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PHYLOGENETIC ANALYSIS OF GROUND CHERRY (*PHYSALIS*) SPECIES

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SUMMARY

Solanaceae is one of the prominent plant families that provide medicine and food. For the diagnosis of its species (*Physalis peruviana* and *Physalis angulata*), the use of molecular markers with reliable and precise nuclear ribosomal DNA (nrDNA) ITS sequences has helped in the presumption of molecular evolution and phylogenetic studies of the plants. It precisely measured the variance between two samples of *Physalis* L. Purified fragments' sequencing using BLAST to align each sequence, evaluate its relationship with other sequences, and confirm the species of different sequences. The genetic analysis program, MEGA V.11, aided in performing multiple sequence alignment and phylogenetic analysis. The first sample showed a relation to the species *Physalis peruviana* voucher Smith 217 (WIS) placed in the GenBank with the serial number DQ314161.1 by 96.91%, which belonged to a strain located in the USA. The researcher registered the species as *Physalis peruviana* isolate AH-ZE1, with serial number OQ616506.1. However, the second sample, after contrasting it with the global species sequences, indicated a connection to the species *Physalis angulata* isolate LHR28I deposited in the Genome Bank with the serial number MK412130.1 at a rate of 98.02%, which belonged to a Spanish strain. The species' registration by the researcher continued as *Physalis angulata* isolate AH-ZE1, sequence number OQ616509.1. The current study results revealed the diagnosis of two species of *Physalis* identified for the first time in Iraq, as they incur registration in the National Center for Biotechnology Information. The recording depended on the molecular characteristics' differences in the ribosomal DNA (rDNA) region, knowing the evolutionary relationship between the *Physalis* species and comparing them to the sequences found in the GenBank for previously defined types. The results also showed that the ITS2 region provided success ability. Therefore, using other DNA barcodes as auxiliary factors to distinguish between *Physalis* species would be beneficial.

Keywords: Ground cherry (*Physalis* L.), phylogeny, molecular identification, ITS2, phylogenetic relationship

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Key findings: The species *Physalis peruviana* with isolate AH-ZE1 attained registration in the GenBank with serial number OQ616506.1. The second species, *Physalis angulate,* with isolate AH-ZE1, also incur recording in the GenBank with serial number OQ616509.1.

INTRODUCTION

The Solanaceae family, considered one of the most important plant families, provides food and contains 2,000 species (Yadav *et al*., 2016). For the past 50 years, a constant improvement has evolved in the agricultural biodiversity of nutritionally valuable Solanaceae family plants (Samuels, 2015). *Physalis* L. is considerably the Solanaceae family's highly crucial genera, which contains more than 120 species (Al-Tamimi and Farhood, 2022).

The *Physalis* species often exist in temperate zones and America's tropical, with only a few species observed in Southeast and Eurasia (Zamora-Tavares *et al*., 2015). China has only five *Physalis* species, mainly found in the country's South, East, and Central regions, as the Chinese Academy of Sciences (CAS) reported in 1978 (Li, 2007). The *Physalis* species are rich in minerals, vitamins, and antioxidants, with antibacterial, anti-cancer, and anti-inflammatory actions (Hong *et al*., 2015).

Considering their highly similar morphological characteristics, the mistaken identification of *Physalis* species is prevalent. Sometimes, there can be skepticism between the species *P. minima, P. pubescens, and P. angulata* because of their application influence in traditional medicines (Feng *et al*. 2016). Different *Physalis* species and their related species contain physiologically diverse active chemical compounds, having varied utilization. Therefore, improper identification of *Physalis* species might result in the inappropriate use of such species, consequently failing to conserve their genetic resources.

Past studies confirmed that the traditional morphological diagnosis often has influences from growth, environment, and heritable variations (Chen *et al*., 2010; Vargas-Ponce *et al*., 2011). Thus, finding an identification method to be reliable and rapid for *Physalis* spp. is essential. DNA barcoding has proven advantageous in species identification because it is a reliable and fast technology. This technique relies on sequence differences within short and standardized chloroplast DNA regions or nuclear DNA regions (Feng *et al*., 2016).

Recent studies have utilized many molecular markers to assess and identify crop plants' phylogenetic relationship and genetic diversity (Barchi *et al*., 2011). DNA markers have also helped study the molecular diversity of *Physalis* species, such as the inter-simple sequence repeat and the simple sequence repeat markers (Zamora-Tavares *et al*., 2015).

The Ribosomal DNA has been considered one of the multigenic families in all eukaryotic genomes. The rRNA gene contains three regions of 28S, 18S, and 5.8S, which make up each separate unit as an exterior transcribed spacer skips and the ITS1 and ITS2 internal transcribed spacers (Giudicelli *et al*., 2017). In rDNA, the regions of Exons are highly conserved in eukaryotic organisms. However, the ITS regions' exposure to varied lengths resulted from insertions/deletions (indels) and point mutations. Studies of molecular evolution and the inference of phylogenetic hypotheses used the ITS sequences at several plant taxonomic levels (Giudicelli *et al*., 2017). The pertinent investigation sought to identify two *Physalis* species introduced into Iraq using molecular markers based on nuclear ribosomal DNA (rDNA) region differences, knowledge of the evolutionary relationship between the *Physalis* species, and by comparing with the sequences found in the GenBank for previously identified species.

MATERIALS AND METHODS

The relevant study comprised the collection of eight samples of two *Physalis* species. The obtained samples from the fields belonging to private sector nurseries in Kerbala

Governorate, Iraq, on September 21, 2022, sustained classification based on visual morphology. It was also evident that these species samples belonged to the family Solanaceae and the genus *Physalis*, and both species samples are self-propagating.

DNA extraction and amplification

DNA isolation from the young leaves of *Physalis* species samples was random, and amplification of ITS2 sequences used a pair of universal primers, i.e., ITS- ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS-5 (5'-GGAAGTAAAAGTCGTAA CAAGG-3') (Sharma and Jana, 2002). The PCR procedure continued in 25 µL volumes, containing 1×PCR Buffer, 1µ Taq DNA, 2.5 mM Mg 2+, 0.5 µM of each primer, 0.4 mM dNTPs polymerase, and 50 ng genomic of the DNA template. The amplification progressed with the following PCR program, i.e., 94 °C for 5 min, followed by 35 cycles of 93 °C for 45 s, 57 °C for 45 s, 73 °C for 1.5 min, and 72 °C for 10 min.

Data analysis

The selected ITS sequences' aligning employed the blast-NCBI, with the genetic distances calculated using the MEGA 11.0 program (Tamura *et al.,* 2013). The calculated averages of the interspecific distance, the minimum interspecific distance, and their averages aided in evaluating the interspecific divergences (Chen *et al.,* 2010). DNA barcoding gaps compared the distributions of intra vs. interspecific variability (Gao *et al.,* 2010). The BLAST method helped evaluate the species authentication efficacy (Feng *et al.,* 2015). In the BLAST method, the ITS regions of *Physalis* species served as query sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The selected ITS2 sequences' alignment also used blast-NCBI, and the genetic distances' formulation applied the MEGA 11.0 program (Tamura *et al*., 2013).

RESULTS

Phylogenetic analysis

The phylogenetic analysis based on the ribosomal DNA ITS regions revealed that the PCR amplification authenticated the initiator ITS ably identified its complementary sequences in the DNA of the two *Physalis* species samples. The molecular size of the bundles in the first species samples was 625 pb, and the molecular size of the second species samples was 633 pb. These results were consistent with past findings, which revealed that for molecular identification purposes, the data recording and analysis continued to transfer on agarose gel at a concentration of 2% (Lumariz *et al.,* 2019). After completing the PCR programs, recorded observations depended on the size and location of the packages (Figures 1 and 2).

Comparing the sequence of the first sample and the existence of matching in different proportions with the global *Physalis* species has the results ascertaining the phylogenetic analysis by drawing the genetic tree based on the sequences of various nitrogenous bases and calculating the genetic distance by the Neighbor-Joining method and using the genetic analysis program, MEGA V.11 (Figure 3).

The nucleotide sequences indicated the confirmed identity of the amplified aggregates through the NCBI blast GenBank website, noticing the highest similarities to the first *Physalis* species sample and the genetic sequence deposited in the GenBank. The comparison transpired between them, with the genetic tree of one of the species of the genus *Physalis'* creation depending on the DNA sequences and the contained DNA sequences in the GenBank. The phylogenetic tree included 11 different species belonging to the same genus *Physalis*. The species under study showed a relationship with the *Physalis peruviana* voucher Smith 217 (WIS) by

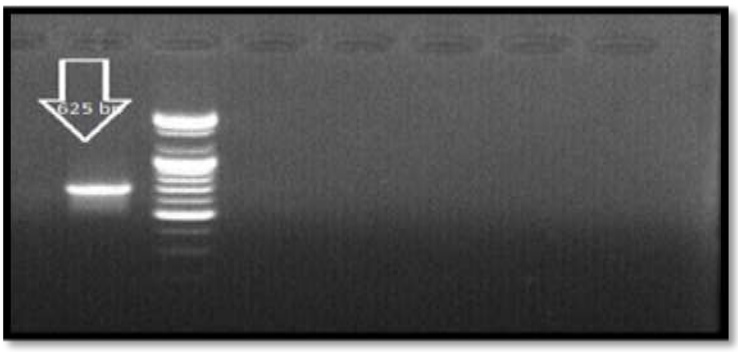

Figure 1. The results of the PCR assay for the ITS gene for the first *Physalis peruviana* samples.

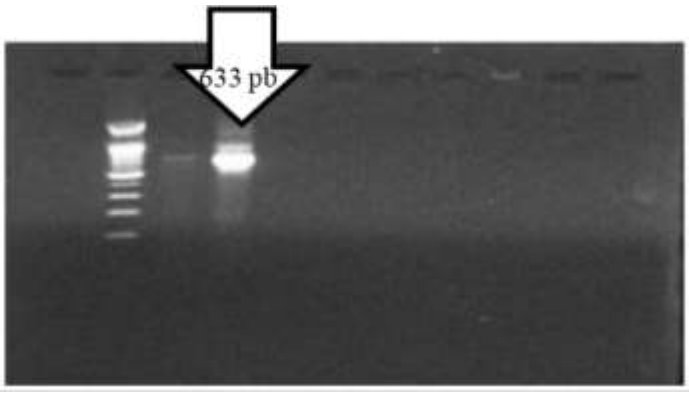

Figure 2. The results of the PCR assay for the ITS gene for the second species, *Physalis angulata* samples.

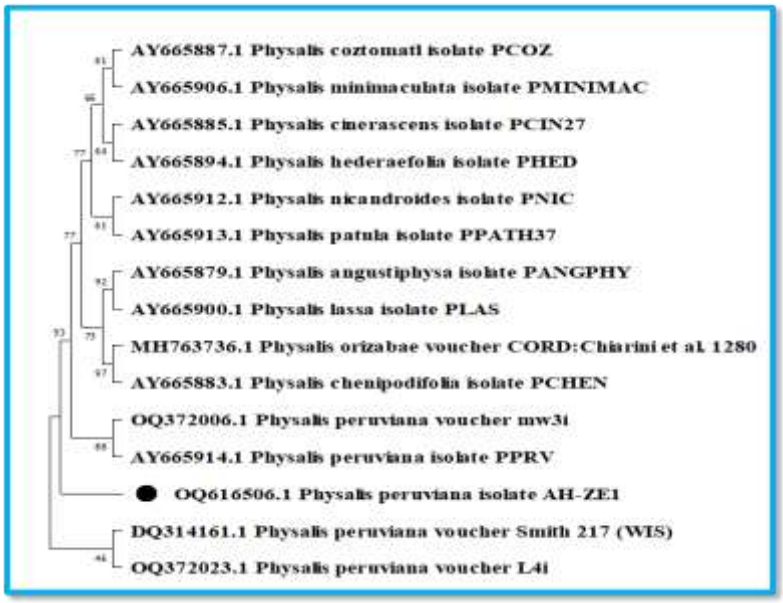

Figure 3. The genetic tree of the species *Physalis peruviana* isolate AH-ZE1 (genetic distance calculation used the Neighbor-Joining method).

96.91%, continued depositing in the GenBank with the serial number DQ314161.1, which also belonged to a *Physalis* strain located in the USA, based on the analyzed ribosomal sequences (Lumariz *et al.,* 2019).

Using the Sequence Demarcation Tool program, the percentage of similarity calculation relied on the sequences of its nitrogenous bases for the ITS-rDNA region between the *Physalis peruviana* isolate AH-ZE1 (marked with a red-edged rectangle) and global isolates and strains of plant species belonging to the same genus obtained from the GenBank data container (Figure 4).

Concerning the second *Physalis* species sample, after comparing it with the global species sequences, a match in different proportions was apparent, with the species under study showing an association with the *Physalis angulata* isolate LHR28I, deposited in the Genome Bank with the serial number MK412130.1 with a rate of 98.02%, which also belonged to a *Physalis* strain found in Spain (Figure 5). These results were in a higher analogy with the findings of Pretz and Deanna (2020).

Using the Sequence Demarcation Tool program, the percentage of similarity formulation depended on the sequences of its nitrogenous bases for the ITS-rDNA regions between the *Physalis angulata* isolate AH-ZE1 (marked with a red-edged rectangle) and global isolates and strains of plant species belonging to the same genus obtained from the GenBank data container (Figure 6).

DISCUSSION

The genus *Physalis* species are beneficial medicinal plants with various significant economic values. Therefore, their identification is highly imperative. However, their identification based on morphological characteristics is difficult because of the morphological similarities. In the present era, the DNA barcoding used for the ribosomal DNA (ITS region) to identify species has become vital and attractive (Chen *et al*., 2010; Motyleva *et al*., 2022). The ITS had many advantages due to some of its better and small-intraspecific variations; however, the high interspecific variation was prominent by using the ITS region between *Physalis* species to allow the detection of a gene divergence (Chen *et al*., 2010). The presented results explained that the ITS2 region demonstrated a higher success capability. Therefore, it might be beneficial to use other DNA barcodes to help discriminate between these *Physalis* species (Harrington *et al*., 2009).

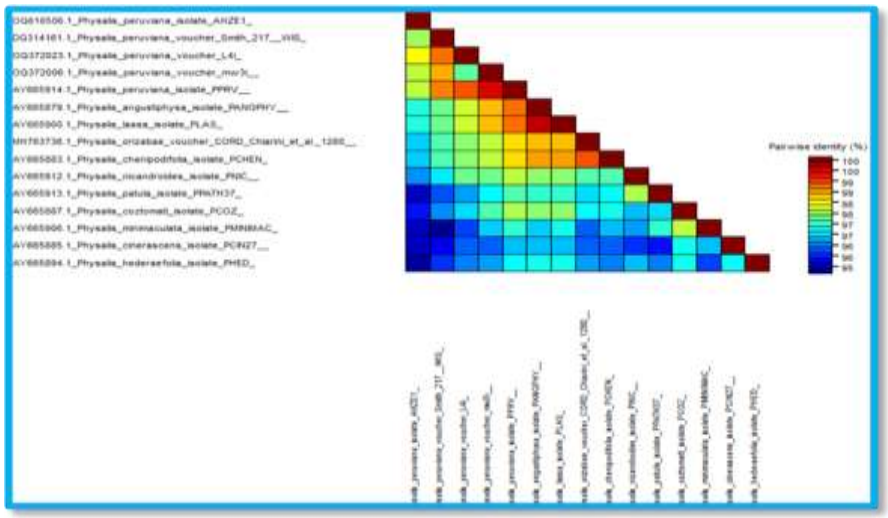

Figure 4. Percent similarity between *Physalis peruviana* isolate AH-ZE1 (marked with a red-edged rectangle) and global isolates and the strains found in the GenBank.

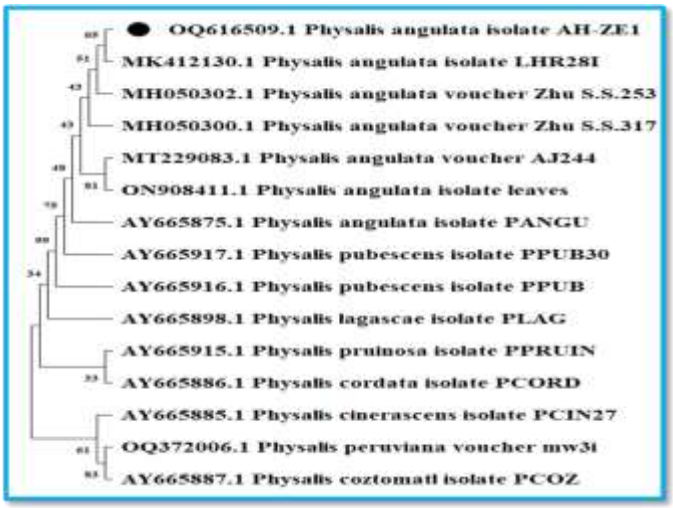

Figure 5. The genetic tree of the species *Physalis angulata* isolate AH-ZE1 (genetic distance calculation used the Neighbor-Joining method).

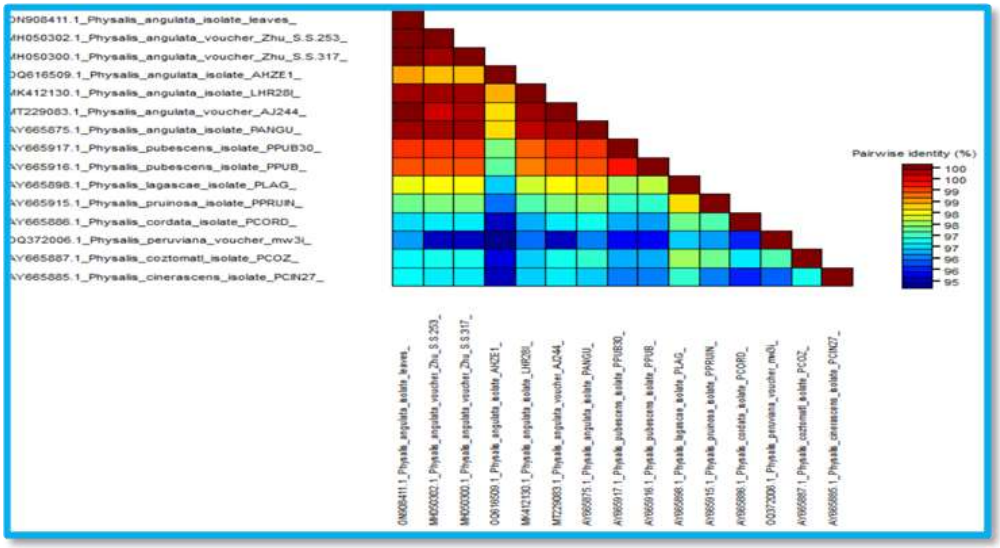

Figure 6. Percent similarity between *Physalis angulata* isolate AH-ZE1 (marked with a red-edged rectangle) and global isolates and the strains found in the GenBank.

According to the results of the BLAST analysis and on account for the nearest-distance methods, ITS leads to high species' discriminability identification success rates. Interestingly, the *Physalis* species, *Physalis peruviana* and *Physalis angulata* have highly similar morphological characteristics and were indistinctive from one another using conventional morphological characteristics differentiation. However, using nuclear ribosomal sequences, the *Physalis* species identification can be easy and accurate by their ITS regions (Feng *et al*., 2016). The results strongly suggested that the nuclear ribosomal sequences' ITS region could better serve as the complementary barcode (Chen *et al*., 2010).

CONCLUSIONS

The practical study succeeded in the molecular diagnosis of two *Physalis* species found in Iraq. These two species (*Physalis peruviana* and *Physalis angulata*) attained registration, obtaining a serial number for each species in the GenBank, noting that the genetic diagnosis of these two endemic species did not have previous studies in Iraq. Molecular diagnostics are also recommendable based on the whole chloroplast genome sequences, which are now becoming more readily available with the development of sequencing technologies.

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