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GENETIC RELATIONSHIP OF *Ougeinia oojeinensis* (Roxb.) Hochr., A THREATENED ENDEMIC SPECIES, BASED ON cpDNA *rbcL* REGIONS

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The cosmopolitan genus *Desmodium* incorporates numerous significant species with medicinal and fodder usage. The genus *Ougeinia* forms sister group with the *Desmodium* clade with low support, of which maximum genera of Desmodieae framed an unresolved and poorly supported clade. In addition, its phylogenetic placement is still uncertain. In this connection, the present study has been planned to assess its genetic relationship with other *Desomodium* species. Thirty-eight *Desmodium* species were explored utilizing non-coding *rbcL* barcode regions in chloroplastic genome. A sequence length of 650 bp with an average 43.30% G+C content was successfully amplified with the *rbcL1F* and *rbcL724R* genes. Nucleotide diversity π and θ were found as 0.682 and 0.238respectively; while haplotype/gene diversity (Hd) was 0.994 which showed high level of genetic variations among the species belong to this genera. Pairwise difference comparison revealed that geographically common species share more similarity. *Ougeinia oujeinensis* showed high similarity with *Tadehagi triquetrum* based on nucleotide homology and phylogenetic analysis. Although, the phylogenetic study showed that the biogeographic circulation of species doesn't exhibit any affiliation inferring species localization.

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But this barcode gene study would have value for correct identification of *O. oojeinensis* and its genetic relationship with other *Desmodium* species.

Keywords: *Desmodium* spp., *Ougeinia oojeinensis*, phylogenic relationship, *rbcL* barcode gene, threatened endemic species

INTRODUCTION

Ougeinia oojeinensis (Roxb.) Hochr. (Syn. *Desmodium oojeinense* (Roxb.) H. Ohashi.; *Ougeinia dalbergioides* Benth.), a native taxon belongs to family Leguminosae and only tree species of genus *Ougeinia*. The name of species *O. oojeinensis* Fwas first published in Annuaire Conserv. Jard. Bot. Genève 13-14: 51 (1909) as per the Royal Botanical Garden, Kew, UK. Later on, its name was changed to *Desmodium oojeinense* (Roxb.) H. Ohashi (RAO *et al.*, 2019). The species is endemic to the India and has now become endangered to Eastern Ghats of India (SANKAR *et al.*, 2008). It is commonly known as Sandan (Hindi) and Bhasmagharba (Sanskrit). Its scattered populations are reported in tropical moist deciduous forests (CHAMPION and SETH 1968). It is notified as threatened species by the ENVIS-WII, India (RAWAT 2008). The plant is source of prominent ethnomedicinal drug used to treat ailments in folk medicines (SAMYAL *et al.*, 2013). Besides, its leaves are used as quality fodder for the cattle of tribal communities. Excessive lopping of *Ougeinia* trees for fuelwood and fodder purpose is the key threat for the depletion of species abundance which affects the seed production and natural regeneration potential. Therefore, conservation strategies should be emphasized to avoid the extinction of this threatened endemic species.

The tribe Desmodieae (subfamily Papilionoideae) comprises 30 genera and 530 species, scattered mainly in tropics, subtropics and temperate zone of the world (ROCIO *et al.*, 2017). *Desmodium* is one the most diverse genus, comprising 275 species (0 2005), of which, approximately 23 species are found in India (https://sites.google.com/site/efloraofindia/species/a-l/f/fabaceae/desmodium). Species of *Desmodium* are highly polymorphic, leading to a confusing and problematic classification, and thus, several complexes or groups of species have been proposed. The morphology based classification leads to ambiguity in identification with respect to shape and/or number of leaflets (MCVAUGH 1987). Thus, the identification based on sole morphological characters can lead to ambiguity. Latest molecular tools are the most authentic and apt techniques for assessment of identification and phylogenetics among species.

DNA-based identification (barcoding) is simple, repeatable, and universal, needs least taxonomic expertise and is free from subjective errors. Authentic characterization and identification of any plant material is the main concern in morphological scientific classification which is the essential goal of DNA barcoding (HEBERT and GREGORY 2005; SPOONER 2009). Mitochondrial cytochrome C oxidase I (*COI* or *coxI*) is basic gene which is applied in animal kingdom for DNA barcodes. However, in terrestrial plants there are several major plastid regions (*rbcL*, *matK*, *rpoB*, *rpoC1*, *trnH-psbA*, *atpF-atpH*, *psbK-psbI*, etc.), of which *rbcL* and *matK* are two-marker combination which are highly used as a core DNA barcode for plants (CBOL 2009). Among these, *rbcL* served easy amplification, sequencing and alignment and the better discriminatory power offered by the *matK* region due to its high rate of substitutions (HOLLINGSWORTH *et al.*, 2011). This quality of *matK* makes it a significant gene for the phylogenetic and systematic studies in plants (KRESS and ERICSON 2007, SHINWARI *et al.*, 2014).

Recent studies have reported genetic analyses of some *Desmodium* species using DNA-based molecular markers (SINGH *et al.*, 2016, RAHMAN *et al.*, 2017).

Very few studies at molecular level have been carried out in *O. oojeinensis*. The genus *Ougeinia* (belongs to Phyllodium group) is sister to the *Desmodium* group but with very low support and all the remaining genera of Desmodieae formed an unresolved and poorly supported clade. In addition to, its phylogenetic placement is still uncertain (JABBOUR *et al.,* 2018). Contemporary phylogenetic methods encompass a range of algorithmic approaches, including distance-based (e.g., Neighbor-Joining), maximum parsimony, maximum likelihood, and Bayesian inference techniques. Distance-based methods offer computational efficiency but may sacrifice accuracy, while maximum parsimony identifies trees with the fewest evolutionary changes. Maximum likelihood and Bayesian inference incorporate explicit models of sequence evolution, providing statistically robust estimations of phylogenetic relationships. These methods are implemented in widely used software such as RAxML (STAMATAKIS, 2014), MrBayes (RONQUIST *et al.,* 2012), and BEAST (BOUCKAERT *et al.,* 2019). The integration of genome-scale data and advanced models continues to enhance phylogenetic resolution (FELSENSTEIN, 2004) which will be helpful in understanding the correct placement of species in traditional taxonomy using the molecular phylogenetic studies. Therefore, the present study was started with the objective to find out suitable barcode gene for correct identification and its genetic relationship with other *Desmodium* species.

MATERIALS AND METHODS

The present study was collaborative efforts of Systematic Botany Discipline, Forest Research Institute (FRI) Dehradun & School of Agricultural Biotechnology, Punjab Agricultural University (PAU), Ludhiana, India. Fresh and tender leaf material from 30-years-old tree of *O. oojeinensis* for molecular studies and branch with flower and fruit (Figure 1a) for submission of voucher herbarium specimen were collected from Botanical Garden, FRI Dehradun, Uttarakhand, India (30º20'20.49"N; 78°00'05.34"E; 665 asml). Voucher specimen was deposited to the DD Herbarium, FRI Dehradun for authentic identification and cross examine the species (Figure 1b). The genomic DNA was extracted by using CTAB method with some modifications given by KUMAR *et al.,* (2003), which include 4% PVP instead of 1%, increased water bath period (2 hours instead of 1 hour at 65ºC) successive repetition of Chloroform : Isoamyl alcohol step and washing of DNA pellets was buffer containing ammonium acetate. The quality of extracted DNA was checked on 0.8% agarose with 1X TBE buffer, staining with EdBr (0.5µg/ml) and the quantity in Biophotometer (Eppendorf-6131, Germany). The final concentration of DNA was expressed in ng/µl. Then finally, dilutions were made using doubled distilled water for a final concentration of 50 ng μL^{-1}.

For molecular study, nuclear ribosomal *ITS* and two chloroplast regions (*rbcL* and *matK*) were utilized in light of the fact that these have been utilized effectively for phylogenetic investigations of closely related plants (SHAW *et al.,* 2005), and furthermore for phylogenetic investigations inside Fabaceae and especially in Papilionoideae (KASS and WINK 1997, HU *et al.,* 2002, NEMOTO *et al.,* 2010). The molecular primers used are presented in Table 1. PCR reaction mixture (25 µL) contained the following: 15 µL of PCR Master Mix (Sisco Research Laboratories PVT LTD, Mumbai, India), 1 µM (1µL) each primer (Eurofins MWG Operon, Germany), 2 µL (±50 ng) gDNA and the leftover volume was adjusted with distilled water. PCR amplification was performed with a thermal cycler (GenePro, Hangzhou Bioer Tech Co. Ltd., China).PCR products were purified by QIA quick gel extraction kit (Qiagen, Maryland, USA). The fragments were isolated under agarose gel electrophoresis and pictured under gel documentation system (UVP GelDoc-IT 310 Imaging System, Upland CA). The sequencing of PCR amplicon was carried out by Eurofins Genomics India Pvt. Ltd., Bangalore. Forward and reverse DNA sequencing response of PCR amplicon was completed utilizing BDT v3.1 Cycle sequencing kit on ABI 3730xl genetic analyzer. The DNAsp v 5.10 was used to calculate the segregating/polymorphic sites (S), and haplotype (gene) diversity (Hd), nucleotide diversity with π (NEI 1987) and θ (WATTERSON 1975). Genotype/cultivar comparisons were performed by Arlequin 3.5.2 programming with the utilization of the population examination tool with the accompanying settings; pairwise differences (π) with Tajima and Nei's method for 100 permutations and 0.05 significance level (EXCOFFIER and LISCHER 2010).

Table 1. Detail of molecular markers used in the present study

S.N.	Primer	Sequence 5′-3′	Reference
1.	rbcL1 (F)	ATGTCACCACAAACAGAAAC	Fay *et al.*,1997
	rbcL724 (R)	TCGCATGTACCTGCAGTAGC	
2.	*matK*390 (F)	CGATCTATTCATTCAATATTTC	Shinwari *et al.*,2014
	*matK*1326 (R)	TCTAGCACACGAAAGTCGAAGT	
3.	*ITS*4 (F)	TCCTCCGCTTATTGATATGC	Rautela 2014
	*ITS*5 (R)	GGAAGTAAAAGTCGTAACAAGG	

The *rbcL* region sequences were BLAST searched at the NCBI, then choosing reference information domain as nucleotide collection (nt/nr) for exceptionally comparative mega-blast search. The taxonomic identities of the isolates were determined after comparing the search results based on maximum identity scores of first ten sequences. To supplement the *Ougeinia oojeinensis*, 37 additional *rbcL* sequences from various *Desmodium* species were obtained from the NCBI database (www.ncbi.nlm.nih.gov). Thus, a total of 38 *rbcL* regions (Table 2) were used in phylogenetic analyses. Additional species include *D. adscendens, D. barbatum, D. bracteatum, D. canadense, D. canescens, D. ciliare, D. cinererum, D. cuspidatum, D. dillenii, D. distortum, D. floridanum, D. gangeticum, D. glabellum, D. gyrans, D. heterocarpon, D. heterophyllum, D. illinoense, D. incanum, D. intortum, D. laxiflorum, D. paniculatum, D. pauciflorum, D. perplexum, D. psilocarpum, D. purpusii, D. rotundifolium, D. sessilifolium, D. strictum, D. styracifolium, D. tortuosum, D. triflorum, D. velutinum, D. viridiflorum, Hylodesmum glutinosum, H. podocarpum, H. nudiflorum* and *Tadehagi triquetrum*. The NCBI accession numbers of additional sequences were shown on phylogenetic trees. Sequences were aligned using BioEdit ver. 7.0.5 sequence alignment editor multiple alignment program Clustal W (THOMPSON *et al.*, 2002). Phylogenetic tree was developed with MEGA v. 7.0 (KUMAR *et al.*, 2016) utilizing the maximum likelihood approach based on Kimura 2- parameter model (KIMURA 1980). The bootstrap consensus tree inferred from 1000 replicates was taken to address the evolutionary relationship of the taxa analyzed (FELSENSTEIN 1985).

Table 2. Geographic location and some rbcL gene features of thirty-eight Desmodium species

S.N.	Species	Accession	Locality	G+C content (%)	*rbcL* gene (bp)
1.	*Desmodium oojeinensis*	DD172249	Dehradun, India	43.30	650
2.	*Desmodium adscendens*	JQ591687.1	Ontario, Canada	42.57	552
3.	*Desmodium barbatum*	JQ591691.1	Ontario, Canada	42.39	552
4.	*Desmodium bracteatum*	KX344699.1	Delhi, India	43.39	577
5.	*Desmodium canadense*	HQ590061.1	Columbus, USA	41.50	600
6.	*Desmodium canescens*	MG246343.1	Ontario, Canada	41.67	552
7.	*Desmodium ciliare*	KJ773442.1	Florida, USA	42.86	1323
8.	*Desmodium cinereum*	LT576812.1	Lancaster, UK	41.97	1389
9.	*Desmodium cuspidatum*	KX385959.1	Florida, USA	42.51	668
10.	*Desmodium dillenii*	MG248861.1	Ontario, Canada	41.97	498
11.	*Desmodium distortum*	JQ591697.1	Ontario, Canada	42.64	516
12.	*Desmodium floridanum*	KJ773443.1	Florida, USA	42.94	1318
13.	*Desmodium gangeticum*	KX119286.1	Lagos, Nigeria	41.75	685
14.	*Desmodium glabellum*	KX385960.1	Florida, USA	42.66	668
15.	*Desmodium gyrans*	KX365338.1	Delhi, India	42.41	646
16.	*Desmodium heterocarpon*	AB925673.1	Fukuoka, Japan	42.56	531
17.	*Desmodium heterophyllum*	KY702614.1	Paris, France	41.22	676
18.	*Desmodium illinoense*	KX385961.1	Florida, USA	42.22	668
19.	*Desmodium incanum*	KY627455.1	Illinois, USA	41.35	561
20.	*Desmodium intortum*	KY702615.1	Paris, France	41.27	676
21.	*Desmodium laxiflorum*	KP095045.1	Guangdong, China	41.81	562
22.	*Desmodium paniculatum*	KJ773445.1	Florida, USA	42.89	1322
23.	*Desmodium pauciflorum*	EU717280.1	Ontario, Canada	42.57	1325
24.	*Desmodium perplexum*	KX385963.1	Florida, USA	43.41	668
25.	*Desmodium psilocarpum*	LT576814.1	Lancaster, UK	41.83	1389
26.	*Desmodium purpusii*	JQ591711.1	Ontario, Canada	41.49	552
27.	*Desmodium rotundifolium*	MG248353.1	Ontario, Canada	41.67	552
28.	*Desmodium sessilifolium*	KX385964.1	Florida, USA	42.22	668
29.	*Desmodium strictum*	KY627442.1	Illinois, USA	41.71	561
30.	*Desmodium styracifolium*	GQ436339.1	Beijing, China	42.67	703
31.	*Desmodium tortuosum*	KX119287.1	Lagos, Nigeria	42.70	1335
32.	*Desmodium triflorum*	KJ773446.1	Florida, USA	42.97	1322
33.	*Desmodium velutinum*	KX119288.1	Nigeria	42.85	1335
34.	*Desmodium viridiflorum*	KY627409.1	Illinois, USA	41.87	530
35.	*Hylodesmum glutinosum*	MG249498.1	Ontario, Canada	41.67	552
36.	*Hylodesmum nudiflorum*	KP643866.1	Washington, USA	41.70	542
37.	*Hylodesmum podocarpum*	KY702619.1	Paris, France	41.42	676
38.	*Tadehagi triquetrum*	JN407292.1	Hong Kong, China	41.55	515

RESULTS AND DISCUSSIONS

The voucher specimen of taxonomically identified plant species, *i.e. Ougeinia oojeinensis* (Figure 1a) was submitted to DD herbarium (Accession number DD172249) (Figure 1b) and the same was subjected to further molecular study. The method given by Kumar *et al.* (2003) with modifications produced ample quantity (451.5-568.4 ng/ul) with wide range of quality gDNA (OD: 1.74-1.85) in the target species. Some modification, *i.e.* 3% CTAB, 50 mM Tris-HCl (pH 8), 1.4 MNaCl, 10 mM EDTA (pH 8), 1% β-mercaptoethanol, 4% PVP and ascorbic acid 3.5% were made due to presence of high amount of polyphenoles and polysaccharides. In present study, three universal barcode genes (i.e. *rbcL, matK* and *ITS*) were used for amplification, of which, *rbcL* (1F & 724R) gene gave satisfactory amplification. A single discrete PCR amplicon band of ~700 bp was observed when resolved on agarose by GelDoc imaging system. The cpDNA *rbcL* region demonstrated sequence length of 650 bp, with an average of 42.30% G+C content. Despite the high degree of similarities between sequences, it was also searched for the presence of any informative variable site/s. The variable (polymorphic/segregating), parsimony informative sites and total numbers of indel sites were found to be 36, 38, 43, 53, 73, 108, 142, 150, 193, 287, 333, 371, 419, 467 and 468. Nucleotide diversity π, θ, segregating/polymorphic sites (S) and Tajima's test statistics (D) was found as 0.682, 0.238, 498 and 7.034, respectively, while haplotype (gene) diversity, Hd was 0.994.

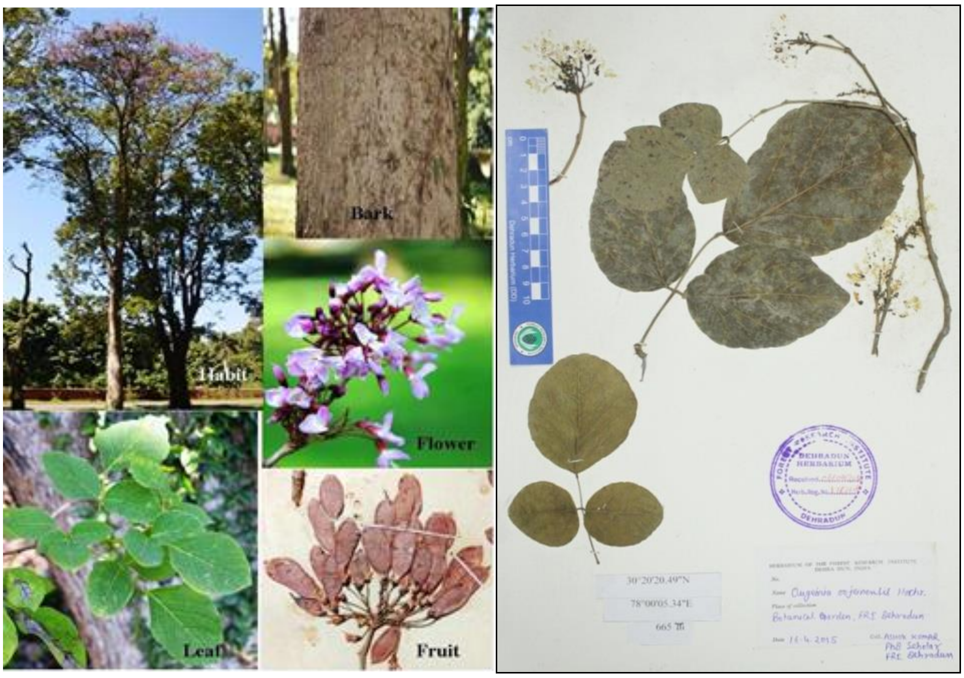

Figure 1. (a) Representative species; (b) Voucher specimen

The *Ougeinia oojeinensis* native to Indian sub-continent is considered as vulnerable a tree species now. Of the 275 species in the genus *Desmodium*, 23 are found in Indian sub-continent. Of these, *O. oojeinensis* is an important tropical forest timber species. Because the majority of the Desmodieae genera formed an unresolved and poorly supported clade based on morphology and taxonomy, *O. oojeinensis* still lacks a correct taxonomic position. Therefore, molecular characterization based on the barcode genes its genetic relationship with other *Desmodium* species are needed. Out of three barcode genes i.e. *rbcL*, *matK* and *ITS*, only *rbcL* gene gave the satisfactory amplification. Universal primer *matK* (390F & 1326R) was not successfully amplified. Though, *ITS* showed amplification but resolution was not optimal even after repetition. Apart from this, *ITS* primers have been mainly used for the identification of fungi. Majority of forestry species contains the microscopic fungi and their spores on and inside the plant parts and during amplification it was difficult to know whether DNA extracted belongs to fungi or plant samples. Therefore, *ITS* was not taken into consideration for phylogenetic analysis.

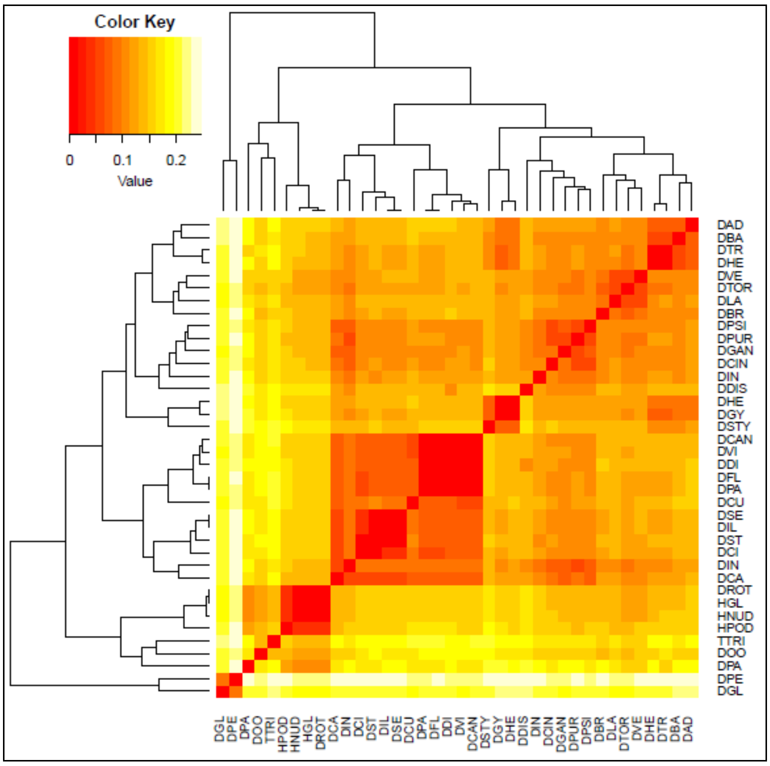

Figure 2. The pairwise difference comparisons of thirty-eight *Desmodium* species. The red, yellow and white colors represent Nei’s distance, within population and between population variations, respectively

The current study clearly supported that the *rbcL* gene is the most widely recognized gene used to establish phylogenetic investigation in plants (COBL 2009), however it has restriction to determine phylogenetic connections beneath the family level (DONOGHUE *et al.,* 1992). As anticipated, a framework consisting of a single or combination of plastid genes did not work well in the targeted taxa that showed low plastid variability measures, but it did work well in certain other taxa (NEWMASTER and RAGUPATHY 2009). As of late, *rbcL* is broadly utilized in developing of barcodes in plants. It could be because of high-quality sequences of *rbcL* which are effectively retrievable across phylogenetically divergent ancestries and it performs well in segregation tests in blend with different loci (COBL 2009).

Table 3. Similarity matrix among Desmodium species

	Species	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1	*D. oojeinensis*																																					
2	*D. triflorum*	4.872																																				
3	*D. floridanum*	4.344	5.099																																			
4	*D. illinoense*	5.098	3.358	4.896																																		
5	*D. velutinum*	3.943	3.539	5.280	4.121																																	
6	*D. strictum*	6.420	3.944	5.144	4.174	4.763																																
7	*D. ciliare*	5.098	3.358	4.896	0.000	4.121	4.174																															
8	*D. viridiflorum*	5.263	3.944	5.233	4.174	4.763	0.003	4.174																														
9	*D. intortum*	5.165	5.261	4.495	4.785	5.160	5.231	4.785	5.145																													
10	*D. sessilifolium*	5.098	3.358	4.896	0.000	4.121	4.174	0.000	4.174	4.785																												
11	*D. heterophyllum*	5.250	5.264	4.522	4.785	5.063	6.364	4.785	6.364	0.008	4.785																											
12	*D. perplexum*	5.080	3.151	4.752	0.034	4.279	4.399	0.034	4.399	4.679	0.034	4.679																										
13	*D. glabellum*	4.709	0.208	4.407	1.942	3.011	3.829	1.942	3.829	6.717	1.942	6.713	1.805																									
14	*D. laxiflorum*	3.763	6.493	5.080	4.805	4.166	3.815	4.805	3.821	4.456	4.805	4.441	5.787	4.043																								
15	*H. glutinosum*	5.274	3.578	3.724	3.478	3.715	3.433	3.478	3.428	3.624	3.478	3.743	3.147	3.780	3.655																							
16	*D. pauciflorum*	4.104	3.153	4.143	4.880	3.928	4.669	4.880	4.799	4.856	4.880	4.748	5.038	3.533	4.632	6.747																						
17	*D. bracteatum*	4.568	4.962	5.201	3.668	5.083	4.372	3.668	4.514	3.461	3.668	3.459	3.597	4.574	3.407	3.466	5.044																					
18	*D. adscendens*	5.062	3.539	3.626	3.801	3.987	3.425	3.801	3.421	3.361	3.801	3.475	3.377	3.659	3.518	0.015	6.637	3.765																				
19	*D. barbatum*	5.122	3.615	3.590	3.853	3.915	3.354	3.853	3.350	3.413	3.853	3.527	3.429	3.716	3.572	0.013	6.597	3.689	0.002																			
20	*D. canadense*	4.842	3.717	3.765	3.574	4.429	4.763	3.574	4.775	4.418	3.574	4.677	3.435	3.766	4.258	3.282	6.652	3.604	3.285	3.356																		
21	*D. canescens*	5.135	3.618	3.590	3.593	3.658	3.644	3.593	3.639	3.425	3.593	3.540	3.220	3.844	3.710	0.015	6.614	3.452	0.013	0.011	3.361																	
22	*D. cuspidatum*	5.151	3.150	4.939	0.006	4.166	4.109	0.006	4.109	4.728	0.006	4.728	0.028	1.858	5.809	3.417	4.944	3.464	3.600	3.653	3.679	3.400																
23	*D. dillenii*	4.901	3.653	3.710	3.636	4.360	4.816	3.636	4.828	4.478	3.636	4.732	3.496	3.706	4.329	3.212	6.597	3.535	3.215	3.282	0.003	3.287	3.468															
24	*D. distortum*	5.177	3.415	3.626	3.646	3.843	3.412	3.646	3.408	3.608	3.646	3.725	3.287	3.632	3.641	0.013	6.651	3.632	0.009	0.007	3.364	0.007	3.451	3.358														
25	*D. gangeticum*	4.801	4.326	3.635	5.103	4.315	4.507	5.103	4.514	4.762	5.103	4.792	4.943	4.514	4.771	5.388	2.611	3.642	5.313	5.273	4.930	5.333	5.037	4.979	5.287													
26	*D. gyrans*	4.224	2.889	4.866	5.198	3.385	4.484	5.198	4.612	4.977	5.198	4.772	5.145	3.427	4.975	4.816	2.793	4.303	4.536	4.484	4.607	4.728	5.240	4.554	4.557	3.540												
27	*D. heterocarpon*	4.985	4.655	3.415	3.521	4.538	3.451	3.521	3.462	3.411	3.521	3.434	3.773	3.838	3.647	4.619	4.664	3.903	4.641	4.570	4.970	4.557	3.713	4.918	4.616	2.978	4.146											
28	*D. incanum*	6.515	4.071	5.137	4.422	4.966	0.007	4.422	0.008	5.141	4.422	6.255	4.634	4.081	4.056	3.415	4.903	4.507	3.407	3.337	4.659	3.621	4.361	4.714	3.393	4.232	4.619	3.457										
29	*D. paniculatum*	5.105	3.216	4.889	0.003	4.244	4.168	0.003	4.168	4.778	0.003	4.778	0.030	1.886	5.878	3.490	4.880	3.523	3.653	3.705	3.460	3.451	0.002	3.521	3.502	4.997	5.198	3.643	4.416									
30	*H. podocarpum*	5.026	5.097	4.592	4.804	5.025	6.452	4.804	6.352	0.013	4.804	0.014	4.811	6.665	4.299	3.566	4.802	3.506	3.361	3.357	4.573	3.369	4.749	4.629	3.550	4.959	4.814	3.381	6.348	4.797								
31	*D. purpusii*	5.167	3.421	3.613	3.653	3.843	3.401	3.653	3.397	3.608	3.653	3.725	3.288	3.632	3.641	0.013	6.651	3.620	0.009	0.007	3.370	0.007	3.457	3.360	0.002	5.276	4.557	4.616	3.382	3.509	3.550							
32	*D. rotundifolium*	5.274	3.578	3.724	3.478	3.715	3.433	3.478	3.428	3.624	3.478	3.743	3.147	3.780	3.655	0.000	6.747	3.466	0.015	0.013	3.282	0.015	3.417	3.212	0.013	5.388	4.816	4.619	3.415	3.490	3.566	0.013						
33	*D. styracifolium*	3.735	3.416	3.523	4.937	6.484	4.127	4.937	4.127	4.125	4.937	4.147	5.013	4.765	4.527	4.640	5.056	4.176	4.569	4.614	4.423	4.929	4.898	4.373	4.782	4.363	3.600	4.736	4.127	4.839	4.069	4.769	4.640					
34	*D. tortuosum*	5.151	3.289	4.737	0.009	4.173	4.220	0.009	4.220	4.699	0.009	4.699	0.029	1.914	5.794	3.418	4.830	3.762	3.605	3.658	3.673	3.405	0.011	3.736	3.456	5.176	5.166	3.461	4.464	0.011	4.719	3.462	3.418	5.000				
35	*H. nudiflorum*	4.936	3.754	4.036	3.597	4.330	4.795	3.597	4.806	4.550	3.597	4.797	3.455	3.646	4.251	3.289	6.658	3.880	3.291	3.320	0.012	3.342	3.429	0.015	3.399	5.022	4.738	5.178	4.690	3.482	4.697	3.405	3.289	4.340	3.695			
36	*T. triquetrum*	3.283	4.511	4.955	4.900	4.821	5.092	4.900	4.988	3.506	4.900	3.361	5.268	3.784	4.619	5.224	3.599	6.225	5.025	5.086	3.508	5.180	5.064	3.446	5.217	4.191	4.297	4.502	5.186	5.009	3.360	5.224	5.224	7.018	5.060	3.647		
37	*D. cinereum*	4.490	3.689	3.624	3.754	5.258	2.598	3.754	2.684	5.244	3.754	5.146	3.729	3.676	4.017	4.209	4.259	2.797	4.346	4.278	6.966	4.360	3.574	6.996	4.187	5.162	3.191	3.367	2.515	3.628	5.171	4.187	4.209	4.183	3.710	7.037	4.831	
38	*D. psilocarpum*	4.477	3.689	3.617	3.754	6.389	2.602	3.754	2.687	5.157	3.754	5.054	3.718	3.665	3.997	4.209	4.266	2.702	4.346	4.278	6.960	4.360	3.565	6.990	4.187	5.071	3.191	3.486	2.519	3.628	5.081	4.187	4.209	4.190	3.699	7.031	4.839	0.003

In order to have insights about the genetic relationships of *Desmodium* species, pairwise similarity and difference comparisons of *rbcL* sequences were conducted (Figure 2). The similarity matrix (Table 3) ranged between lowest 0.000 (USA species) and highest 7.032 (UK and USA species) values. The distance among *D. illinoense*, *D. ciliare*, *D. sessilifolium*; and *H. glutinosum* and *D. rotundifolium* have lowest (0.000) pairwise difference, therefore the highest similarity, while *H. nudiflorum* and *D. cinereum* have highest (7.032) pairwise difference, and therefore the lowest similarity. Accordingly, it seems that geographically common species share more sequence similarities than relatively distant species. The low level of genetic variations in cpDNA *rbcL* regions in *Desmodium* species analyzed could have arisen due to indel variations. Variable (polymorphic/segregating), parsimony informative and total numbers of the insertion or the deletion of bases in the DNA (indel sites) were 36, 38, 43, 53, 73, 108, 142, 150, 193, 287, 333, 371, 419, 467 and 468, respectively.

Total nucleotide frequencies were 28.5% for Adenine (A), 29.2% for Thymine (T), 19.8 for Cytosine (C) and 22.5% for Guanine (G) in its amplified length with the total nucleotide of 650. The best fit substitution model was found Maximum Likelihood (ML) for the tree construction as model with the lowest BIC scores (Bayesian Information Criterion) was considered to describe the substitution pattern the best. Nucleotide sequences for *rbcL* gene of 37 *Desmodium* species were found on NCBI GenBank database and comparatively analyzed for evolutionary relation (Figure 3). *Desmodium oojeinensis* was found very close to *Tadehagi triquetrum* based on nucleotide homology and phylogenetic analysis. *Desmodium* species were grouped into two clusters.

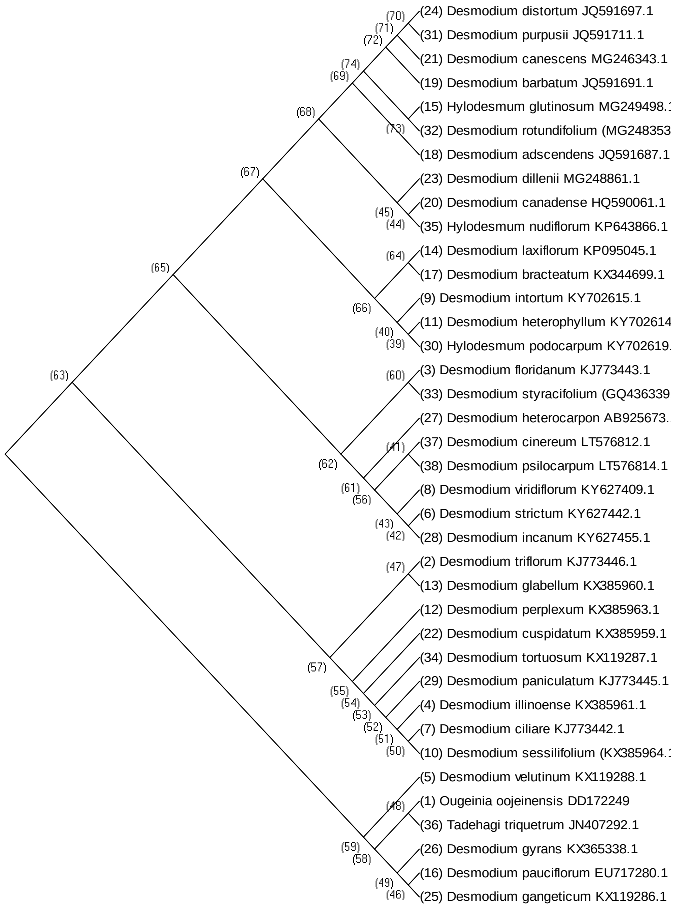

Figure 3. Phylogenetic tree showing the evolutionary relationship of *Desmodium* species including Indian *Ougeinia* based on *rbcL* regions

The minor cluster (cluster-I) consists of six species including *O. oojeinensis*. The major cluster is divided into two sub clusters, sub-cluster ‘a’ and sub-cluster ‘b’. Sub-cluster ‘a’ (cluster-II) consists of nine species and sub-cluster ‘b’ consists of twenty-three species. Sub-cluster ‘b’ is further divided into two sub sub-clusters, sub sub-cluster ‘i’ and sub sub-cluster ‘ii’. Sub sub-cluster ‘i’ (cluster-III) consists of eight species. Sub sub-cluster ‘ii’ again grouped into two clusters i.e. cluster IV (five species) and cluster V (ten species).

The nucleotide diversity and parsimony informative sites in *Desmodium* species were viewed as high. Past examinations have likewise observed similar results. For instance, in SSR analysis, the Turkish olive cultivars analyzed were not grouped according to their locations of cultivation and individuals collected from the same region indicated 100% identity (IPEK *et al.*, 2012). The phylogenetic analysis indicated that germplasm could be relatively more conserved in cluster II, IV and V. Notwithstanding, grouping of different species in the similar group could show the chance of germplasm exchanges between geographical areas. Likewise, KAYA *et al.* (2018) showed that *Olea europaea* genotypes from various nations grouped together irrespective of their geographical origin. *Melia azedarach* cultivars having the same origin had also been found distributed in different clusters (CHAUHAN *et al.*, 2018).

CONCLUSIONS

In this study, *Desmodium* species demonstrated a high degree of genetic variations in terms of cpDNA *rbcL* regions. This could show the genetic flimsiness of plastid genomes. In addition, no specific relationship was seen between biogeographic distribution and species localization. These multitudes of studies demonstrate that geographical origin was not so effective as to permit any inference for phylogenetic relationships to be made. Further assessment with more species is required for a better understanding of the evolutionary history of *Desmodium* species with the use of chloroplast genomes.

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GENETSKA VEZA *Ougeinia oojeinensis* (Roxb.) Hochr., UGROŽENE ENDEMIČKE VRSTE, NA OSNOVU cpDNA rbcL REGIONA

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Izvod

Kosmopolitski rod Desmodium obuhvata brojne značajne vrste sa medicinskom i stočnom upotrebom. Rod Ougeinia formira sestrinsku grupu sa kladom Desmodium sa slabom podrškom, od kojih je maksimalan broj rodova Desmodieae uokvirivao nerešenu i slabo podržanu kladu. Pored toga, njegov filogenetski položaj je još uvek neizvestan. U vezi sa tim, ova studija je planirana da proceni njegov genetski odnos sa drugim vrstama Desmodium. Trideset osam vrsta Desmodium je istraženo koristeći nekodirajuće rbcL barkod regione u hloroplastičnom genomu. Sekvenca dužine 650 bp sa prosečnim sadržajem G+C od 43,30% uspešno je amplifikovana sa genima rbcL1F i rbcL724R. Nukleotidna raznovrsnost p i th je pronađena kao 0,682 i 0,238 respektivno; dok je haplotipska/genska raznovrsnost (Hd) bila 0,994, što je pokazalo visok nivo genetskih varijacija među vrstama koje pripadaju ovom rodu. Poređenje parnih razlika pokazalo je da geografski uobičajene vrste imaju veću sličnost. *Ougeinia oujeinensis* je pokazala veliku sličnost sa *Tadehagi triquetrum* na osnovu nukleotidne homologije i filogenetske analize. Iako je filogenetska studija pokazala da biogeografska cirkulacija vrsta ne pokazuje nikakvu povezanost koja bi ukazivala na lokalizaciju vrste. Međutim, ova studija bar-kod gena bi imala vrednost za tačnu identifikaciju *O. oojeinensis* i njegovu genetsku vezu sa drugim vrstama Desmodium.

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