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Comparison between some genera of *Grammosciadium* and *Torilis* belonging to the (Apiaceae) family using nucleotide sequence of Matk gene into a protein

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Abstract

Background: The amino acid composition of certain species of the *Grammosciadium* and *Torilis* genera, belonging to the Apiaceae or Umbelliferae family, is investigated. This family of flowering plants is named after the genus Apium and is commonly referred to as the celery or carrot family. These plants are found in the wild in temperate regions and the northern regions of Iraq.

Methods: The research was carried out in the Molecular Genetics Laboratory at the College of Education for Pure Sciences, University of Diyala, Iraq. A total of 40 plant samples were collected from various places in Iraq, with 10 samples obtained for each species. The pliable specimens were enclosed in nylon pouches, stored in the refrigerator, and conveyed to the laboratory for the explicit objective of DNA extraction, with the subsequent data being duly documented. The molecular systematic research on Apioideae has involved international collaboration. The ongoing investigations will focus on analyzing the remaining 200 or so genera that have not been reviewed previously, particularly those that have been difficult to classify phylogenetically. Additionally, the current tribes and clades will be re-examined and improved. The current and upcoming updates to all of these well-known groups, which will include both molecular and morphological information, will finally lead to the creation of a new classification for the subfamily.

Results: The results demonstrated a distinction in nucleotide sequences of the Matk gene across two genera as well as between species belonging to the same genus. Specifically, there were variations in the arrangement of 20 amino acids. The pH value of 14.03 was recorded as the maximum for citric acid in *G. daucoides*.

Conclusion: The commonality lies in the relative quantities of certain amino acids. Members of the same genus, as well as people belonging to the same species.

Introduction

The Apiaceae (Umbelliferae) family is widely distributed globally, with the highest diversity found in temperate climate regions and rarely in tropical regions. It encompasses 466 genera and 3800 species [1, 2]. The family comprises a range of 300–455 genera and 3000–3700 species, as stated in reference [3]. The study encompassed a total of four participants. According to the findings of research [4, 5], the order Apiales comprises around 6,000 species distributed among 522 genera. The Apiaceae family, under this order, is composed of four subfamilies: Apioideae, Azorelloideae, Mackinlayoideae, and Saniculoidae.

Grammosciadium, a genus, was classified within the Careae clan. Additionally, the Scandiceae clan was subdivided into three groups within the Subtribe clan, namely Daucinae, Scandicinae, and Torilidinae.

Based on the findings of molecular genetics investigations [6, 7, 8, 9], the genus *Torilis* was classified into the subfamily Torilidinae. Cite both [10] from information acquired from sources. Published botanical records provide quantitative analysis of plant species, revealing a total of 3300 species distributed among 980 genera and 136 families of flowering plants. Among these families, five major families stand out in terms of species diversity. One of these families is Apiaceae, which encompasses 155 species found in various regions and environments of Iraq. According to the source [11], the Apiaceae family is known to have 466 genera and 3800 species worldwide. It is worth noting that Asia has the highest number of genera within the Apiaceae family. Previously known as the Umbelliferae family, this family is associated with carrots and consists of 434 genera and 3780 species [12]. Acids possess distinctive characteristics. Amines are colorless ionic compounds that exhibit various degrees of solubility in cold and hot water as well as alcohol. Additionally, they possess a high melting point. The solubility qualities and high melting points of these substances can be attributed to their nature as hybrid ions, also known as zwitterions. They are present in plants either independently or combined with each other to create proteins and different peptide compounds. They are categorized into There are a total of 20 amino acids that make up proteins. These amino acids can be found either freely in plant tissue or as a result of the hydration of proteins or peptides. The quantity of these acids differs among tissues, contingent upon the metabolic processes occurring within them. Typically, glutamic acid, aspartic acid, glutamine, and asparagine Amino acids are found in plants in higher amounts compared to other acids, and they are commonly observed in plant extracts. It has been highlighted in references [13, 14] that amino acids serve as the smallest structural unit in protein synthesis, acting as the fundamental building blocks for all proteins. Amino acids are chemical substances that consist of an amine group and a carboxyl group. These amino acids are classified as alpha amino acids, which are the building blocks of proteins. There are 20 amino acids that occur naturally. These amino acids are formed through the breakdown of proteins, either through chemical processes or enzyme reactions, which are sometimes referred to as chemical mechanisms [15].

Only the nucleic acids responsible for heredity have the capability to combine and create proteins. The significance of nucleic acids lies in their role as the primary regulators of protein synthesis. He asserted that nucleic acids are high-molecular-weight polymers found in all living systems, comprising 5–15% of the dry weight of these systems. Acids serve as repositories of genetic information and facilitate its transmission to the organism.

Methods

Specimens collection

The research was carried out in the Molecular Genetics Laboratory of the Department of Biology at the College of Education for Pure Sciences, University of Diyala, Iraq. A total of 40 plant samples were collected from various places in Iraq, with 10 samples obtained for each species. The pliable specimens were packed in nylon pouches and stored in the refrigerator, thereafter, being sent to the laboratory for the specific objective of DNA extraction.

DNA extraction

The research was carried out in the Molecular Genetics Laboratory of the Department of Biology at the College of Education for Pure Sciences, University of Diyala, Iraq. A total of 40 plant samples were collected from various places in Iraq, with 10 samples obtained for each species. The pliable specimens were enclosed in nylon pouches and stored in the refrigerator, subsequently transported to the laboratory for the specific objective of DNA extraction.

Makt gene amplification

The primers utilized for the amplification of the Makt gene were Matk-f (5'-ACTGTATCGCACTATGTATCA - 3') and Matk-R (5'-GCATCTTTTACCCARTAGCGAAG - 3') [16]. The PCR mixture had a total volume of 25 µL, comprising 5 µL of master mix, 10 µL of forward primer, 10 µL of reverse primer, 1.5 µL of DNA, and 16.5 µL of deionized water. The programmed thermoplastic polymer undergoes an initial denaturation at a temperature of 95°C for a minimum duration of 3 minutes. Following this are 35 cycles of denaturation at 95°C for 4 minutes, annealing at 50°C for 40 seconds, and extension at 72°C for 40 seconds. Finally, there is a final extension at 72°C for 40 seconds. The PCR results were subjected to electrophoresis on a 1% agarose gel and then treated with 0.5 µl of ethidium bromide for staining. Captured with UV lighting on the LG 2020 Gel Documentation System. Subsequently, ten specimens of PCR results from each species were dispatched to Macrogen, located in South Korea, for the purpose of direct sequencing.

Sequencing of Matk gene

The nucleotide sequence alignment of the Matk gene was conducted for samples from the species *Grammosciadium daucoides*, *Grammosciadium Platycarpum*, *Torilis arvensis*, and *Torilis chrysocarpa*, utilizing the seventh edition of the Bioedit program. This gene corresponds to the protein.

Results

It was found that the nucleotide sequence of the Matk gene was different between samples from the *Grammosciadium* and *Torilis* genera. A total of 20 amino acids were isolated and distinguished. The amino acids exhibited differences in the classification of the two genera, *Grammosciadium* and *Torilis*. The species *G. daucoides* exhibited a composition of 20 amino acids, with serine acid having the highest percentage at 14.03% and histidine and tyrosine acids having the lowest percentage. As it reached a rate of 1.16. Regarding the species *G. patycarpum*, As seen in Figures 1, 2, and 5, The taxonomic classification of *Torilis* belongs to the rank of genus. The species *T. arvensis* consists of 20 amino acids, with the largest proportion of bile acid at 12.5% and the lowest proportion of glucose acid. As it reached a rate of 0.59. Regarding the species *T. chrysocarpa*, the amino acid composition consisted of 20 different amino acids, with the greatest percentage being 12.19% for oleic acid. The acids with the lowest percentage were Alanine (Ala), Glycine (Gly), and Cysteine (Cys). As it reached a velocity of 0.81. Furthermore, it is worth noting that there is a notable resemblance between the two species, *G. platycarpum* and *G. daucoides*, in terms of the composition of amino acids. Specifically, both species exhibit an identical proportion of 4.67% for the amino acids lysine and arginine. Additionally, while examining the amino acid composition of *G. daucoides*, it was revealed that it has a significant amount of glutamine and glutamic acid.

The ratio of 2.33 is observed for the amino acids Ala and Val, whereas the ratio of 3.50 is shown for the amino acids Arg, Gly, and Lys.

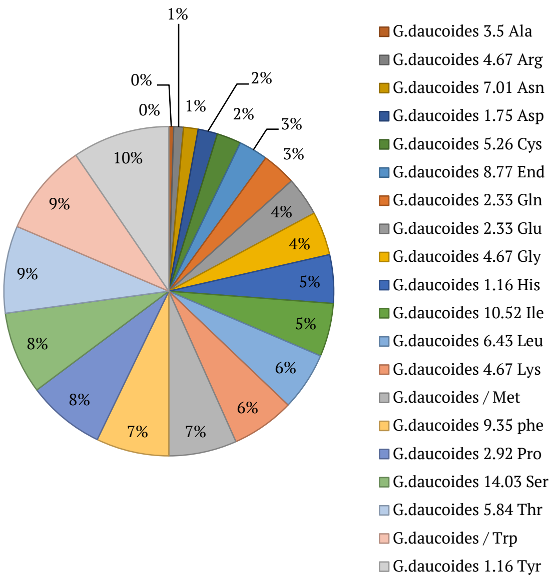

Figure 1: shows the percentage concentrations of the amino acids sequence of the species *G. daucoides.*

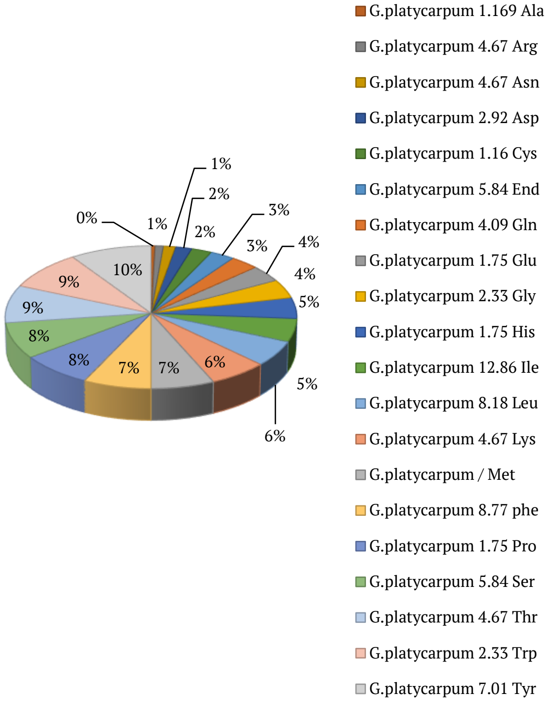

Figure 2: shows the percentage concentrations of the amino acids sequence of the species *G. platycarpum.*

Additionally, a ratio of 4.67 is observed for the amino acids His and Tyr. The amino acid ratios in type *G. platycarpum* were as follows: (Arg, Asn, Lys, Thr) had a ratio of 4.67; (Asp, Val) had a ratio of 2.92; (End, Ser) had a ratio of 5.84; (Glu, His, Pro) had a ratio of 1.75; and (Gly, Trp) had a ratio of 2.33. In *T. arvensis*, (Asn, Trp) had a ratio of 4.16, (Asp, His, Val) had a ratio of 1.78, and

(Cys, Gln, Pro) had a ratio of 3.75. In *T. chrysocarpa*, (Ala, Cys, Gly) had a ratio of 0.81; (Arg, Asp, Tyr) had a ratio of 4.87; (End, Lys) had a ratio of 7.31; and (His, Trp) had a percentage of 1.62. (Phe, Ser) had a percentage of 8.94, and (Thr, Val) had a percentage of 4.06 [20], as seen in Figures 3 and 4.

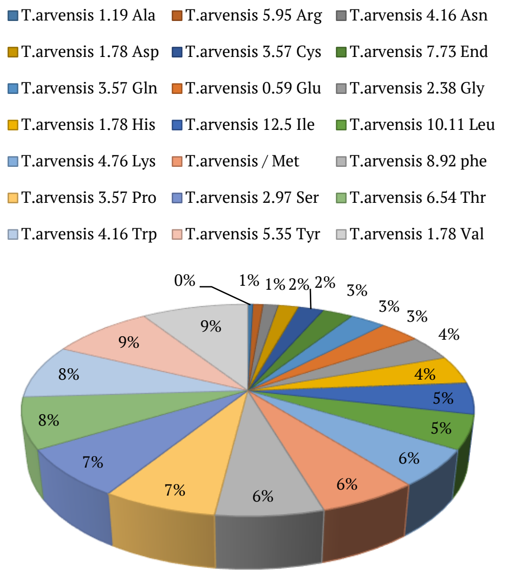

Figure 3: shows the percentage concentrations of the amino acids sequence of the species *T. arvensis.*

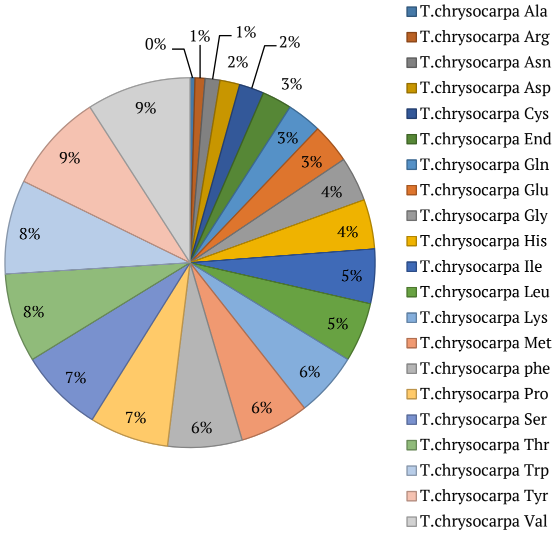

Figure 4: shows the percentage concentrations of the amino acids sequence of the species *T. chrysocarpa.*

Discussion

The extraction of DNA was conducted to determine the nucleotide sequences of various genetic regions of mtDNA. Then, because they show a lot of nucleotide substitution, these sequences were used to figure out the genetic link between different genera or between genetically different groups within the same genus [17]. The specific kind is *G. platycarpum*. The amino acid composition of the sample consisted of 20 different amino acids. Among these, the largest proportion was seen for isoleucine (Ile) acid, which accounted for 12.86% of the total. The lowest percentage was observed for the amino acids histidine, proline, and cysteine. The value achieved a rate of 1.16 [18]. The amino acids *T. arvensis* (Asn, Trp) increased by 4.16, (Asp), (His, Val) increased by 1.78, and (Cys, Gln, Pro) increased by 3.75. *T. chrysocarpa* (Ala, Cys, Gly) increased by 0.81, (Arg, Asp, Tyr) increased by 4.87, (End, Lys) increased by 7.31, and (His, Trp) had a percentage of 1.62, (Phe, Ser) had a percentage of 8.94, and (Thr, Val) had a percentage of 4.06 [19]. The amino acids *T. arvensis* (Asn,Trp) increased by a factor of 4.16, (Asp), (His, Val) increased by a factor of 1.78, and (Cys, Gln, Pro) increased by a factor of 3.75. *T. chrysocarpa* (Ala, Cys, Gly) increased by a factor of 0.81, (Arg, Asp, Tyr) increased by a factor of 4.87, (End, Lys) increased by a factor of 7.31, and (His, Trp) had a percentage of 1.62, (Phe, Ser) had a percentage of 8.94, and (Thr, Val) had a percentage of 4.06 [20].

The genetic link between different genera or genetically diverse groups within a genus was evaluated by analyzing the nucleotide sequences of several genetic regions of mtDNA. This analysis was conducted due to the significant frequency of nucleotide substitution seen in these locations [18]. The amino acid composition of the sample consisted of 20 different amino acids, with the greatest proportion being oleic acid, which accounted for 12.86%. The lowest percentage was observed for the amino acids histidine, proline, and cysteine. The value achieved a rate of 1.16 [19].

Including them would mostly lead to the merging of the Angelica and Apium clades. We emphasize the temporary nature of these relationships, as just half of the approximately 400 genera acknowledged in Apioideae have been included in DNA research. However, a significant number of the other genera are classified under the extensive tribe Apidae [21]. Until they are thoroughly examined, it is hard to determine their relationship. Nevertheless, it is highly probable that a considerable portion of them will belong to the apioid superclade.

Although the links derived from different DNA markers are usually consistent, it has been challenging to define many of these newly identified tribes and clades based on clear morphological or anatomical characteristics [22]. It is anticipated that forthcoming research will contribute to this existing knowledge. However, if this is not the case, we would need to acknowledge that the reclassification of Apioideae at the higher taxonomic level will need to rely on molecular data rather than physical characteristics. We acknowledge that this is a controversial matter; without clear and distinctive physical features, it is challenging to identify or differentiate several of the tribes included here.

Efforts to create a family tree for the entire subfamily using traditional characteristics have been challenging. While analyzing the physical and anatomical traits of Scandiceae, Oenantheae, and basal Apioideae has provided valuable insights into their family trees, these findings have limited applicability to broader taxonomic levels [22].

Given that many systematic botanists are unhappy with the current diagnostic criteria used in the higher-level classification of Apioideae and the lack of agreement on connections seen in current taxonomic approaches, it is not surprising that this conclusion was reached. Essentially, it is difficult to clearly define many of the tribes and subtribes now acknowledged in Apioideae using physical characteristics alone. Expecting to find physical traits that these tribes and other significant groups supported by molecular research share may be unrealistic. The molecular systematic research on Apioideae has involved international collaboration. The ongoing investigations will focus on analyzing the remaining 200 or so genera that have not been reviewed previously, particularly those that have been difficult to classify phylogenetically. Additionally, the current tribes and clades will be re-examined and improved. The current and upcoming updates to all of these well-known groups, which will include both molecular and morphological information, will finally lead to the creation of a new classification for the subfamily.

Author Contributions

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Resources: Ghassan Radam Idan Al.Taie , Nagham Thayer Salman, Ali Mala Khedir Galalaey
Software: Ghassan Radam Idan Al.Taie , Nagham Thayer Salman, Ali Mala Khedir Galalaey
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Conflict of Interest

The authors declare that there is no conflict of interest.

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