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Original article

Plastome of *Saraca asoca* (Detarioideae, Fabaceae): Annotation, comparison among subfamily and molecular typing

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ABSTRACT

Saraca asoca (Roxb.) Willd. (subfamily Detarioideae, family Fabaceae) is a perennial evergreen sacred medicinal tree classified under 'vulnerable' by the IUCN. The chloroplast (cp) genome/plastome which follows uniparental inheritance contains many useful genetic information because of its conservative rate of evolution. The assembled cp genome of *S. asoca* which maps as a conserved circular structure revealed extensive rearrangement in gene organization, comprising total length 160,003 bp including LSC, SSC, IRa, and IRb, and GC content was 35.26%. Herein a set of *rbc*L and *mat*K gene were established using molecular phylogenetic analyses for molecular typing of *S. asoca*.

1. Introduction

Saraca asoca (Roxb.) de Wilde [family Fabaceae, subfamily Detarioideae (APG IV, 2016; LPWG, 2017)], commonly known as '*asoca*' (Fig. 1A-B), indigenous to Assam, E. Pakistan, Upper Burma, Malaya, Ceylon and South India (Singh et al., 2015), is one of the most sacred tree of the Indian subcontinent (Murthy et al., 2008; Mollik et al., 2010). Apart from its various pharmacological significance e.g. antimicrobial (Seetharam, et al., 2003; Shirolkar et al., 2013), anticancer (Cibin et al., 2012), anti-inflammatory (Cibin et al., 2012; Saha, et al., 2012), antiarthritic (Preethi and Krishnakumar, 2011) activities, the barks, leaves, flowers, and seeds of '*asoca*' have extensively been used against uterine infections and as astringent in the cases of the internal haemorrhoids in modern as well as in the Indian traditional systems of medicine (Nudrat et al., 2005; Singh et al., 2015).

The continuous development in the next-generation sequencing (NGS) platforms (Shendure et al., 2017), and bioinformatics tools (Yang and Rannala, 2012) including cloud computing for genomic data analysis (Kwon et al., 2015; Langmead and Nellore, 2018) during last two decades have (a) greatly propelled to sequencing of the organellar genome e.g. mitochondria (Kozik et al., 2019), chloroplast (Daniell et al., 2016) and whole genome (Chen et al., 2018), (b) revolutionized the understanding of various biological disciplines (Ali et al., 2020) e.g. tree of life (Philippe et al., 2005;

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Fig. 1. *Saraca asoca*. A. The tree in the flowering stage. B. An enlarged view of inflorescence.

Rokas, 2006), evolution of plant genomes (Wendel et al., 2016), gene families and gene function (Leebens-Mack et al., 2019), conservation biology (Johnson and Koepfli, 2014; Wambugu et al., 2018), and (c) alleviate the enhancement of the agronomic traits (Rogalski et al., 2015; Daniell et al., 2016; Lima et al., 2016). The over-exploitation of *S. asoca* from the wild habitat due to increasing commercial demand of the bark of '*asoca*' as crude drug material leads it to vulnerable (IUCN, 2019); hence, the characterization of plastome/whole chloroplast (cp) genome of *'asoca'* and its genetic comparison will facilitate the development of DNA markers for diversity assessment, conservation, and in unraveling function of genes and gene families to produce its enhanced agronomic traits through genetic engineering.

2. Materials and methods

2.1. Leaf sampling and DNA sequencing

The green young leaves material of *S. asoca* was collected [voucher information: 'MAA & TKPAN-116′ (BHAG, KSUH)] from the tree growing at conservatory of the botanical garden, Tilka Manjhi Bhagalpur University (TMBU), Bhagalpur, India, without harming the plant, were used for the extraction of DNA using # DNeasy Plant Mini Kit (QIAGEN). The *de novo* sequencing (as a single end run of 51 bp) was performed at Illumina platform, Illumina Pipeline 1.3.2 (Nie et al., 2012) was used for base calling.

2.2. Cp genome assembly and annotation

The raw reads were first filtered using fastqc. The high-quality reads were then assembled using spades (Bankevich et al., 2012), and annotated using the online tool GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq.html) at *Tamarindus indica* L. (GenBank NC_026685.1) as reference (Hansen et al., 2007; Tillich et al., 2017). The repeat structure and small inversion (Maia et al., 1991; Timme et al., 2007; Yang et al., 2010; Doorduin et al., 2011; Castro et al., 2013; Beier et al., 2017) in cp genome were analyzed.

2.3. Comparison of cp genome and phylogenetic analysis

The cp genome of *S. asoca* was compared with the five other complete **Detarioideae** (Fabaceae) cp genomes including *Crudia harmsiana* Wild., (NC_036743.1), *Daniellia pilosa* (J. Léonard) Estrella, (NC_036744.1), *Guibourtia leonensis* J. Leonard, (NC_036742.1) and *Tamarindus indica* L. (NC_026685.1) by aligning using Kalign (Lassmann and Sonnhammer, 2005) and UPGMA analysis (Sneath and Sokal, 1973) employing MEGA X (Kumar et al., 2018) followed by the verification of the taxon proximity under UPGMA tree with MAUVE alignment.

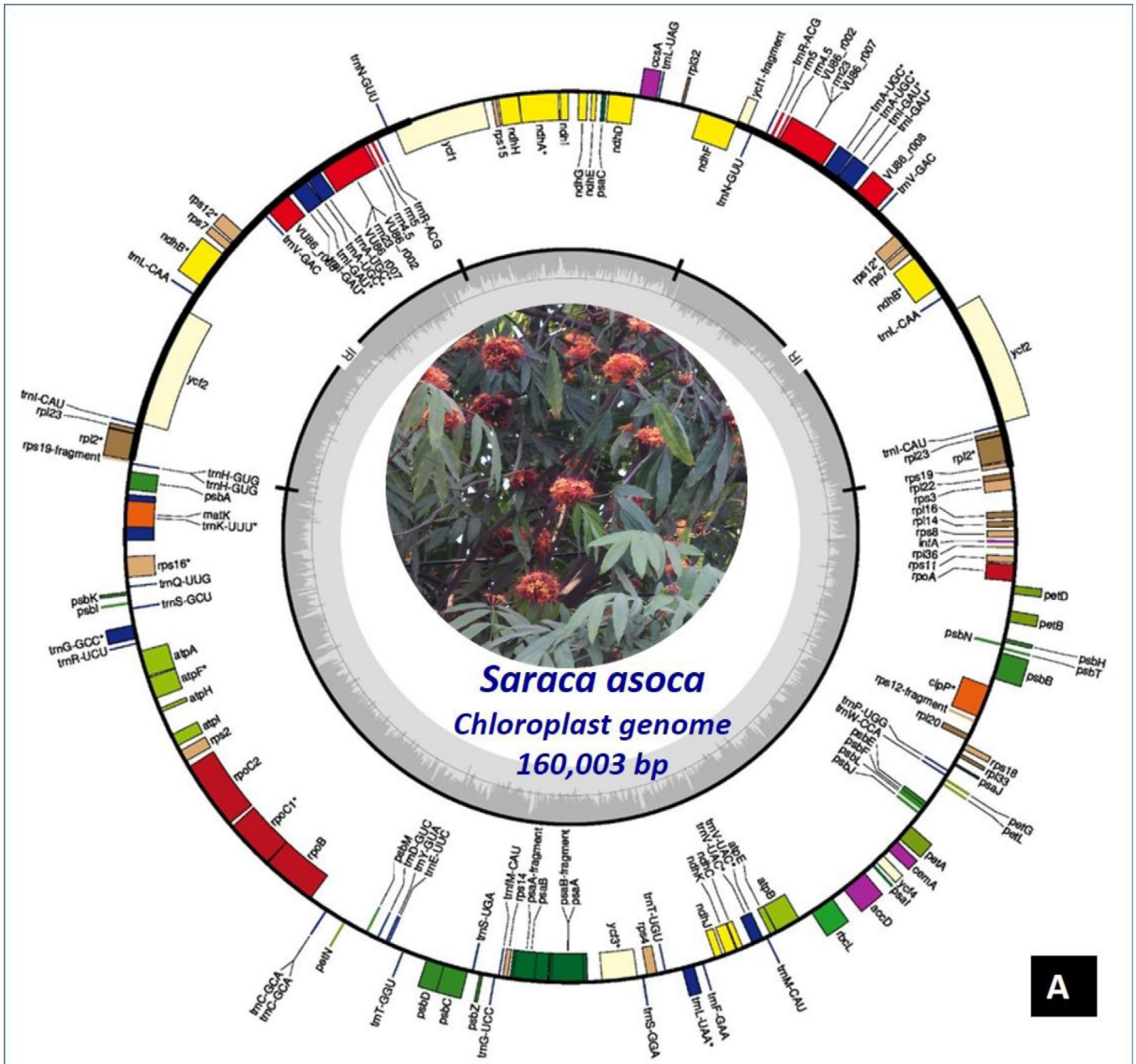
The plant DNA barcoding genes i.e. *rbc*L and *mat*K of adulterant species (a) *Bauhinia variegata* L. (GU135196, GU135033), (b) *Mesua ferrea* L. (KY654490, JN114759), (c) *Polyalthia longifolia* (Sonn.) Thwaites (JX856748, AY518786), (d) *Shorea robusta* Gaertn. (KY654498, KY973059) and (e) *Trema orientalis* (L.) Blume (KY654502, AB924756) were retrieved from the GenBank, and analyzed together with the sequences of the *S. asoca* (KY678341, KC592386). The sequences were aligned (Thompson et al., 1994), and the molecular phylogenetic analyses by Maximum Evolution method (Rzhetsky and Nei, 1992) rooted using outgroup *Sarcandra glabra* [Clade: Angiosperms, Order: Chloranthales, Family: Chloranthaceae (KP208901, JN407112) were performed using MEGA X (Kumar et al., 2018).

3. Results and discussion

The assembled cp genome maps as a conserved circular structure (Fig. 2A), comprising total length 160,003 bp including LSC, SSC, IRa, and IRb, and GC content was 35.26% (NCBI GenBank accession number: MN866115) as similar to those of other angiosperms (Daniell et al., 2016). The cp genome possessed 111 genes (97 CDS, 29 tRNA, 4 rRNA genes) (Fig. 2B). Twelve of the CDS and eight of the tRNAs contain introns; 18 of these contain single intron, and two genes (*ycf3* and *clpP*) possess 2 introns each (Fig. 2A).

The tandem and dispersed repeats were analyzed for *S. asoca* cp genome. It is evident that the number of tandem and dispersed repeats were more in 15–20 bp and 31–40 bp category, respectively (Fig. 3A). The repeat structures of *S. asoca* and other five species of Fabaceae were analyzed by REPuter and were compared. The forward and palindrome repeats were common in these species (Fig. 3B-C). A total of 70 different SSR loci repeated more than 1 time (Table 1), contribute to the A–T richness of cp genome. The repeat regions play very significant roles in genome recombination (Yang et al., 2010). The SSRs are highly polymorphic due to higher mutation rate that affects the number of repeat units (Tsai et al., 2008).

The comparison of cp genome of *S. asoca* with the five other complete **Detarioideae** (Fabaceae) cp genomes e.g. *C. harmsiana*, *D. pilosa*, *G. leonensis*, and *T. indica* revealed extensive rearrangement in gene organization (Fig. 4). Further, the ME tree from the set of the GenBank accession number [*Bauhinia variegata* [(Clade:



Photosynthesis	Photosystem I	*psaA*	*psaB*	*psaC*	*psaI*	*psaJ*
	Photosystem II	*psbA*	*psbB*	*psbC*	*psbD*	*psbE*
		psbF	*psbH*	*psbI*	*psbJ*	*psbK*
		psbL	*psbM*	*psbN*	*psbT*	*psbZ*
	Cytochrome b/ f complex	*petA*	**petB*	**petD*	*petG*	*petL*
		petN				
	ATP synthase	*atpA*	*atpB*	*atpE*	**atpF*	*atpH*
		atpI				
	Cytochrome c synthesis	*ccsA*				
	stability of photosystem I	**ycf3*	*ycf4*			
	NADPH dehydrogenase	**ndhA*	**ndhB*	*ndhC*	*ndhD*	*ndhE*
		ndhF	*ndhG*	*ndhH*	*ndhI*	*ndhJ*
		ndhK				
	Rubisco	*rbcL*				
Transcription and translation	Transcription	*rpoA*	*rpoB*	**rpoC1*	*rpoC2*	
	Ribosomal proteins	*rps2*	*rps3*	*rps4*	*rps7*	*rps8*
		rps11	**rps12*	*rps14*	**rps16*	*rps18*
		rps19	*rpl2*	*rpl14*	**rpl16*	*rpl20*
		rpl22	*rpl23*	*rpl32*	*rpl33*	*rpl36*
RNA	Ribosomal RNA	*rrn5*	*rrn4.5*	*rrn16*	*rrn23*	
	Transfer RNA	**trnA-UGC*	*trnC-GCA*	*trnD-GUC*	*trnE-UUC*	*trnF-GAA*
		**trnG-UCC*	*trnH-GUG*	*trnI-CAU*	**trnI-GAU*	**trnK-UUU*
		trnL-CAA	*trnL-UAG*	*trnM-CAU*	*trnN-GUU*	*trnP-UGG*
		trnQ-UUG	*trnR-ACG*	*trnR-UCU*	*trnS-CCC*	*trnS-GCU*
		trnS-GGA	*trnS-UGA*	*trnT-GGU*	*trnT-UAA*	*trnT-UGU*
		trnV-GAC	**trnV-UAC*	*trnW-CCA*	*trnY-GUA*	
Other	RNA processing	*MatK*				
	Carbon metabolism	*cemA*				
	Fatty acid synthesis	*accD*				
	Proteolysis	**clpP*				
	Initiation factor	*infA*				
Unknown function	conserved reading frames	*ycf1*	*ycf2*			

B

Fig. 2. A. The cp geneome map *Saraca asoca*, B. the genes of different groups are color-coded.

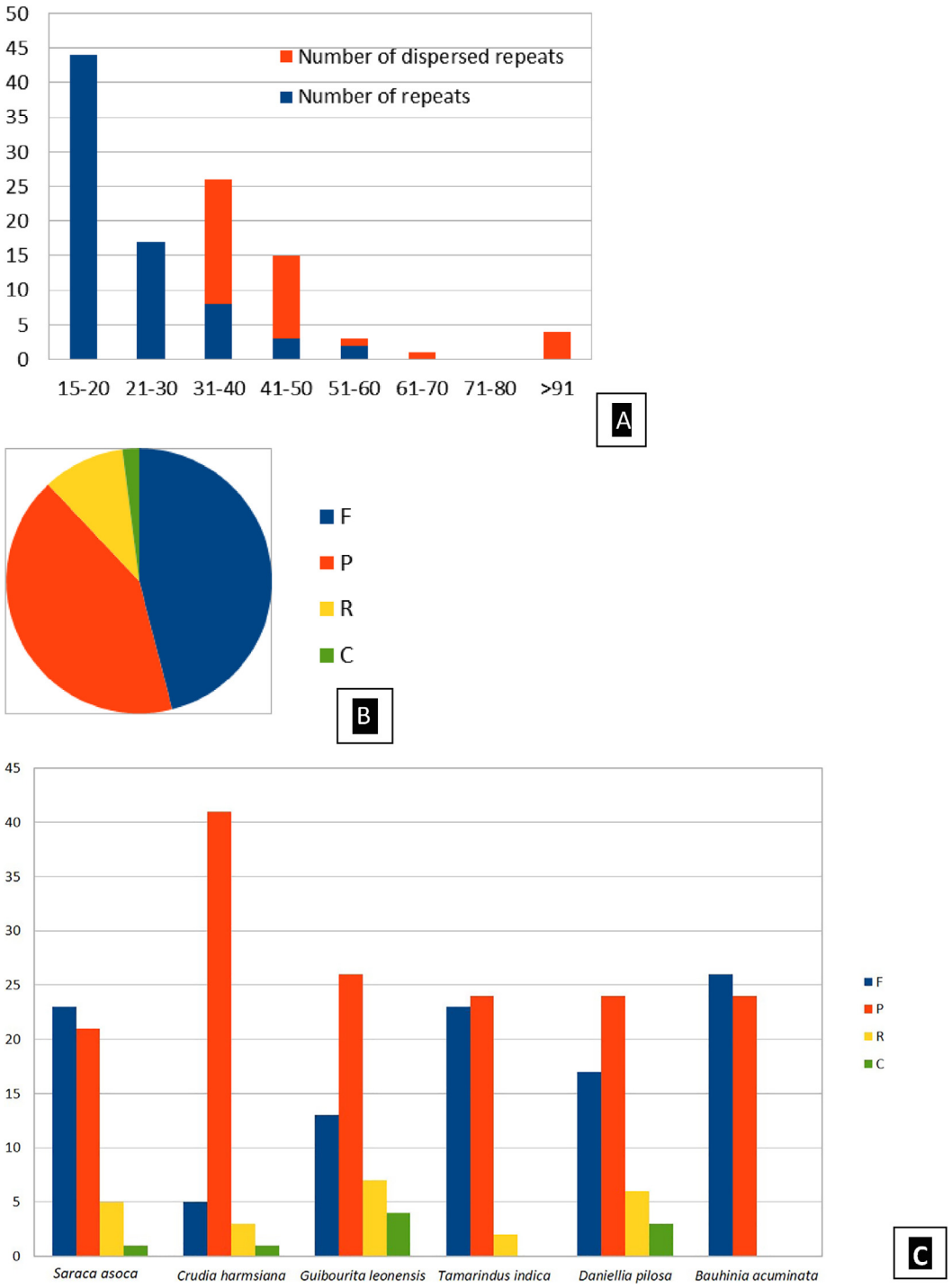


Fig. 3. The repeat structure analysis of the cp genome *S. asoca*. [A. The frequency of repeat by length; B. The repeat type; C. Comparison among six sequenced Fabaceae cp genomes (F: forward, P: palindrome, R: reverse, C: complement orientations)].

Rosids, Order: Fabales, Family: Fabaceae; GenBank accession number: GU135196, GU135033)], *Mesua ferrea* [Clade: Rosids, Order: Malpighiales, Family: Calophyllaceae; GenBank accession number: KY654490, JN114759)], *Polyalthia longifolia* [Clade: Magnoliids, Order: Magnoliales, Family: Annonaceae; GenBank accession number: JX856748, AY518786)], *Shorea robusta* [Clade: Rosids, Order: Malvales, Family: Dipterocarpaceae; GenBank accession number: KY654498, KY973059] and *Trema orientalis* [Clade: Rosids, Order: Rosales, Family: Cannabaceae; GenBank accession number: KY654502, AB924756)] of *rbc*L and *mat*K [-the cp genes used in the plant DNA barcoding (CBOL, 2009)] with the sequence of *S. asoca* (KY678341, KC592386/MN866115) revealed the optimal tree with the sum of branch length 0.57133802 (Fig. 5), and have potential to be used as molecular typing of *S. acoca* from its adulterants (Hegde et al., 2018) as NMR spectroscopy (Urumarudappa et al., 2016) and *rbc*L-ISSR based DNA barcodes (Hegde et al., 2018) are least user-friendly.

Table 1
The SSR loci of *S. asoca* cp genome.

S.	Type	SSR	Size	Starts	End
1	P1	(T)10	10	2993	3002
2	p2	(TA)6	12	3933	3944
3	p2	(CT)6	12	9477	9488
4	p2	(TA)6	12	9799	9810
5	p1	(A)11	11	11,185	11,195
6	p1	(A)10	10	11,518	11,527
7	p1	(T)10	10	14,444	14,453
8	p1	(A)10	10	15,746	15,755
9	c	(T)11seq (T)14	105	16,111	16,215
10	c	(T)15 seq (A)10	109	17,236	17,344
11	p1	(T)12	12	18,454	18,465
12	p1	(T)13	13	18,906	18,918
13	p1	(A)11	11	19,421	19,431
14	p1	(A)10	10	48,187	48,196
15	p1	(A)14	14	50,369	50,382
16	p1	(T)14	14	51,455	51,468
17	p2	(TA)8	16	51,746	51,761
18	p1	10(A)	10	53,129	53,138
19	p1	(T)10	10	53,454	53,463
20	p1	(T)14	14	54,019	54,032
21	c	(AT)7 seq (T)11	119	59,271	59,389
22	p1	(T)10	10	59,795	59,804
23	c	(AT)6 seq (AT)6 seq (AT)7	163	60,249	60,411
24	p3	(TAT)5	15	60,634	60,648
25	p1	(A)10	10	61,434	61,443
26	c	(T)11 g(A)10	22	63,236	63,257
27	p1	(T)12	12	65,351	65,362
28	p1	(T)10	10	65,958	65,967
29	p4	(TTAA)6	24	69,696	69,719
30	p1	(T)12	12	73,240	73,251
31	p1	(T)10	10	74,761	74,770
32	c	(A)10 seq (A)9	89	76,392	76,480
33	c	(A)10 seq (AT)6	58	77,232	77,289
34	p1	(T)11	11	77,940	77,950
35	p1	(A)11	11	79,224	79,234
36	p1	(T)10	10	80,731	80,740
37	p1	(G)10	10	82,977	82,986
38	p1	(A)15	15	84,348	84,362
39	p1	(C)11	11	84,762	84,772
40	p2	(AT)6	12	85,254	85,265
41	p1	(A)10	10	91,245	91,254
42	p1	(T)10	10	91,781	91,790
43	p1	(T)13	13	92,481	92,493
44	p1	(A)14	14	93,452	93,465
45	p1	(A)12	12	93,799	93,810
46	p2	(AT)10	20	94,894	94,913
47	p2	(TA)6	12	96,515	96,526
48	p2	(AT)6	12	96,648	96,659
49	p1	(A)12	12	101,516	101,527
50	p1	(T)10	10	103,439	103,448
51	p2	(TA)7	14	103,785	103,798
52	p1	(T)15	15	105,822	105,836
53	p1	(T)10	10	106,166	106,175
54	p2	(AT)6	12	106,328	106,339
55	p1	(T)12	12	108,073	108,084
56	p1	(T)10	10	109,129	109,138
57	c	(A)11 seq (T)10	38	109,557	109,594
58	p1	(A)13	13	112,044	112,056
59	p2	(AT)6	12	112,200	112,211
60	c	(AT)7 t(TA)7	29	112,852	112,880
61	p1	(A)10	10	114,163	114,172
62	p1	(A)13	13	115,814	115,826
63	p1	(T)10	10	116,265	116,274
64	p1	(T)10	10	117,561	117,570
65	p1	(T)10	10	120,635	120,644
66	p1	(A)10	10	121,197	121,206
67	p1	(T)10	10	126,128	126,137
68	p1	(T)13	13	130,202	130,214
69	p1	(A)11	11	131,334	131,344
70	p1	(T)10	10	133,319	133,328

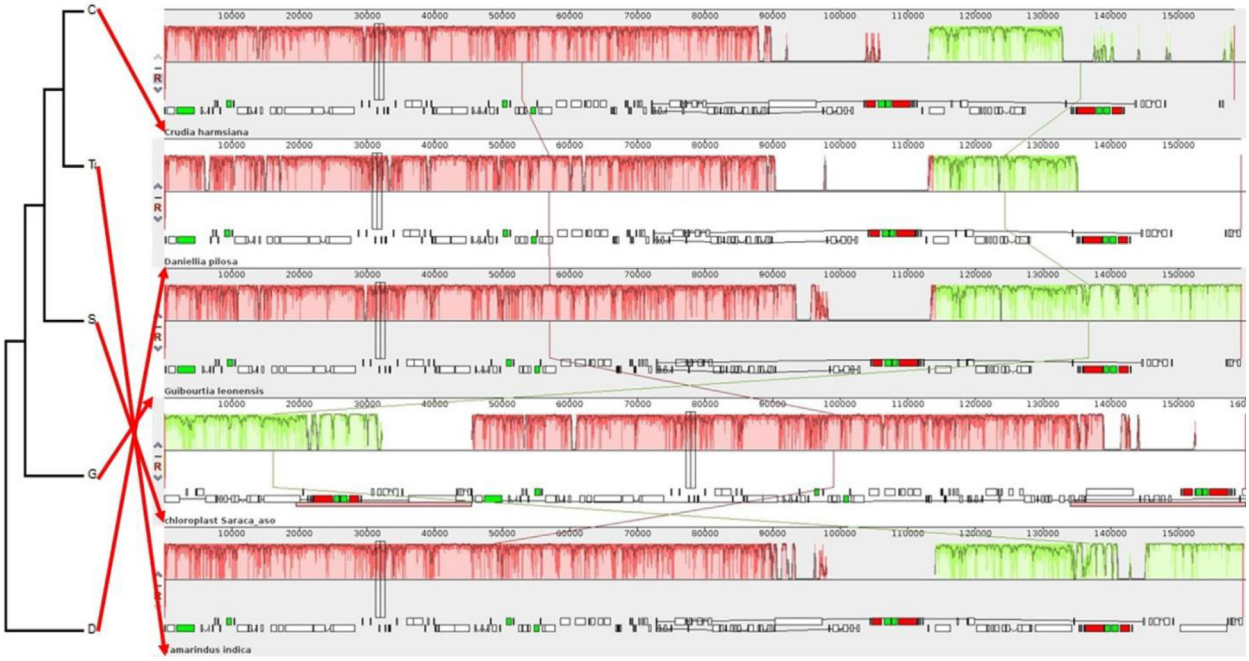


Fig. 4. The MAUVE alignment of cp genomes of five different Detarioideae, showing genomic rearrangement.

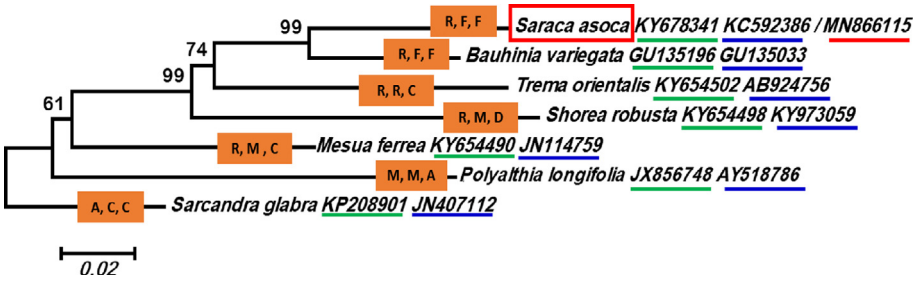


Fig. 5. The minimum evolution tree based on combined sequence of *rbc*L and *mat*K gene representative species of Rosids. (R, F, F: Clade: Rosids, Order: Fabales, Family: Fabaceae; R, M, C: Clade: Rosids, Order: Malpighiales, Family: Calophyllaceae; M, M, A: Clade: Magnoliids, Order: Magnoliales, Family: Annonaceae; R, M, C: Clade: Rosids, Order: Malvales, Family: Dipterocarpaceae; R, R, C: Clade: Rosids, Order: Rosales, Family: Cannabaceae).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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