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 *rbcL* and *matK* gene were established using molecular phylogenetic analyses for molecular typing of *S. asoca*.

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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 signif-

icance e.g. antimicrobial (Seetharam et al., 2003; Shirodkar 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;...
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 NDNeasy 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 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 repeats were more in 15–20 bp and 31–40 bp category, respectively (Fig. 3A). The repeat structures of 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 forward and palindrome repeats were common in these 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 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 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. rbcl and matK of adulterant species (a) Bauhinia variegata L. (GU135196, GU135033), (b) Mesua ferrea L. (KV654490, JN114759), (c) Polyalthia longifolia (Sonn.) Thwaites (JK856748, AY518786), (d) Shorea robusta Gaertn. (KV654498, KY973059) and (e) Trema orientalis (L.) Blume (KV654502, AB924756) were retrieved from the GenBank, and analyzed together with the sequences of the S. asoca (KV678341, 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 used for MEGA X (Kumar et al., 2018).
Fig. 2. A. The cp genome map Saraca asoca, B. the genes of different groups are color-coded.
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: Magnoliidae, 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 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. asoca from its adulterants (Hegde et al., 2018) as NMR spectroscopy (Urumarudappa et al., 2016) and rbcL-ISSR based DNA barcodes (Hegde et al., 2018) are least user-friendly.

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)].
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)11        | 105  | 16,111   | 16,215 |
| 10  | c    | (T)15        | 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   | 51,465 |
| 17  | p2   | (TA)8        | 16   | 51,746   | 51,761 |
| 18  | p1   | (A)10        | 10   | 53,129   | 53,138 |
| 19  | p1   | (T)10        | 10   | 53,543   | 53,563 |
| 20  | p1   | (T)14        | 14   | 54,019   | 54,032 |
| 21  | c    | (AT)7        | 119  | 59,271   | 59,389 |
| 22  | p1   | (T)10        | 10   | 59,795   | 59,804 |
| 23  | c    | (AT)6        | 163  | 60,294   | 60,411 |
| 24  | p3   | (TA)5        | 15   | 60,634   | 60,648 |
| 25  | p1   | (A)10        | 10   | 61,434   | 61,443 |
| 26  | c    | (T)11        | 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        | 89   | 76,392   | 76,480 |
| 33  | c    | (A)10        | 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        | 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        | 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 |
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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