. Med. Ther.*, vol. 22, no. 1, 2022, doi: 10.1186/s12906-022-03710-1.

[11] M. Rozan and E. Boriy, "Chemical composition, phytochemical Profile, antioxidant activity of Eruca sativa seeds, and utilization of defatted seeds in the production of functional biscuits," *Egypt. J. Food Sci.*, vol. 0, no. 0, 2022, doi: 10.21608/ejfs.2022.131392.1130.

[12] A. Rayes Kamel, A. Selim, S. Mohammed, and D. N. A. L. Al Sayed Abdel Ghaffar, "Chemical, Nutritional Analysis, Quality of Essential Oil of Eruca sativa and It ̇s Potential Antimicrobial Activity," *J. Specif. Educ. Stud. Res.*, vol. 8, no. 4, pp. 827–857, 2022.

[13] S. Bouacidaa *et al.*, "Effect of marination with Eruca vesicaria longirostris leaves on Turkey meat properties during storage and consumer acceptance," *J New Sci*, vol. 12, no. 1, pp. 253–264, 2020.

[14] P. Golkar and V. Nourbakhsh, "Analysis of genetic diversity and population structure in Nigella sativa L. using agronomic traits and molecular markers (SRAP and SCoT)," *Ind. Crops Prod.*, vol. 130, 2019, doi: 10.1016/j.indcrop.2018.12.074.

[15] M. Shakeel, M. Inayatullah, and H. Ali, "Checklist of insect pollinators and their relative abundance on two canola (Brassica napus) cultivars in Peshawar, Pakistan," *J. Entomol. Zool. Stud. JEZS*, vol. 326, no. 36, 2015.

[16] M. Shakeel *et al.*, "Insect pollinators diversity and abundance in Eruca sativa Mill. (Arugula) and Brassica rapa L. (Field mustard) crops," *Saudi J. Biol. Sci.*, vol. 26, no. 7, 2019, doi: 10.1016/j.sjbs.2018.08.012.

[17] B. Khaliq *et al.*, "Eruca sativa seed napin structural insights and thorough functional characterization," *Sci. Rep.*, vol. 11, no. 1, 2021, doi: 10.1038/s41598-021-02174-6.

[18] C. Y. Zhang *et al.*, "Comparative analyses of the chloroplast genomes of patchouli plants and their relatives in Pogostemon (Lamiaceae)," *Plants*, vol. 9, no. 11, 2020, doi: 10.3390/plants9111497.

[19] X. Du *et al.*, "The complete chloroplast genome sequence of yellow mustard (Sinapis alba L.) and its phylogenetic relationship to other Brassicaceae species," *Gene*, vol. 731, 2020, doi: 10.1016/j.gene.2020.144340.

[20] C. L. Henriquez *et al.*, "Molecular evolution of chloroplast genomes in Monsteroideae (Araceae)," *Planta*, vol. 251, no. 3, 2020, doi: 10.1007/s00425-020-03365-7.

[21] W. K. Taia, "Modern Trends in Plant Taxonomy," *Asian J. Plant Sci.*, vol. 4, no. 2, 2005, doi: 10.3923/ajps.2005.184.206.

[22] R. L. Hong, L. Hamaguchi, M. A. Busch, and D. Weigel, "Regulatory elements of the floral homeotic gene AGAMOUS identified by phylogenetic footprinting and shadowing," *Plant Cell*, vol. 15, no. 6, 2003, doi:

10.1105/tpc.009548.
[23] I. Khan, Z. K. Shinwari, N. B. Zahra, S. A. Jan, S. Shinwari, and S. Najeebullah, "DNA barcoding and molecular systematics of selected species of family Acanthaceae," *Pakistan J. Bot.*, vol. 52, no. 1, 2020, doi: 10.30848/PJB2020-1(27).
[24] I. S. Masad, A. Al-Fahoum, and I. Abu-Qasmieh, "Automated measurements of lumbar lordosis in T2-MR images using decision tree classifier and morphological image processing," *Eng. Sci. Technol. an Int. J.*, vol. 22, no. 4, pp. 1027–1034, Aug. 2019, doi: 10.1016/j.jestch.2019.03.002.
[25] P. D. N. Hebert, S. Ratnasingham, and J. R. DeWaard, "Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species," *Proc. R. Soc. B Biol. Sci.*, vol. 270, no. SUPPL. 1, 2003, doi: 10.1098/rsbl.2003.0025.
[26] S. Anvarkhah, M. Khajeh-Hosseini, M. H. Rashed-Mohassel, and A. D. E. . Panah, "Identification of three species of genus Allium using DNA barcoding. ," *Int. J. Agric. Crop Sci.*, vol. 5, no. 11, p. 1195, 2013.
[27] I. K. K. Al-Qaisi, "The uranium dimension in the strains of maize and its cross hybrids based on the indicators of the RAPD," 2014.
[28] P. Golkar and M. A. Bakhtiari, "Evaluation of genetic diversity in the world collection of Eruca sativa L. using oil content, fatty acids and molecular markers," *Ind. Crops Prod.*, vol. 148, 2020, doi: 10.1016/j.indcrop.2020.112280.
[29] B. Zhu, F. Qian, Y. Hou, W. Yang, M. Cai, and X. Wu, "Complete chloroplast genome features and phylogenetic analysis of Eruca sativa (Brassicaceae)," *PLoS One*, vol. 16, no. 3 March, 2021, doi: 10.1371/journal.pone.0248556.
[30] C. Guijarro-Real *et al.*, "Large scale phenotyping and molecular analysis in a germplasm collection of rocket salad (Eruca vesicaria) reveal a differentiation of the gene pool by geographical origin," *Euphytica*, vol. 216, no. 3, 2020, doi: 10.1007/s10681-020-02586-x.
[31] N. Bibi, F. M. Abbasi, M. A. Rabbani, and S. A. Jan, "ASSESSMENT OF GENETIC VARIATION AMONG DIVERSE TARAMIRA (ERUCA SATIVA MILL.) GERMPLASM BASED ON MOLECULAR MARKERS," *Pakistan J. Bot.*, vol. 54, no. 2, 2022, doi: 10.30848/PJB2022-2(5).
[32] M A Innis, D H Gelfand, J J Sninsky, and T J White., *PCR protocols-a guide to methods and applications*. Academic Press, London , 1990.
[33] M. Nei and W. H. Li, "Mathematical model for studying genetic variation in terms of restriction endonucleases," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 76, no. 10, 1979, doi: 10.1073/pnas.76.10.5269.
[34] C. Qian *et al.*, "Population dynamics of Agriophyllum squarrosum, a pioneer annual plant endemic to mobile sand dunes, in response to global climate change," *Sci. Rep.*, vol. 6, 2016, doi: 10.1038/srep26613.
[35] M. D. Shakara, *Genetics*, First edition. Amman - Jordan: Amman, Dar Al Masirah for Publishing and Distribution, 1999.

***Conflict of Interest Statement**:The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*