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Molecular Similarities and Differences Associated with Determining the Six Species from Asteraceae Family.

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Abstract: Six species from the Asteraceae family were studied using the nuclear region: ITS. This method was used to discover the molecular similarities and differences associated with determining the genetic structure. As a result of DNA amplification and sequencing, the ITS ribosomal analysis showed genetic relationships between the species, which were distributed across several clades. A member of the Asteraceae family was studied for the first time at the molecular level in Iraq, its molecular identification was confirmed, and it was registered in the NCPI as a new species in Iraq, specifically in Baghdad Governorate.

Keywords: molecular study, Asteraceae family, DNA , Aster family.

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Introduction

Molecular studies are considered among the most important studies for species identification because they are more accurate and comprehensive in the process of separation and differentiation. The development of modern scientific instruments and life techniques represented by the invention of the scanning electron microscope (S.E.M) and the transmission electron microscope (T.E.M), as well as the precision in studying the karyotype, played an effective role in advancing studies and solving many complex problems. [1]

The Asteraceae family is one of the largest families of flowering plants with a global distribution in terms of species and economic importance. It consists of more than 1,600 genera and over 25,000 species, belonging to 17 tribes and three subfamilies. Most members of the Asteraceae family are shrubs, herbaceous plants, subshrubs, vines, or rarely trees. The family has been divided into several groups and tribes, differing among researchers and their study locations [2,3,14] were the first to organize the members of the Asteraceae family into tribes and several subtribes based on morphological traits such as stems, leaves, flowers, and other characteristics.

The ITS region is a powerful tool for studying the Asteraceae family, as it aids in evolutionary analysis, species identification, and conservation efforts. These studies contribute to a deeper understanding of the evolutionary dynamics and biodiversity of this diverse plant family. For further reading, the provided references are excellent starting points for exploring the application of ITS in Asteraceae research. The internal transcribed spacer (ITS) region in ribosomal DNA is a common molecular marker in plant classifications, especially for studies involving the Asteraceae family. ITS sequences are useful for evolutionary analysis, species identification, and understanding evolutionary relationships. Here is an overview of the use of ITS in molecular studies of the Asteraceae family and for understanding evolutionary relationships. ITS sequences are widely used to clarify the evolutionary relationships between genera and species within the Asteraceae family. Studies often reveal previously unrecognized relationships and clarify taxonomic ambiguities.[4].

Similarly, species identification can help ITS distinguish between closely related species, especially in complex groups where morphological traits may overlap. It is particularly useful for identifying ambiguous species.

The ITS region provides insights into the evolutionary history of the family, including patterns of diversity and hybridization events.[5].

ITS data can benefit conservation strategies by assessing genetic diversity within endangered species groups of the Asteraceae family.[6]

Among all nuclear regions, ITS is one of the most widely used DNA segments in molecular classification of plants at the genus and species levels due to its high potential accuracy for relationships between species and genera. [7,8,9] .

Materials and methods

The study was conducted in the graduate laboratories of the College of Science, Department of Biology Sciences at the University of Baghdad, with the assistance of the Al-Amin Center in Najaf Governorate, and DNA sequencing was performed at the Macrogen Center in South Korea. The species under study were: *Pseudognaphalium luteoalbum, Senecio vulgaris, Glebionis coronaria, Cotula australis ,symphyotrichum subulatum and Erigeron Canadensis*

Primers

The primers were diluted with the addition of (ddH2o) water and according to the attached leaflet from the US provider (IDT) [Table1], the required concentration was prepared by taking 20 µl of the primer solution and completing the volume to 100 µl by adding di-distilled water to make it ready for use. The best ice pack is to take 20 microliters and dissolve it in 80 microliters of double-distilled water andreturn the box containing the primers to the freezer, which is kept in arefrigerator.

DNA extraction

Here, reference is made to the kit used for extraction, and reference is made to following the manufacturer's recommendations for the purpose of extraction.

Polymerase chain reaction (PCR)

The polymerase chain reaction technique was used to amplify the (ITS 1) region using the ITSA and ITSB primers provided by the American company (IDT), where the reaction the program as following: denaturation 94c, Annealing 54c, Extension72c, Final extension72c, and number of 30 cycle.

Analysis of agarose gel electrophoresis:

Gel electrophoresis methods were performed according to [10, 15].

Knowledge of sequencing is a useful tool for resolving ambiguities and overlaps, finding evolutionary relationships between related species, and completing the sequence. The analysis program (UPGAM) has been used to study the relationships between some species of the composite family that were placed under study.

Results and discussion

Phylogenetic analysis relies on one of the most important indicators used by researchers to clarify the historical and evolutionary relationships between organisms[11]. Recently, there has been a notable increase in research focusing on finding evolutionary relationships in plants of the Asteraceae family. To obtain the phylogenetic tree that illustrates the proximity and divergence relationships between plants, and to complete the analysis, the gene and region must be identified. Therefore, the ribosomal ITS region must be determined.[12, 16].

DNA extraction

DNA was extracted from five leaves of different species belonging to various strains of the Asteraceae family, which were collected during the study period only. The electrophoresis results showed the efficiency of the extraction method using the Genomic DNA Mini Kit.

Amplification of the ITS region by polymerase chain reaction:

The results showed the amplification of DNA extracted from leaves of some species and genera of the Asteraceae family using the primer pair ITS A, ITS B to amplify the ITS region, the nitrogenous base sequence of the target area. The amplification results showed a polymerase product of 800 base pairs in length for all species, with a similar genetic pattern as in Figure(1).

Figure(1):Polymerization products using the ITS starter pair of (6) genuse of Asteraseae family. M - Ladder with a weight of 100 base pairs - (base pair = bp).

ITS region sequence analysis in the studied species:

The sequence in the ribosomal ITS region was studied and a highly supportive genetic tree was obtained. The analysis was performed using the UPGAM program. A genetic tree consisting of two main branches was obtained. The first contains the two species *pseudognaphalium luteoalbum* and *senecio vulgaris* with a high degree of data reliability and is considered a sister to the second branch, while the species *glebionis coronaria* was isolated as a single branch alone and a sister to the first branch. The second branch in turn contains the two species *symphyotrichum subulatum* and erigeron Canadensis and their data were highly reliable. The secondary branch was isolated and is considered a sister to the first and second branches, which is *cotula australis*, which was recorded in the ncpi, making it the first record of this species in Iraq. Figure (2 , 3) .This is consistent with[12, 13].

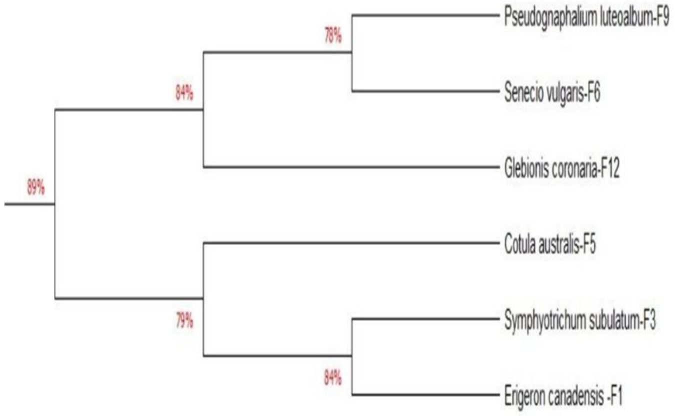
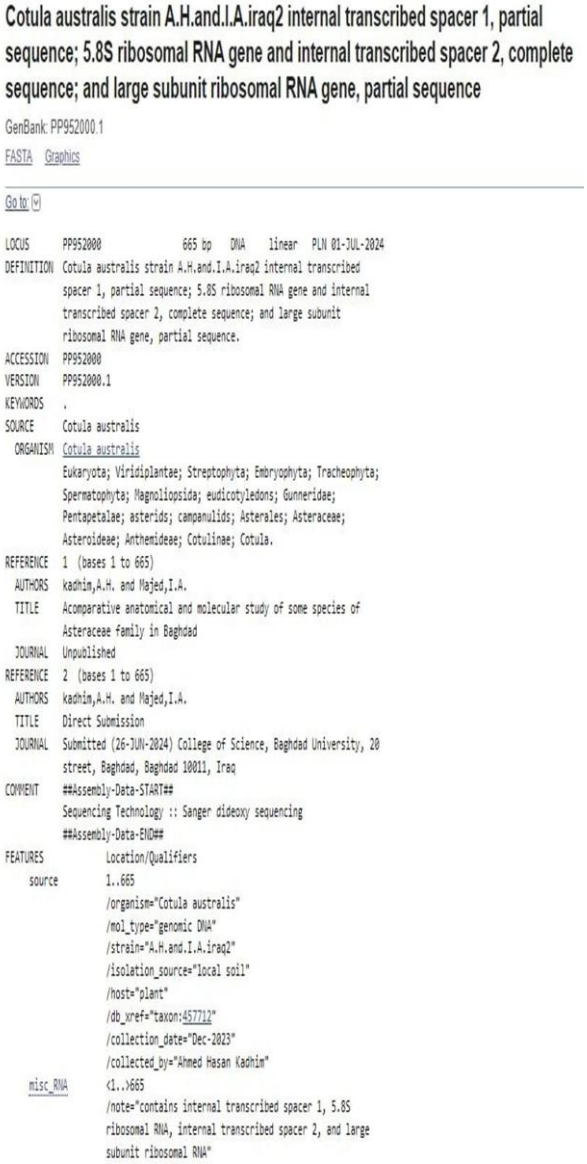


Figure (2): Phyllogenetic realationship treeof the studied species in the ITS region using UPGAM analysis software.



Cotula australis strain A.H.and.I.A.iraq2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: PP952000.1

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LOCUS       PP952000               665 bp    DNA     linear   PLN 01-JUL-2024
DEFINITION  Cotula australis strain A.H.and.I.A.iraq2 internal transcribed
            spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal
            transcribed spacer 2, complete sequence; and large subunit
            ribosomal RNA gene, partial sequence.
ACCESSION   PP952000
VERSION     PP952000.1
KEYWORDS    .
SOURCE      Cotula australis
  ORGANISM  Cotula australis
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae;
            Pentapetalae; asterids; campanulids; Asterales; Asteraceae;
            Asteroideae; Anthemideae; Cotulinae; Cotula.
REFERENCE   1  (bases 1 to 665)
  AUTHORS   kadhim,A.H. and Majed,I.A.
  TITLE     Acomparative anatomical and molecular study of some species of
            Asteraceae family in Baghdad
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 665)
  AUTHORS   kadhim,A.H. and Majed,I.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-JUN-2024) College of Science, Baghdad University, 20
            street, Baghdad, Baghdad 10011, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /note="contains internal transcribed spacer 1, 5.8S
                     ribosomal RNA, internal transcribed spacer 2, and large
                     subunit ribosomal RNA"
```

Figure(3): Image showing the species registration information at the National Center for Biotechnology Information.

Conclusion

The ITS region was used as an effective tool to analyze the molecular relationships among six species of the Asteraceae family. Genetic sequencing results revealed clear genetic similarities and differences, which aided in species classification and the study of their evolutionary relationships. The analysis demonstrated that some species are closely related within evolutionary groups, while others occupy distinct taxonomic positions. One of the studied species, *Cotula australis*, was recorded for the first time in Iraq as a new site at the molecular level. This study provides important data that contributes to enriching the genetic database of the Asteraceae family, particularly in Iraq. The results of the study can be used in future research related to the conservation of genetic diversity and accurate plant taxonomy.

References

1. Heywood, V.H.; Brummitt, R.K.; Culham, A. and Seberg, O. Brassicaceae. Pp. 68-71. In: Flowering Plant Families of the World. New York, Firefly Books (2007).
2. Khaleel, A. and Al-Dobaissi, I.A. (2022). Trichomes Morphological diversity in some species from related tribes of Asteraceae family in Iraq. Iraqi journal of science. 63(6):2362-2372
3. MEKHLFI, T.; 2023. Physio-chemical study of a medicinal plant of the Aster family (Chamomile) cultivated in the Algerian desert. univ-ouargla.dz
4. Goertzen, L. R.; Cannone, J. J.; Gutell, R. R. and Jansen, R. K. (2003). ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny of the Asteraceae. Molecular Phylogenetics and Evolution, 29(2), 216-234.
5. Masuda, Y.; Yukawa, T. and Kondo, K. (2009). Molecular phylogenetic analysis of members of Chrysanthemum and its related genera in the tribe Anthemideae, the Asteraceae in East Asia on the basis of the internal transcribed spacer (ITS) region and the external transcribed spacer (ETS) region of nrDNA. Chromosome Botany, 4(2), 25-36.
6. Shen, F.; Qin, Y.; Wang, R.; Huang, X.; Wang, Y.; Gao, T.; He, J.; Zhou, Y.; Jiao, Y.; Wei, J.; Li, L. and Yang, X.; (2023). Comparative genomics reveals a unique nitrogen-carbon balance system in Asteraceae.
7. Staggemeier VG, Diniz-Filho JA, Forest F, Lucas E (2015) Phylogenetic analysis in Myrcia section Aulomyrcia and inferences on plant diversity in the Atlantic rainforest. Annals of Botany, 115, 747–761.
8. Yasir, L. B. and Almaliky, B. S. A. (2024). Morphology, molecular identification, pathogenicity and first report of fusarium equiseti associated with root rot and wilt disease of catharanthus roseus in baghdad, iraq. iraqi journal of agricultural sciences, 55(6), 2153-2163.
9. Hussein, A. and Jubrael, H. (2021). Aflp marker in genetic diversityassessment of fig (ficus carica l.) populations in kurdistan region – iraq. iraqi journal of agricultural sciences, 52(4), 859-867.
10. Russell, D. W. and Sambrook, J. (2001). Molecular cloning: a laboratory manual (Vol. 1, p. 112). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
11. Al-Masoudi, R.K. and Al-Judy N.J. (2024). Sequencing Analysis of the ITS Region for Some Fusarium Species Isolated from Infected Tomato Plants. Iraqi Journal of Science, 65(12), 6894-6907. https://doi.org/10.24996/ijs.2024.65.12.8
12. Lundberg, J. and Bremer, K. (2003). A phylogenetic study of the order Asterales using one morphological and three molecular data sets. International Journal of Plant Sciences, 164(4), 553-578.
13. Ebadi, M. and Eftekharian, R. (2021). Morphological and genetic diversity of Senecio vulgaris L.(Asteraceae) in Iran. Acta Botanica Croatica, 80(2), 125-130.
14. Obaid, H. H.; Abdul, N. T. B. A. G.; Halim, Z. H. and Sagban, M. N. A. (2017). Cytotoxic effect of saponins extracted from Yucca on human breast cell line (HBL-100) in vitro. Iraqi journal of biotechnology, 16(3).
15. Al-Salihi, A. A.; Al-Saadi, B. Q. and Al-Anbari, N. N. (2017). Genotypes relationship of Growth hormone gene polymorphism with some productive and reproductive trait in Awassi sheep.
16. Mustafa, E. A.; Hamdoon, S. M. and Shehab, E. Y. (2021). Molecular detection and identification of Enterococcus faecium isolated from dental root canals. Iraqi Journal of Science, 1447-1451.