Bangladesh J. Plant Taxon. **28**(1): 125–130, 2021 (June) https://doi.org/10.3329/bjpt.v28i1.54212

MOLECULAR AUTHENTICATION OF *EUPHORBIA SCHIMPERIANA* SCHEELE USING INTERNAL TRANSCRIBED SPACER SEQUENCES OF NUCLEAR RIBOSOMAL DNA

MESFER M. ALQAHTANI[1*], M. AJMAL ALI[2*], M. OLIUR RAHMAN[3], FAHAD M. AL-HEMAID, SIDANAND V. KAMBHAR[4] AND JOONGKU LEE[5]

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

Keywords: Molecular signature; *Euphorbia schimperiana*; ITS; nrDNA; Phylogenetic relationships.

Abstract

The Internal Transcribed Spacers (ITS) sequences of nuclear ribosomal DNA (nrDNA) are commonly used in plant molecular phylogenetics for the molecular based taxonomic identification and DNA barcoding because of shorter length and easy to amplify by using the universal primers, and further has discrimination ability to distinguish the taxon at lower taxonomic level. The present molecular phylogenetic analysis of ITS nrDNA sequences focuses to determine the taxonomic status of an unresolved medicinally important species *Euphorbia schimperiana* Scheele of the family Euphorbiaceae reported from Saudi Arabia. The combined length of the entire ITS region in *E. schimperiana* is 644 nucleotides. The study reveals that *E. schimperiana* shows a close proximity with the members of the subgenus *Esula.*

Introduction

The Euphorbiaceae is a large family of flowering plants with about 300 genera and 7,500 species. The genus *Euphorbia* L. *sensu lato* belonging to the family Euphorbiaceae comprises nearly 2,000 recognized taxa with global distribution. It is considered as the largest genus of flowering plants (Govaerts *et al*., 2000; Frodin, 2004). In Saudi Arabia, *Euphorbia* is represented by 42 species (Abedin *et al*., 2001). The four main molecular phylogenetic studies of *Euphorbia* to date have revealed the overall phylogeny of the genus, with a major point of consensus being the recognition of four subgeneric clades: *Rhizanthium*, *Esula*, *Euphorbia*, and *Chamaesyce* (Steinmann and Porter, 2002; Bruyns *et al*., 2006; Park and Jansen, 2007; Zimmermann *et al*., 2010).

The Internal Transcribed Spacers (ITS) of Nuclear Ribosomal DNA (nrDNA) in plants is being extensively used for phylogenetic studies, molecular discrimination of raw drug material and DNA barcoding (Ali *et al*., 2014). The DNA sequence of *Euphorbia schimperiana* has not been done before and is not available in the GenBank, moreover, the molecular evolutionary

[1]Department of Biological Sciences, Faculty of Science and Humanities, Shaqra University, P.O. Box 1040, Ad-Dawadimi 11911, Saudi Arabia (mesferalqahtani@hotmail.com)
[2]Corresponding author. Email: ajmalpdrc@gmail.com, majmalaliksu@gmail.com
[3]Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh
[4]Post Graduate Department of Botany, KLE Society's, Basavaprabhu Kore College, Chikodi-591 201, Belagavi, Karnataka, India
[5]Department of Environment and Forest Resources, Chungnam National University, Daehak-ro, Yuseong-gu, Daejeon, Republic of Korea
*The first and second authors contributed equally to this study

relationships of the Saudia Arabian *E. schimperiana* is lacking; thus molecular evolutionary study on *E. schimperiana* from Saudi Arabia is very much needed. Hence, this study has been undertaken to determine evolutionary relationships and molecular signature of the medicinally important *E. schimperiana* based on nrDNA ITS sequences.

Materials and Methods

Plant materials:

Leaf material of *E. schimperiana* was collected from the herbarium specimen [Voucher information: Al-Baha, 26.10.1978, A. R. Dawood *s.n.* (RIY)] lodged at National Herbaium and GenBank, National Agriculture and Animal Resources Research Center, Ministry of Agriculture, Riyadh, Saudi Arabia, and the taxonomic identification of the species was confirmed through the consultation of Flora of Saudi Arabia (Abedin *et al.*, 2001).

Extraction of genomic DNA, amplification and sequencing of nrDNA ITS gene:

The leaf material was crushed with liquid nitrogen using 'Qiagen Tissue Lyser' (# 85300). The robotic workstation 'QIAcube' (# 9001292) using 'DNeasy Plant Mini Kit' (# 69104) was used for automated purification of the total genomic DNA. The nuclear ribosomal DNA ITS sequences (ITS1-5.8S-ITS2) were amplified in the thermal cycler (Applied Biosystems Veriti) via Polymerase Chain Reaction (PCR) using the primers (White *et al.*, 1990) [forward primer ITS1 (5'GTCCACTGAACCTTATCATTTAG3') and the reverse primer ITS4 (5'TCCTCCGCTTATT GATATGC3')] and PCR Mix (# K-2011, Bioneer, Daejeon, Republic of Korea). The DNA sequencing of the amplified product was performed using kit (# 4337455, BigDye Terminator cycle sequencing kit, Perkin-Elmer, Applied Biosystems) in DNA Analyzer (Perkin-Elmer, Applied Biosystems, # ABI PRISM 3730XL).

Phylogenetic analyses:

ITS sequences of nrDNA of 34 species of the genus *Euphorbia* including two sequences of Outgroup (Table 1) were retrieved from GenBank database of National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The sequence alignment was performed using Clustal X version 1.81 (Thompson *et al.*, 1997), and then the alignment was subsequently adjusted manually using BioEdit (Hall, 1999).

The gaps in the sequence alignment were treated as missing data in phylogenetic analysis. The sequence generated in the present study was submitted to NCBI GenBank (accession number KC432622). The Maximum Parsimony (MP) analysis with 1000 bootstrap replicates was performed using MEGA X (Kumar *et al.*, 2018).

Results and Discussion

The combined length of the entire ITS region (ITS1, 5.8S and ITS2) in *Euphorbia schimperiana* was 644 nucleotides. The length of the ITS1 region and GC contents were 256 nucleotides and 63% respectively, the 5.8S gene was 162 nucleotides long, and the length of the ITS2 region and the GC contents were 226 nucleotides and 68% respectively. The length of the ITS1 region and GC contents in *E. schimperiana* was found consistent with some other earlier studies on the family Euphorbiaceae (Steinmann and Porter, 2002; Barres *et al.*, 2011).

The parsimony analysis of the whole ITS region resulted into two maximally parsimonious trees (MPTs) with a total length of 1,335 steps, a consistency index (CI) of 0.495 (0.490 CI excluding uninformative characters), a homoplasy index (HI) of 0.522 (0.510 HI excluding uninformative characters), rescaled consistency index (RC) of 0.362 and a retention index (RI) of 0.731. One of the MPTs is shown in Fig. 1 in which the numbers above the lines indicate the

bootstrap support in 1000 replicates. The taxa included in the analyses are from all the four subgenera of *Euphorbia* i.e. *Rhizanthium*, *Esula*, *Euphorbia*, and *Chamaesyce*. A perusal of phylogenetic tree clearly indicates that the ingroup is monophyletic, and all the subgeneric clades are well resolved with strong bootstrap support, and *E. schimperiana* nested within the clade of the subgenus *Esula* (Fig. 1).

Table 1. List of taxa used for phylogenetic analyses with accession number retrieved from NCBI GenBank.

Group	Subgenus		Taxon	GenBank Accession number
Ingroup	*Rhizanthium*	1.	*Euphorbia antso* Denis	AF537579
		2.	*E. atrispina* N.E. Br.	AF537568
		3.	*E. balsamifera* Ait.	AF537571
		4.	*E. clava* Jacq.	AF537569
		5.	*E. namuskluftensis* L.C. Leach	AF537562
		6.	*E. obesa* Hook. f.	AF537566
	Esula	7.	*E. aphylla* Brouss. *ex* Willd.	AF537540
		8.	*E. characias* L.	GU984304
		9.	*E. dendroides* L.	AF537539
		10.	*E. exigua* L.	GU984325
		11.	*E. mauritanica* L.	AF537531
		12.	*E. orthoclada* Baker	DQ204876
		13.	*E. peplus* L.	AF537532
		14.	*E. regis-jubae* J. Gay	AF537541
		15.	*E. schimperi* C. Presl	AF537537
		16.	*E. schimperiana* Scheele	JN207816
	Euphorbia	17.	*E. abdelkuri* Balf. f.	AF537458
		18.	*E. beharensis* Leandri	AJ508983
		19.	*E. cylindrifolia* Marn.-Lap. & Rauh	AJ508955
		20.	*E. drupifera* Thonn.	AF537480
		21.	*E. epiphylloides* Kurz	AF537484
		22.	*E. milii* Des Moul.	AJ508974
		23.	*E. ramipressa* Croizat	AF537481
		24.	*E. teke* Schweinf. *ex* Pax	AF537485
	Chamaesyce	25.	*E. fulgens* Karw. *ex* Klotzsch	AF537404
		26.	*E. graminea* Jacq.	AF537410
		27.	*E. heterophylla* L.	GU214931
		28.	*E. ipecacuanhae* L.	AF537397
		29.	*E. leucocephala* Lotsy	GU214932
		30.	*E. misera* Benth.	AF537383
		31.	*E. pulcherrima* Willd. *ex* Klotzsch	GU214943
		32.	*E. sphaerorhiza* Benth.	AF537412
Outgroup		33.	*Dichostemma glaucescens* Pierre	AF537584
		34.	*Neoguillauminia cleopatra* (Baill.) Croizat	AF537581

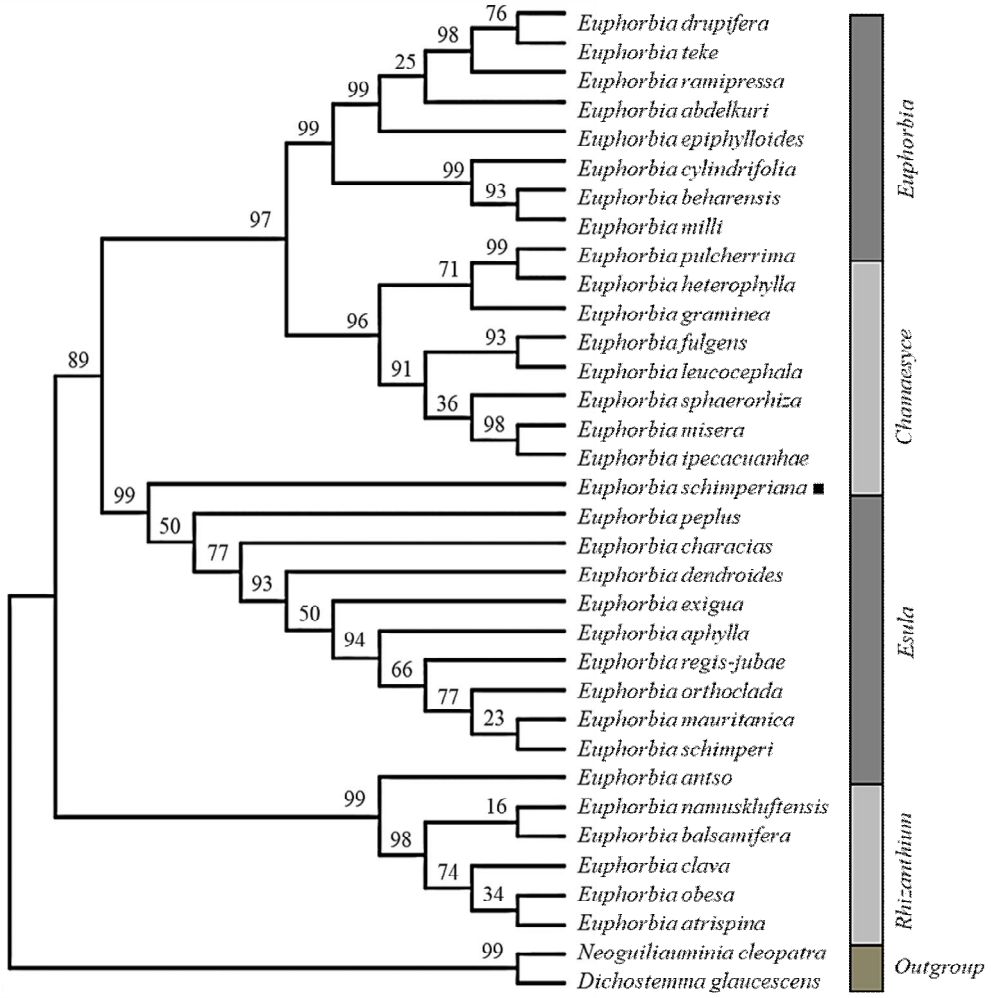

Fig. 1. Molecular phylogenetics of *Euphorbia schimperiana* inferred from nrDNA ITS sequences using the Maximum Parsimony method.

In the present investigation of the nrDNA ITS sequence of *E. schimperiana* with the members of sect. *Tirucalli,* subsect. *Pachycladae,* sect. *Aphyllis,* sect. *Cymatospermum,* sect. *Esula,* sect. *Paralias,* sect. *Chylogala,* sect. *Helioscopia* and sect. *Myrsinites* belonging to the subgenus *Esula* reveals the grouping of the taxon in the phylogenetic tree according to previously recognized sections of the subgenus *Esula,* and this result is found to be congruent with the previous study of molecular phylogeny of *Euphorbia* subg. *Esula* sect. *Aphyllis* (Barres *et al*., 2011) based on nrDNA and cpDNA markers. In the present study, *E. schimperiana* shows a close proximity with the members of the subgenus *Esula*.

This is the first report of inferring the nrDNA ITS based phylogenetic relationships and establishment of molecular signature of the *E. schimperiana,* a medicinally important plant reported to be used as a laxative and vermifuge (Abulafatih, 1987). Recently, four bioactive

compounds were isolated from *E. schimperiana* and the species was found to possess potential antioxidant activity (Shaker *et al*., 2015). Therefore, the molecular authentication of *E. schimperiana* will be of immense importance in molecular validation of raw herbal drug material.

The proper identification of medicinal plants is required to ensure the purity, quality and safety of drugs (Jayasinghe *et al.*, 2009). Hence, in addition to the morpho-taxonomical key based conventional methods of identification of raw plant drug materials, the DNA-based methods have been developed for the proper identification of medicinal plants (Sucher and Carles, 2008). The attempts are being made to use several candidate DNA barcode regions to identify species. In absence of a universal plant DNA barcode as in animal systems, a number of candidate genes located in the chloroplast genome such as *psb*A-*trn*H have been suggested to be used as DNA barcodes (Kress *et al.*, 2005; Shaw *et al.*, 2005; Chase *et al*., 2007; Kress and Erickson, 2007). The ITS2 region has been suggested to use as a standard DNA barcode (Chen *et al.*, 2010; Yao *et al.*, 2010). The assessments of 871 species in 66 genera of the family Euphorbiaceae have demonstrated that ITS/ITS2 is a potential barcode in delimitation of Euphorbiaceous species (Pang *et al.*, 2010), and in our study ITS has been found instrumental in molecular signature of *E. schimperiana*.

Acknowledgement

Joongku Lee is thankful to Chungnam National University, Daejeon, Republic of Korea for the support.

References

Abedin, S., Mossa, J.S., Al-Said, M.S. and Al-Yahya, M.A. 2001. Euphorbiaceae. *In*: Chaudhary, S. (Ed.), Flora of Saudi Arabia. Ministry of Agriculture and Water, National Herbarium, National Agriculture and Water Research Center, Riyadh, Saudi Arabia, **II**(1): 291–395.

Abulafatih, H.A. 1987. Medicinal Plants in Southwestern Saudi Arabia. Economic Botany **41**(3): 354–360.

Ali, M.A., Gyulai, G., Norbert, H., Balázs, K., Al-Hemaid, F.M.A., Pandey, A.K. and Lee, J. 2014. The changing epitome of species identification - DNA barcoding. Saudi J. Biol. Sci. **21**(3): 204–231.

Barres, L., Vilatersana, R., Molero, J., Susanna, A. and Galbany-Casals, M. 2011. Molecular phylogeny of *Euphorbia* subg. *Esula* sect. *Aphyllis* (Euphorbiaceae) inferred from nrDNA and cpDNA markers with biogeographic insights. Taxon **60**: 705–720.

Bruyns, P.V., Mapaya, R.J. and Hedderson, T. 2006. A new subgeneric classification for *Euphorbia* (Euphorbiaceae) in southern Africa based on ITS and *psbA-trnH* sequence data. Taxon **55**: 397–420.

Chase, M.W., Cowan, R.S., Hollingsworth, P.M., Berg, C.V.D., Madriñán, S., Petersen, G., Seberg, O., Jørgsensen, T., Cameron, K.M., Carine, M., Pedersen, N., Hedderson, T.A.J., Conrad, F., Salazar, G.A., Richardson, J.E., Hollingsworth, M.L., Barraclough, T.G., Kelly, L. and Wilkinson, M. 2007. A proposal for a standardised protocol to barcode all land plants. Taxon **56**: 295–299.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. and Leon, C. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS ONE **5**(1): e8613.

Frodin, D.G. 2004. History and concepts of big plant genera. Taxon **53**: 753–776.

Govaerts, R., Frodin, D.G. and Radcliffe-Smith, A. 2000. World checklist and bibliography of Euphorbiaceae (with Pandaceae), Vol. **4**. Royal Botanic Gardens, Kew, UK, 415 pp.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acid Symp. Ser. **41**: 95–98.

Jayasinghe, R., Niu, L.H., Coram, T.E., Kong, S., Kaganovitch, J., Xue, C.C.L., Li, C.G., Pang, E.C.K. 2009. Effectiveness of an innovative prototype subtracted diversity array (SDA) for fingerprinting plant species of medicinal importance. Planta Medica **75**: 1180–1185.

Kress, W.J. and Erickson, D.L. 2007. A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. PLoS ONE **2**: e508.

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. and Janzen, D.H. 2005. Use of DNA barcodes to identify flowering plants. Proc. Nat. Acad. Sci. USA **102**: 8369–8374.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. **35**(6): 1547–1549.

Pang, X., Jingyuan, S., Yingjie, Z., Caixiang, X. and Shilin, C. 2010. Using DNA barcoding to identify species within Euphorbiaceae. Planta Medica **76**(15): 1784–1786.

Park, K.R. and Jansen, R.K. 2007. A phylogeny of Euphorbieae subtribe Euphorbiinae (Euphorbiaceae) based on molecular data. J. Plant Biol. **50**: 644–649.

Shaker, K.H., Al Shehri, B.M., Oteef, M.D.Y. and Mahmoud, M.F. 2015. Antioxidant compounds from *Euphorbia schimperiana* Scheele in Aseer Region, Saudi Arabia. Int. J. Pharm. Sci. Rev. Res. **32**(1): 117–122.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W.S., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. and Small, R.L. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am. J. Bot. **92**: 142–166.

Steinmann, V.W. and Porter, J.M. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndh*F sequence data. Ann. Miss. Bot. Gard. **89**: 453–490.

Sucher, N.J. and Carles, M.C. 2008. Genome-based approaches to the authentication of medicinal plants. Planta Medica **74**: 603–623.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, G.D. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24**: 4876–4882.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninksky, J.J. and White, T.J. (Eds), PCR protocols: a guide to method and amplifications. Academic Press, San Diego, California, pp. 315–322.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P. and Chen, S. 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS ONE **5**(10): e13102.

Zimmermann, N.F.A., Ritz, C.M. and Hellwig, F.H. 2010. Further support for the phylogenetic relationships within *Euphorbia* L. (Euphorbiaceae) from nrITS and *trn*L-*trn*F IGS sequence data. Pl. Syst. Evol. **286**: 39–58.

(Manuscript received on 9 March, 2021; revised on 19 May, 2021)