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Identification and reconstruction of phylogeny among three species of *Cleome* using nrDNA-ITS sequence

Salim Khan[1], Fahad Al-Qurainy[1], Mohammad Nadeem[1], Mohamed Tarroum[1], Saleh Alansi[1], Abdel-Rhman Gaafar[1], Aref Alshameri[1], Hassan O. Shaikhaldein[1], Abdalrhaman M. Salih[1], Norah Arrak Alenezi[1]

[1]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh- 11451, Saudi Arabia

Abstract

Three species of genus *Cleome*, *C. arabica*, *C. ramosissima* and *C. amblyocarpa* were identified using nuclear ribosomal DNA internal transcribed spacer sequence (nrDNA-ITS). The phylogenetic tree was made using different methods viz., neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood for accuracy of the results, and congruence finding in phylogeny. *Cleome ramosissima* and *C. amblyocarpa* were found to be clustered in one group according to the sequence similarity. However, *C. arabica* clustered in a separate clade. The nrDNA-ITS marker clearly differentiated and showed phylogenetic relationships among the three species. Thus, nrDNA-ITS marker could be effectively used for other species of *Cleome* for their identification and phylogenetic studies.

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Introduction

The genus *Cleome* (family: Cleomaceae) occurs widely in subtropical and tropical countries, and has more than 200 species (Chweya and Mnzava, 1997; Short, 2010). The *Cleome* species are annual herbs or shrubs. The different species of *Cleome* contain various secondary metabolites of considerable medicinal value. *Cleome arabica* is widespread in North Africa, which is commonly known as spider flower. *Cleome arabica* is used in folk medicine for the treatment of inflammation and scabies (Ahmad et al., 1990; Tsichritzis et al., 1993) as well as rheumatic pains (Bouriche and Arnhold, 2010). It also possesses antifungal and antimicrobial activities (Takhi et al., 2011) and cytotoxic activities (Nagaya et al., 1997); it also provides high amounts of antioxidants (Selloum et al., 1997). For example, the siliqua fractions of *C. arabica* showed phytotoxic activity on germination and seedling growth of different plants (Ladhari et al., 2013).

Cleome arabica and *C. amblyocarpa*, both are medicinal herbs used in folk medicine as leaves of both are a valuable source of active substances and nutrients (Khlifi et al., 2021). *Cleome ramosissima* and *C. amblyocarpa* have a good antioxidant activity (Al-Humaidi et al., 2019). For example, the methanol extracts of the aerial parts of *C. ramosissima* exhibited antihyperglycemic activity (Ezzat et al., 2014). Various *Cleome* species are found in the natural habitat of Saudi Arabia, including *C. amblyocarpa*, *C. arabica*, *C. ramosissima*, *C. viscosa*, *C. chrysantha* and *C. rupicola* (Rahman et al., 2004). From the nomenclatural point of view, *C. amblyocarpa* has generally been confused with *C. arabica*. However, these herbs are characterized by rigid stems, small trifoliate leaves, and fruits. Both species can be identified based on the shape of siliquae (Pottier-Alapatite, 1979).

Various molecular markers are available for the identification of plant species including RAPD (Khan

***CONTACT** Salim Khan, salimkhan17@yahoo.co.in, ☎ +966-014675875: +966-4678301 Department of Botany and Microbiology, College of Science, King Saud University, Riyadh- 11451, Saudi Arabia

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et al., 2010), ISSR (Balasaravanan et al., 2006), SSR (Tuler et al., 2015) etc. However, DNA sequence based markers such as nrDNA-ITS (Khan et al., 2012) and chloroplast markers (Khan et al., 2013) are more reproducible. The phylogenetic study of *Cleome* species has not been studied till now despite the fact that some species of this genus are medicinally very important. Therefore, in the present study, three species of *Cleome* were identified using the internal transcribed spacer sequence (ITS) of ribosomal DNA, and studied their phylogenetic relationships.

Materials and Methods

Plant collection

The leaves of three species of *Cleome* were collected in silica gel from three different geographical regions of Saudi Arabia including Rabua (*Cleome ramosissima*), Wadi Hanifa (*Cleome arabica*), and Rawdat Khuraim (*Cleome amblyocarpa*). The identification of these species was done by a well-versed taxonomist of the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

DNA isolation and purification

The high quality and quantity genomic DNA was isolated from the leaves using the protocol described by Khan et al. (2007). The RNA was removed from the genomic DNA by treating with10 µg/mL of RNase.

PCR amplification and sequencing

The universal primers were used for the amplification of internal transcribed spacer (ITS) sequence of nuclear ribosomal DNA (nrDNA). The primer was got synthesized from Macrogen, South Korea. The reaction was performed in a 25 µL volume in a master mixture (GE Healthcare, UK). The reaction was performed in triplicate for reproducibility of the results. The sequencing was performed in the sense and antisense directions at Macrogen, South Korea. The sequence obtained was analyzed with the ABI Sequence Navigator Software (Perkin- Elmer/Applied Biosystems). After analysis of the sequences, they were deposited in the GenBank database (http:// www.ncbi.nlm.nih.gov/). The MEGA X software was used for the reconstruction of phylogeny following Kumar (2018).

Results and Discussion

Molecular identification is necessary to differentiate plant species. Morphological markers have some limitations as they are affected by environmental factors, plant growth stages, and also are limited in number (Eagles et al., 2001). Moreover, biochemical markers are affected by various extraction methodologies, types of plant tissues, and different plant growth stages (Mondini et al., 2009).

In the present study, some species of *Cleome* were collected from different geographical regions of Saudi Arabia for their identification using internal transcribed spacer sequence of RNA (nrDNA-ITS). The ITS locus was isolated, purified and sequenced. The ITS sequence of these species in the BLAST search showed similarity to other species of *Cleome* available in the Genbank database. The nrDNA-ITS sequences of *Cleome arabica* (KF805109), *Cleome amblyocarpa* (KF850548), and *Cleome ramosissima* (KF850556) have been submitted to the NCBI GenBank database. The plant species, *Wislizenia palmeri* (KF217242) and *Wislizenia refracta* (KF217247) were used as outgroup downloaded from the GeneBank database (https://www.ncbi.nlm.nih.gov/)**.** The evolutionary history was inferred by using the methods such as maximum likelihood (Tamura and Nei, 1993], neighbor-joining (Saitou N. and Nei M. (1987), and maximum parsimony (**Figures 1, 2 and 3**).

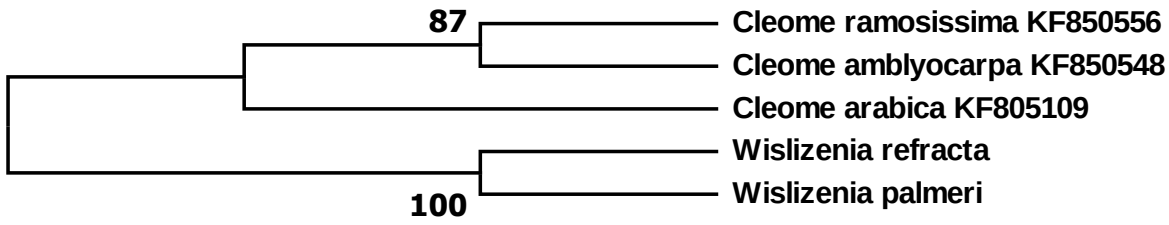

Figure 1. A phylogram based on nrDNA-ITS locus using the maximum likelihood method. Bootstrap values > 50% were shown on branches

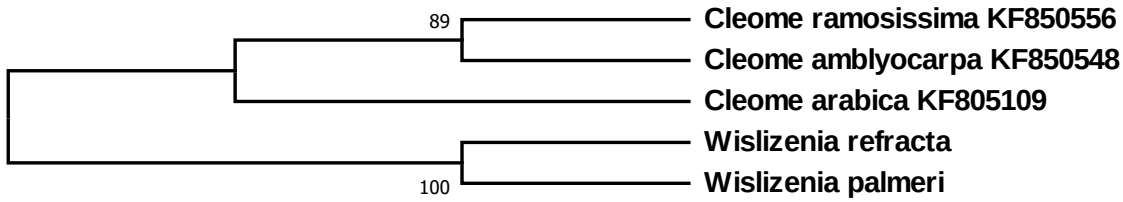

Figure 2. A phylogram generated based on nrDNA-ITS using the neighbor-joining method

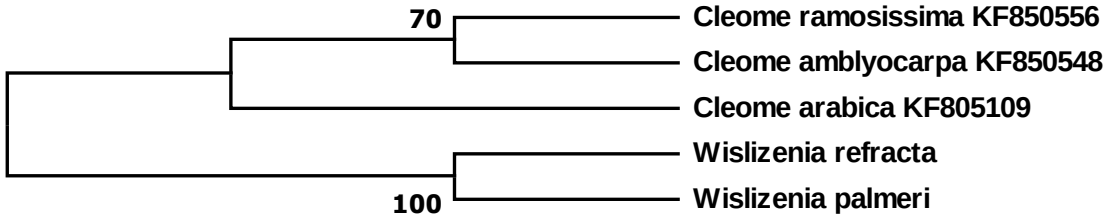

Figure 3. A phylogram generated based on nrDNA-ITS using the maximum parsimony method

The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of taxa analyzed (Felsenstein, 1985). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. There were 659 positions in total in the final dataset. Evolutionary analyses were conducted using the MEGA X tool (Kumar, 2018). All three species of genus *Cleome* showed closeness to each other as shown in Figs. 1, 2 and 3. The species *C. amblyocarpa* and *C. Ramosissima* showed more closeness based on ITS markers to each other compared to *C. arabica*.

The ITS markers have already been used successfully for identification of *Ochradenus arabicus* (Resedaceae), an endemic medicinal plant of Saudi Arabia (Khan et al., 2012). Similarly, phylogenetic relationship was worked out using the nrDNA-ITS sequence in *Senecio asirensis*, *Nepeta deflersiana* and some rare species of Saudi Arabia (Khan et al., 2013; Al-Qurainy et al., 2013; 2014). The development of ITS marker is easy, reproducible and can be developed from a small amount of tissue.

Conclusion

The ITS markers developed here for the *Cleome* species, could be used for the identification of other species using the fresh or dry plant material collected from natural habitats or purchased from herbal markets. However, DNA based markers (nrDNA-ITS) are reproducible compared to morphological and biochemical markers. Thus, such markers should be effectively developed for other plant species for their identification and phylogenetic studies.

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