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DNA BARCODING FOR DISCRIMINATION OF *CHROZOPHORA OBLONGIFOLIA* AND *RICINUS COMMUNIS* FROM TAIF, KSA

SHAWKAT M. AHMED[1,2*] AND AHMED NOURELDEEN[1,3]

[1]*Biology Department, Faculty of Science, Taif University, Taif, Saudi Arabia*
[2]*Biology Department, Faculty of Education, Ain Shams University, Cairo, Egypt*
[3]*Plant Protection, Faculty of Agriculture, Mansoura University, Mansoura, Egypt*
[*]*Corresponding author's email: s.shawkat@tu.edu.sa, Ph: +966535923238*

Abstract

The use of highly discriminative approaches for the identification and characterization of genotypes (especially with known medicinal value) is essential for plant conservation and appropriate use. Therefore, molecular characterization and phylogenetic analysis of *Chrozophora oblongifolia* L. and *Ricinus communis* L. collected from Taif were presented using ITS, ITS2, *rbcLa* and *matK* sequences. *rbcLa* region recorded higher percentage of the variable sites within the two species. Tajima test and Transition/Transversion bias (R) showed no molecular evolution within *C. oblongifolia* genome, whereas, they indicated an accelerated evolution within *R. communis* genome. The intraspecific divergence of ITS and ITS2 was lower than that of *matK* and *rbcLa* loci. The phylogentic analysis proved that the *rbcLa* site showed a higher intraspecific difference than ITS2 in *C. oblongifolia.* Although, accessions of *R. communis* from the same country or continent showed limited genetic diversity, they did not get together and revealed intraspecific divergence. For a rapid and accurate identification of Euphorbiaceous species having medicinal importance, our analyses enforced the employment of the ITS2 mini-barcode as a universal barcode.

Key words: *Chrozophora*; *Ricinus*; ITS; ITS2; *matK*; *rbcLa*.

Introduction

DNA barcoding is an accurate taxonomic method using one or a few short, standardized DNA loci. The internal transcribed spacer (ITS) locus as a complementary marker and *rbcLa* + *matK* as core barcodes were selected as the standard barcode for the identification and characterization of plants. However, the three-barcode system encountered some difficulties in identifying other plants, especially medicinal species (Hebert *et al*., 2003; Hollingsworth *et al.,* 2009). Among these selected barcoding loci and due to its high rate of successful amplification and discrimination power among all tested regions, the ITS2 site was considered as a helpful minibarcode for projects involving a large number of environmental samples as mentioned by Chen *et al*., (2010) and Tang *et al*., (2016). Successful characterization and identification for a target species subsequently depends on a comprehensive DNA barcode database using samples from several geographic sites.

Genus *Chrozophora* comprises 7-8 species belonging to family Euphorbiaceae, that are generally herbs and shrubs. This genus exists in Mediterranean regions, West Africa and South Asia and possess several biological activities such as *C. oblongifolia* has antimicrobial and antioxidant activity. The whole plant is utilized for wounds to improve healing, other parts such as seeds and leaves are utilized as a laxative, a depurative agent and in treating skin diseases. In Saudi Arabia, the plant is also used against purifying blood and jaundice (Ramzi *et al*., 2011; Dipankar *et al*., 2011). Compared to other medicinal plants, it is clear that the genetics of *C. oblongifolia* has not been studied much.

On the other side, the castor bean (*Ricinus communis*) occurs in subtropical and tropical countries as an oilseed crop. It was thought that *R. communis* has originated in eastern Africa (Vavilov, 1951; Zeven & Zhukovsky, 1975). Castor oil is an essential industrial raw material for great products such as lubricants, coatings, paints, soaps, plastics, cosmetics, and medications for skin affections (Brigham, 1993). Castor seeds contain poison ricin, ribosome-inactivating proteins (RIPs), that can be extracted and used as a bioweapon, moreover, castor oil has been sophisticated as a component of biodiesel in Brazil (Endo *et al*., 1987; da Silva *et al*., 2006). Its plants are considered invasive weeds because they spread in indiscriminate areas such as river banks, roadsides and the agricultural field fringes. *R. communis* is considered as self- pollinated or cross-pollinated by wind (Meinders & Jones, 1950; Brigham, 1967), with outcrossing the dominant reproduction method. Although, *R. communis* is an agro-economically important plant, it was clear that it possesses low genetic diversity and no geographic patterns of genetic association based on several molecular markers such as SSR, SNP, EST-SSR, AFLP, SCOT, RAPD, ISSR and TRAP that have been used in assessing its genetic diversity (Allan *et al*., 2008; Bajay *et al*., 2009; Foster *et al*., 2010; Kallamadi *et al*., 2015; Thatikunta *et al*., 2016; Wang *et al*., 2017; Simões *et al*., 2017). However, Agyenim-Boateng indicated that the wild samples of *R. communis* in Southern China were rich in the genetic variability through SRAP analysis and their clusters were closely related to regions in contrast to previous results which showed the absence of a geographically organized genetic population (Agyenim-Boateng *et al*., 2019). *R. communis* genetics still needs further molecular study through DNA barcoding approach.

Given all of the above, our objectives of this study were: 1) to assess molecular characterization for *C. oblongifolia* and *R. communis* collected from Taif using four DNA barcodes; 2) to determine the genetic variance on the basis of its ancestry; 3) to examine evolutionary rate of the two species under study.

Materials and Methods

Plant materials: *C. oblongifolia* and *R. communis* (family Euphorbiaceae) were collected from the highlands of Taif, KSA. They were identified according to Collenette (1999).

Extraction of DNA and The PCR sequencing: Young leaves were utilized for the extracting of the genomic DNA depending on CTAB method (Doyle & Doyle, 1987). The primers for the four loci (ITS, ITS2, matK and rbcL) were introduced in Table 1. PCR products of the four DNA barcodes of *C. oblongifolia* and *R. communis* were sequenced at Macrogen Inc., South Korea. All obtained sequences were submitted to NCBI-GenBank (Their accessions are mentioned in Table 2).

Statistics and phylogenetic trees: Sequences of *C. oblongifolia* and *R. communis* were subjected to BLAST to retrieve the most related species of Euphorbiacea from GenBank database of NCBI. In total, loci of ITS, ITS2, *matK* and *rbcLa* retrieved 178 accessions from GenBank database, 4 accessions were belonging to *C. oblongifolia* and 129 accessions for *R. communis* (Table 2), while the others were different Euphorbiaceous species. For alignment, MUSCLE method was performed (Edgar, 2004; Tamura *et al*., 2013). The statistics; sequence length, (%) variable sites, Average evolutionary divergence and transition/transversion bias (R) and nucleotide exchange rates were calculated by Maximum Likelihood method (Tamura *et al*., 2013) utilizing MEGA6 software. 1000 bootstrap replicates have been performed through the phylogenic trees building (Saitou & Nei, 1987) using the Neighbor-Joining method.

Table 1. List of DNA barcoding primers.

Locus	Primer name		Primer sequences (5'–3')	Ann. temp.
ITS	AB101	F	ACGAATTCATGGTCCGGTGAAGTGTTCG	52˚C
	AB102	R	TAGAATTCCCCGGTTCGCTCGCCGTTAC	
ITS2	ITS-S2F	F	ATGCGATACTTGGTGTGAAT	52˚C
	ITS4	R	TCCTCCGCTTATTGATATGC	
rbcLa	rbcLa	F	ATGTCACCACAAACAGAGACTAAAGC	52˚C
	rbcLa	R	GTAAAATCAAGTCCACCRCG	
matK	matK-KIM1	F	ACCCAGTCCATCTGGAAATCTTGGTTC	52˚C
	matK-KIM3	R	CGTACAGTACTTTTGTGTTTACGAG	

Table 2. GenBank accessions numbers of *C. oblongifolia* and *R. communis.*

Species	ITS	ITS2	*matK*	*rbcLa*
Chrozophora	-	LC503611	-	LC503612
Ricinus	LC503619	LC503620	LC503621	LC503622

Results and Discussion

***Chrozophora oblongifolia*:** For molecular characterization of *C. oblongifolia*, the length of sequence, variable sites % and ratio of GC of the two candidate sites (ITS2 and *rbcLa*) were obtained. After BLAST, ITS2 retrieved three accessions from Saudi Arabia, whereas, *rbcLa* sequence retrieved only one accession from Japan.

Statistical data revealed that sequence length of *rbcLa* was greater than that of ITS2, whereas its GC ratio was lower than ITS2. The percentage of variable sites was higher in *rbcLa* region and subsequently it was more divergent than ITS2 (Table 3). ITS2 locus reported that transitions did not occur, whereas transversions ranged from 9.13 to 16.61 with Transition/Transversion bias (R) equal to 0.00 (Table 4) demonstrating very little or no molecular evolution within *C. oblongifolia* genome. Moreover, the relative evolutionary rate test of Tajima (Tajima, 1993) confirmed the previous result by accepting the null hypothesis of equal evolution rates between *C. oblongifolia* from Taif and the other related accessions collected from different regions of Saudi Arabia (P-value was higher than 0.05) (Table 5). We did not obtain sufficient data about *rbcLa* region because there was only one accession retrieved from the Genbank.

The genetic variation analysis of plant species and their relatives from other countries is a critical aspect of conserving biodiversity. Advanced sequencing technologies can achieve complete screening of plant biodiversity, because they discover and test a major number of molecular markers at a comparatively low price. These markers have been widely utilized in species with or without an available reference genome for genomic selection, linkage map structure and the investigation of plant genetic diversity (Verma *et al*., 2015; Pavan *et al*., 2017).

Here, ITS2 and *rbcLa* sequences represented the genetic divergences of *C. oblongifolia* and its related accessions retrieved from GenBank. The phylogentic tree of ITS2 demonstrated that all *C. oblongifolia* collected from KSA were grouped together (Fig. 1), whilst *rbcLa* tree exhibited intraspecific divergence between the two accessions from Taif and Japan (Fig. 2). The phylogentic analysis showed that the *rbcLa* site exhibited a higher intraspecific variance than ITS2 This reflected the benefit of using ITS2 due to its conserved secondary structure which is related to the low intraspecific variability (Keller *et al*., 2010).

Table 3. Statistics of DNA barcodes for *C. oblongifolia* and *R. communis*.

Parameter	Species	ITS	ITS2	*matK*	*rbcLa*
Sequence length	*Chrozophora*	-	323	-	523
	Ricinus	620	289	770	517
GC ratio	*Chrozophora*	-	63.0	-	45.0
	Ricinus	56.0	60.0	32.0	46.0
Number of the related accessions retrieved from the GenBank	*Chrozophora*	-	3	-	1
	Ricinus	28	51	28	22
% Variable sites after alignment within all related species	*Chrozophora*	-	0.13	-	0.48
	Ricinus	13.0	0.01	10.1	14.7
Average evolutionary divergence over all sequence pairs (the overall mean distance)	Chrozophora	-	0.00	-	0.01
	Ricinus	0.01	0.01	0.12	0.18

Table 4. Mean nucleotide substitution rates in the four loci for *C. oblongifolia* and *R. communis.*

Species	Locus	Transition				Transversion				(R)
		A→G	G→A	T→C	C→T	A or G→T	T or C→A	A or G→C	T or C→G	
C. oblongifolia	ITS2	0.00	0.00	0.00	0.00	9.13	9.25	16.61	15.01	0.00
R. communis	ITS	17.6	11.3	11.3	6.49	4.61	5.49	8.02	8.54	0.84
	ITS2	41.8	28.6	17.8	11.8	0.00	0.00	0.00	0.00	3657.3
	matK	8.56	19.9	8.06	18.6	8.38	7.28	3.62	3.13	1.04
	rbcLa	16.6	18.4	14.3	18.5	4.60	4.18	3.56	3.77	2.08

Table 5. Tajima relative rate tests for *C. blongifolia* and *R. communis*.

Species	Locus	Outgroup	Test group		RI	RD	RA	RB	$\chi 2$	*P* value
			(A)	(B)						
C. oblongifolia	ITS2	Riyadh	Taif	Makkah-Almadinah Road	320	0	0	0	0.00	>0.05
		Makkah-Almadinah Road	Taif	Riyadh	320	0	0	1	1.00	>0.05
R. communis	ITS	Indonesia	Taif	India	367	0	59	0	59.0	<0.05
		UK	Taif	Switzerland	219	0	17	5	6.55	<0.05
		KSA	Taif	China	496	1	93	0	93.0	<0.05
	ITS2	KSA	Taif	Yemen	283	0	0	2	2.00	>0.05
		Lebanon	Taif	UAE	287	0	0	0	0.00	>0.05
		Indonesia	Taif	India	143	0	2	1	0.33	>0.05
		Tunisia	Taif	Egypt	285	0	1	1	0.00	>0.05
		UK	Taif	Switzerland	209	0	3	0	3.00	>0.05
		Venezuela	Taif	USA	276	0	1	1	0.00	>0.05
	matK	India	Taif	Yemen	374	0	228	0	228.0	<0.05
		India	Taif	Pakistan	390	0	240	0	240.0	<0.05
		China	Taif	Japan	390	0	240	0	240.0	<0.05
		Tunisia	Taif	Egypt	369	0	224	1	221.0	<0.05
		South Africa	Taif	East African savanna	388	0	240	0	240.0	<0.05
		Venezuela	Taif	USA	372	0	227	1	224.0	<0.05
		Costa Rica	Taif	Cuba	363	0	225	0	225.0	<0.05
	rbcLa	India	Taif	Pakistan	496	1	16	0	16.0	<0.05
		China	Taif	Japan	495	0	16	1	13.24	<0.05
		South Africa	Taif	Egypt	495	1	16	0	16.0	<0.05
		Costa Rica	Taif	USA	496	0	17	0	17.0	<0.05

- The Tajima relative rate test was performed to test the equality of evolutionary rate of *Chrozophorao blongifolia* and *Ricinus communis* and the other related species depending on different outgorups
- RI represents the identical regions in sequences, RD represents the divergent regions in the three sequences, RA represents the unique differences in the sequence A, RB represents the unique differences in the sequence B
- $\chi 2$ greater than 3.841 ($p<0.05$) points out the acceleration in evolution, *P* value (>0.05) refuse evolution (acceptation of the null hypothesis of equal rates between lineages)

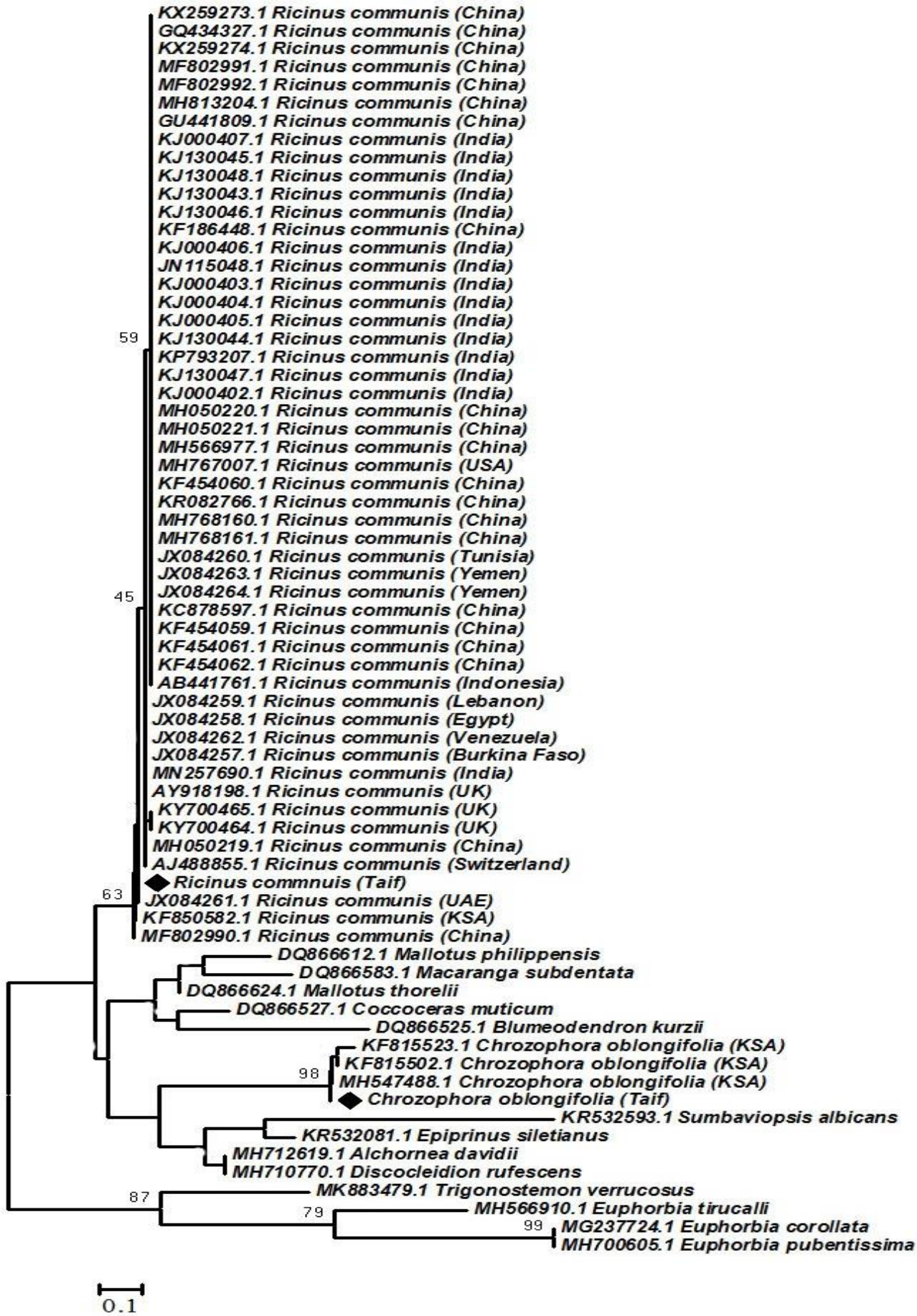

Fig. 1. Phylogenetic analysis of *C. blongifolia* and *R. communis* based on ITS2 by Neighbor-Joining method.

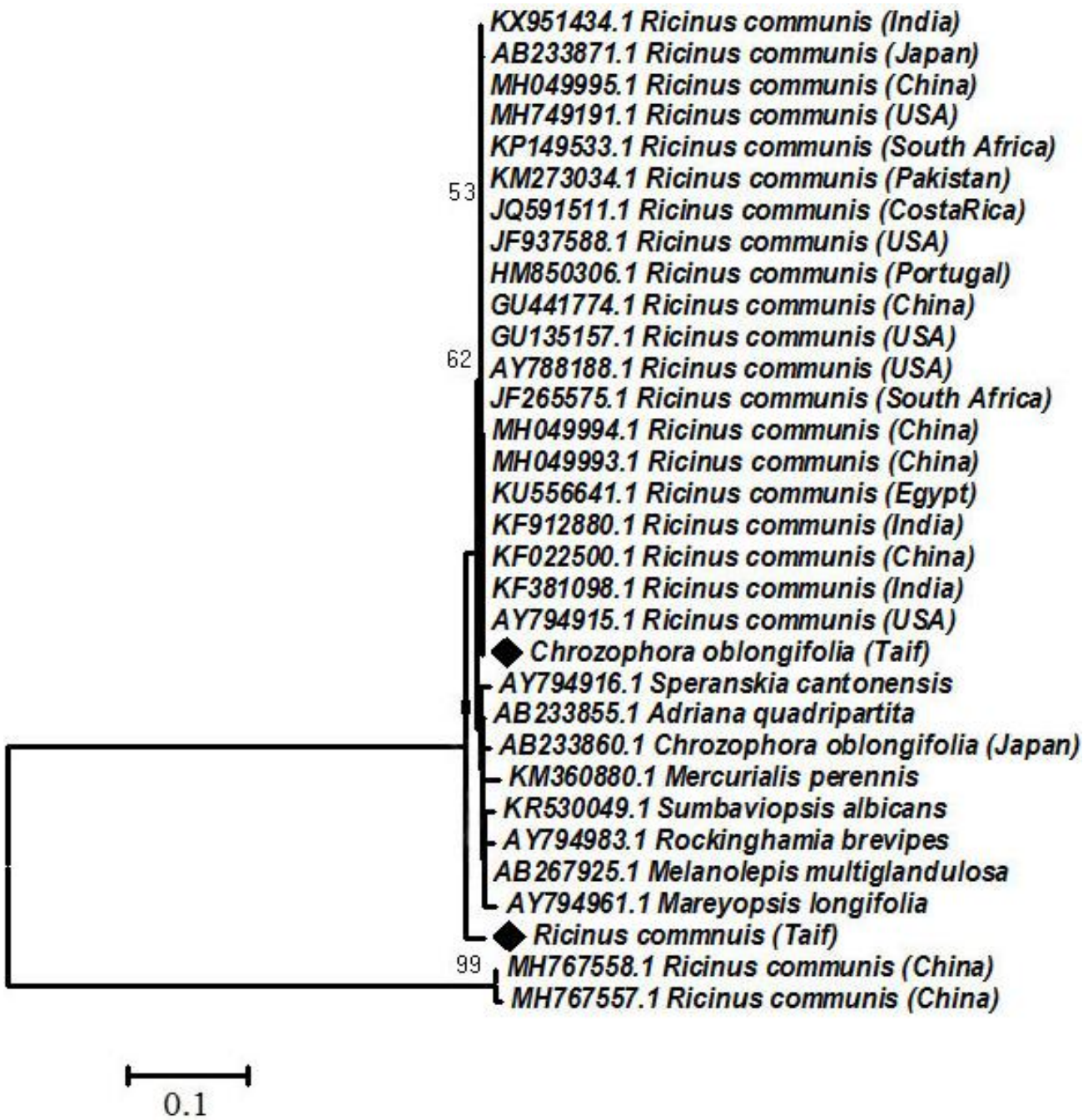

Fig. 2. Phylogenetic analysis of *C. blongifolia* and *R. communis* based on *rbcLa* by Neighbor-Joining method.

***Ricinus comuunis*:** The sequence of *R. communis* genome represents an important resource for studying the genome evolution (Musshoff & Madea, 2009). *R. communis* from Taif highlands was examined to determine and compare the efficiencies of PCR and sequencing of ITS, ITS2, *matK* and *rbcLa* for molecular characterization. After BLAST, the four regions retrieved 28, 51, 28 and 22 accessions belonging to *R. communis* from various countries. The sequence length, GC ratio and variable sites % of the four candidate loci were obtained from the MUSCLE alignment results (Table 3). Statistics showed that *matK* recorded the highest sequence length (770 bp) followed by ITS (620 bp), rbcLa (517 bp), ITS2 (289 bp). ITS and ITS2 had the highest GC ratio. *rbcLa* region recorded higher percentage of the variable sites than the others and was subsequently more divergent than that of the other sequences.

All sequences reported that transition rates occurred more than those of transversion (Table 4) and Transition/ Transversion bias (R) values demonstrated a noticeable molecular evolution within *R. communis* genome collected from Taif. The results of Tajima relative evolutionary rate for ITS, *matK* and *rbcLa* were similar to those of the Transition/Transversion method. Except ITS2, the three loci reject the null hypothesis of equal evolution rates between *R. communis* from Taif and the other related accessions retrieved from GenBank ($p<0.05$) indicating accelerated evolution within *R. communis* genome (Table 5). This might be due to the outcrossing process through cross-pollination by wind. Outcrossing is believed to be the "norm" in the wild plants and is usually supported by self-incompatibility (Richards, 1996).

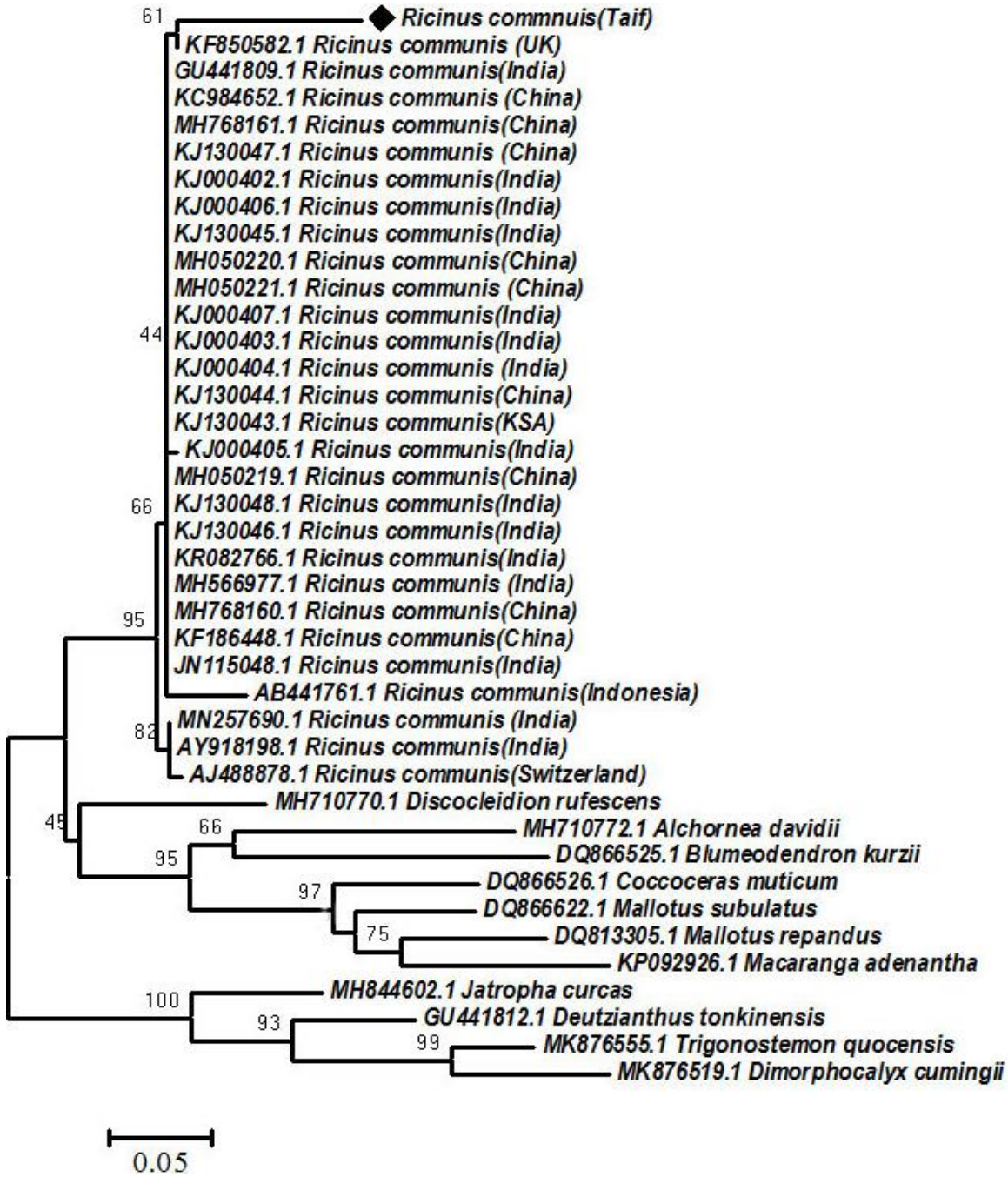

Fig. 3. Phylogenetic analysis of *R. communis* based on ITS by Neighbor-Joining method.

For phylogenetic analysis, four parameters represented the genetic differences of *R. communis* accessions. Within accessions of *R. communis*, the intraspecific distance (overall mean distance) of ITS and ITS2 was lower than that of *matK* and *rbcLa* loci (Table 3 & Figs. 1-4). According to the construction of the phylogenetic trees of ITS and ITS2 by the neighbor-joining method, all *R. communis* accessions were joined in a monophyletic clade and the other Euphorbiaceous species were divided into three clades. Accessions belonging to the Arabian Peninsula, Africa, China and India revealed obvious intraspecific divergence. Based on the ITS2 phylogenetic tree, *R. communis* (Taif) occurred in a separate sub-clade showing little variability from the other origins (Fig. 1), whereas, it grouped with the accession from UK in ITS tree (Fig. 3). On the contrary, *R. communis* accessions were divided into two groups based on the construction of the phylogenetic trees of *rbcLa* and *matK* (Figs. 2 & 4). The first one gathered accessions retrieved from GenBank, and the other group combined *R. communis* (Taif) with accessions from USA and China. Although, accessions from the same country or continent showed limited genetic diversity, they did not get together and revealed intraspecific divergence. *R. communis* (Taif) was found in a separate sub-clade showing noticeable polymorphism from other accessions based on *rbcLa* sequence (Fig. 2). Our results based on DNA barcodes confirmed those of Allan *et al*., (2008), Foster *et al*., (2010) and others who used various molecular aspects that *R. communis* had low genetic diversity, may be due to population bottlenecks. Furthermore we detected some geographically based patterns of genetic relatedness.

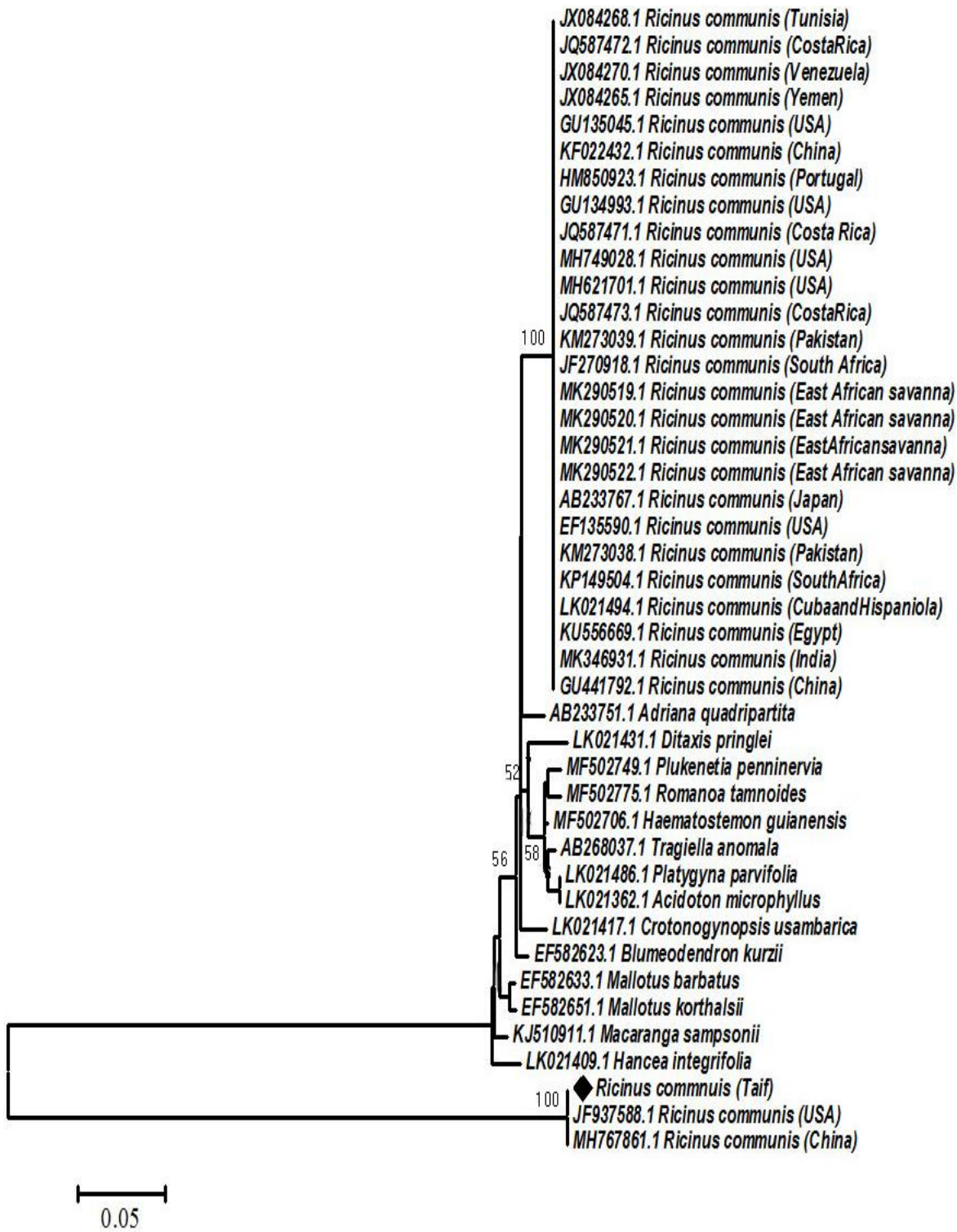

Fig. 4. Phylogenetic analysis of *R. communis* based on *matK* by Neighbor-Joining method.

Overall, characterization and phylogenetic analyses of the castor bean showed that limited genetic variation detected for nuclear genomic sequences (ITS and ITS2) was consistent with the low plastid genetic diversity (*rbcLa* and *matK)*. The genetic divergence distribution of ITS was compatible to that of ITS2 for *R. communis* and the other Euphorbiaceous species. They were divided into separate clades revealing the suitability of ITS and ITS2 as DNA barcodes for distinguishing different species of Euphorbiaceae. Although, the ITS locus contained enough variable sites for species identification in many specimens, it could not be amplified in *C. oblongifolia*, this may be due to the variability of ITS and including variable insertions or deletions at this taxonomic level. The Chinese Plant BOL Group considered ITS2 to be a useful alternative to ITS for easy amplification and sequencing. Thus, the ITS2 locus might be a suitable barcode for medicinal plants due to its secondary structure (Han *et al*., 2013).

Unfortunately, *rbcLa* and *matK* exhibited confused authentication power for various species of Euphorbiaceae. Both *rbcLa* and *matK* showed obvious difference of *C. oblongifolia* and *R. communis* (Taif) from their related accessions. Thus, they were somewhat unsuitable genetic loci for authentication of *C. oblongifolia* and *R. communis*, because of the absence of a clear intraspecific relatedness. Kress *et al*., mentioned that, although *rbcLa* as a source for barcoding and most commonly sequenced for phylogenetic studies, it has little contribution to species level identification. Moreover, universality of *matK* site was demonstrated to be low in some researches which may restrict its employment as a barcode. Although, high rates of nucleotide substitutions in *matK* made it a suitable barcode marker for species identification, but it was difficult to amplify and sequence them regularly across varied lineages (Anon., 2009; Kress *et al*., 2009).

Conclusions

Characterization of *C. oblongifolia* and *R. communis* from Taif was done depending on four specific loci (ITS, ITS2, *rbcLa* and *matK*). Based on statistical analyses and phylogenetic study using ITS2 sequence, we conclude that *C. oblongifolia* and *R. communis* were close to their related accessions from different locations. The short ITS2 site served as an effective barcode compared to the long ITS site in plant identification. Both *rbcLa* and *matK* revealed distinct divergence of *C. oblongifolia* and *R. communis* from the retrieved accessions. A combination of *rbcLa* and *matK* was recommended to be used as a barcode for Euphorbiaceous plants.

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References

Agyenim-Boateng, K.G., J. Lu, Y. Shi, D. Zhang and X. Yin. 2019. SRAP analysis of the genetic diversity of wild castor (*Ricinus communis* L.) in South China. *PLoS One*, 14(7): e0219667.

Allan, G., A. Williams, P.D. Rabinowicz, A.P. Chan, J. Ravel and P. Keim. 2008. Worldwide characterization of castor bean germplasm (*Ricinus Communis* L.) using AFLPs and SSRs. *Genet. Resour. Crop Evol*., 55: 365-378.

Bajay, M.M., J.B. Pinheiro, C.E.A. Batista, M.B.M. Nobrega and M.I. Zucchi. 2009. Development and characterization of microsatellite markers for castor (*Ricinus communis* L.), an important oleaginous species for biodiesel production. *Conserv. Genet. Resour*., 1(1): 237-239.

Brigham, R. 1967. Natural outcrossing in dwarf-internode castor. *Ricinus communis* L. *Crop Sci*., 7: 353-355.

Brigham, R. 1993. *Castor: Return of an old crop*. Wiley & Sons, New York, USA.

Anonymous. 2009. CBOL Plant Working Group. A DNA barcode for land plants. *Proc. Natl. Acad. Sci. USA*., 106: 12794-12797.

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

Collenette, S. 1999. *A Checklist of Botanical Species in Saudi Arabia*. International Asclepiad Society, Burgess Hill, England.

da Silva Nde, L., M.R. Maciel, C.B. Batistella and R.M. Filho. 2006. Optimization of biodiesel production from castor oil. *Appl. Biochem. Biotechnol*., 129-132: 405-414.

Dipankar, C., S. Murugan and P. Uma Devi. 2011. Review on Medicinal and Pharmacological Properties of Iresine Herbstii, *Chrozophora rottleri* and *Ecbolium linneanum*. *Afr. J. Trad. Complement. Alter. Med*., 8(S): 124-129.

Endo, Y., K. Mitsui, M. Motizuki and K. Tsurugi. 1987. The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. The site and the characteristics of the modification in 28 S ribosomal RNA caused by the toxins. *J. Biol. Chem*., 262: 5908-5912.

Foster, J., J.A. Gerard, P.C. Agnes, D.R. Pablo, R. Jacques, J.J. Paul and K. Paul. 2010. Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). *BMC Plant Biol*., 1-11.

Han, J., Z. Yingjie, C. Xiaochen, L. Baoshen, Y. Hui, S. Jingyuan, C. Shilin and M. Fanyun. 2013. The Short ITS2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length ITS. *Biol. Med. Res. Int*., pp. 7.

Hebert, P., A. Cywinska, L.B. Shelley and R.D. Jeremy. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond*., B 270: 313-321.

Hollingsworth, P.M., L.L. Forrest, J.L. Spouge, M. Hajibabaei, S. Ratnasingham, M. van der Bank, M.W. Chase, R.S. Cowan, D.L. Erickson, A.J. Fazekas, S.W. Graham, K.E. James, K.J. Kim, W.J. Kress, H. Schneider, J. Alphenstahl, S.C.H. Barrett, C. van den Berg, D. Bogarin, K.S. Burgess, K.M. Cameron, M. Carine, A. Clark, J.J. Clarkson, F. Conrad, D.S. Devey, C.S. Ford, T.A.J. Hedderson, M.L. Hollingsworth, B.C. Husband, L.J. Kelly, P.R. Kesanakurti, J.S. Kim, Y.D. Kim, R. Layahe, H.L. Lee, D.G. Long, S. Madriñán, O. Maurin, I. Meusnier, S.G. Newmaster, C.W. Park, D.M. Percy, G. Petersen, J.E. Richardson, G.A. Salazar, V. Savolainen, O. Seberg, M.J. Wilkinson, D.K. Yi and D.P. Little 2009. A DNA barcode for land plants. *Proc. Natl. Acad. Sci.*, 106(31): 12794-12797.

Kallamadi, P.R., V. Nadigatla and S. Mulpuri. 2015. Molecular diversity in castor (*Ricinus communis* L.). *Ind. Crops Prod*., 66: 271-281.

Keller, A., F. Förster, T. Müller, T. Dandekar, J. Schultz and M. Wolf. 2010. Including RNA secondary structures improves accuracy and robustness in reconstruction of phylogenetic trees. *Biol. Direct*, 5: 4.

Kress, W.J., D.L. Erickson, F.A. Jones, N.G. Swenson, R. Perez, O. Sanjur and E. Bermingham. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proc. Natl. Acad. Sci. USA*., 106: 18621-18626.

Meinders, H.C. and M.D. Jones. 1950. Pollen shedding and dispersal in the castor plant *Ricinus communis* L. *Agron. J*., 42: 206-209.

Musshoff, F. and B. Madea. 2009. Ricin poisoning and forensic toxicology. *Drug Test Anal*., 1: 184-191.

Pavan, S., A.R. Marcotrigiano, E. Ciani, R. Mazzeo, V. Zonno and V. Ruggieri. 2017. Genotyping-by-sequencing of a melon (*Cucumis melo* L.) germplasm collection from a secondary center of diversity highlights pat-terns of genetic variation and genomic features of different gene pools. *BMC Genom.*, 18: 59.

Ramzi, A.A., K. Sabine, H. Manuela, W. Kristian and L. Ulrike. 2011. Assessment of selected Yemeni medicinal plants for their *In vitro* antimicrobial, anticancer and antioxidant activities. *Pharm. Biol*., 49(2): 200-210.

Richards, J. 1996. Breeding systems in flowering plants and the control of variability. *Folia Geobot. Phytotax*., 31(3): 283-293.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*., 4: 406-425.

Simões, K.S., S.A. Silva, E.L. Machado and M.S. Silva. 2017. Genetic divergence in elite castor bean lineages based on TRAP markers. *Genet. Mol. Res*., 16 (3): gmr16039776

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol*., 30: 2725-2729.

Tang Y., Y. Wu, R. Huang, N. Chao, Y. Liu, P. Xu, K. Li, D. Cai1 and Y. Luo 2016. Molecular identification of *Uncaria* (Gouteng) through DNA barcoding. *Chin Med*., 11: 3.

Thatikunta, R., A.S. Sankar, J. Sreelakshmi, G. Palle, C. Leela and C.V.D. Rani. 2016. Utilization of in silico EST-SSR markers for diversity studies in castor (*Ricinus communis* L.). *Physiol. Mol. Biol. Plants*, 22(4): 535-545.

Vavilov, N.I. 1951. *The origin, variation, immunity and breeding of cultivated plants*. Chronica Botanica, Waltham MA, USA.

Verma, S., S. Gupta, N. Bandhiwal, T. Kumar, C. Bharadwaj and S. Bhatia. 2015. High-density linkage map construction and mapping of seed trait QTLs in chickpea (*Cicer arietinum* L.) using Genotyping-by-Sequencing (GBS). *Sci. Rep*., 5: 17512.

Wang, M.L., M. Dzievit, Z. Chen, J.B. Morris and J.E. Norris. 2017. Genetic diversity and population structure of castor (*Ricinus communis* L.) germplasm within the US collection assessed with EST-SSR markers. *Genome*, 60: 193-200.

Zeven, A.C. and P.M. Zhukovsky. 1975. *Dictionary of cultivated plants and their centres of diversity*. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands.

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