Insights into plastome of *Fagonia indica* Burm.f. (Zygophyllaceae): organization, annotation and phylogeny

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**ARTICLE INFO**

Article history:
Received 21 September 2021
Revised 3 November 2021
Accepted 4 November 2021
Available online 12 November 2021

**Keywords:**
Fagonia indica
Zygophyllaceae
NGS
Plastome
Fabids

**ABSTRACT**

The enhanced understanding of chloroplast genomics would facilitate various biotechnology applications; however, the chloroplast (cp) genome / plastome characteristics of plants like *Fagonia indica* Burm.f. (family Zygophyllaceae), which have the capability to grow in extremely hot sand desert, have been rarely understood. The *de novo* genome sequence of *F. indica* using the Illumina high-throughput sequencing technology determined 128,379 bp long cp genome, encode 115 unique coding genes. The present study added the evidence of the loss of a copy of the IR in the cp genome of the taxa capable to grow in the hot sand desert. The maximum likelihood analysis revealed two distinct sub-clades i.e. Krameriaceae and Zygophyllaceae of the order Zygophyllales, nested within fabids.

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1. Introduction

The cp (chloroplast) genome / plastome encodes several key proteins involved in the photosynthesis and in other metabolic processes important for the interactions of plants with the environment as well as defense against invading pathogens (Bobik and Burch-Smith, 2015). The availability of organelle or even whole genome sequence data in different databases repositories are gradually increasing because of the advancement of massively parallel next-generation DNA sequencing platforms and development of bioinformatics resources during last two decades; as a result, the characterization of over 5000 chloroplast (cp) genome sequences / plastome until September 2021 available in the GenBank, have revolutionized the application of plastome genomics (Ali et al., 2020), genetic engineering to enhance plant agronomic traits (Cosa et al., 2001; Ruf et al., 2001; Dufourmant et al., 2004, 2005; Liu et al., 2007; Zhou et al., 2008; Singh et al., 2010; Lee et al., 2011; Jin et al., 2011, 2012, 2015; Zhang et al., 2015), synthesis of enzymes and biomaterials (Jin et al., 2011; Viitanen et al., 2004; Verma et al., 2010), enhancing nutrition (Shintani et al., 1998; Schneider, 2005; Apel et al., 2009; Jin et al., 2014), biopharmaceuticals (Grabowski et al., 2006; El Kaoutari et al., 2013; Kwon et al., 2013; Shenoy et al., 2014; Kohli et al., 2014; Holtz et al., 2015; Kwon et al., 2015), biomedical products (Daniell et al., 2016), and in understanding the genetic diversity, and phylogeny (Daniell et al., 2016; Brozynska et al., 2016; Jansen et al., 2007; Moore et al., 2010; Elshikh et al., 2020).

*Fagonia indica* Burm.f. (Family: Zygophyllaceae, Order: Zygophyllales, Clade: Fabids) (APG IV, 2016) is a densely to sparsely branched thorny herb approximately 60 × 100 cm in height and width respectively (Fig. 1), possess anticancer activity (Lam et al., 2014), is widely distributed in Asian and African deserts (El Hadidi, 1985; Basto, 2002; Beier et al., 2004; Beier, 2005) to the inner zone of Empty Quarter (-hottest sand desert) (Mandavil, 1986). A thorough survey of published reports revealed that the cp genomes of plants, like *F. indica*, which have the capability to...
grow in extremely hot sand desert, have been rarely characterized. The present report deals the complete cp genome sequence of *F. indica*, and discuss its genome organization including gene content and repeat features, phylogeny, and compare with the representative plants of major habitats to detect similarity and variations.

2. Materials and methods

2.1. Plant material, DNA extraction and de novo genome sequencing

The fresh leaves of *F. indica* were collected from the desert of Riyadh region, Saudi Arabia. The genomic DNA extracted using the Qiagen DNeasy Kit (Qiagen, Hilden, Germany) was subsequently used to construct short-insert libraries according to the manufacturer’s manual (Illumina, Inc., San Diego, USA), and sequenced as a single-end run of 51 bp using the DNA Illumina sequencing platform (Quail et al., 2012).

2.2. The cp genome assembly and annotation

The sequence raw reads were filtered using fastqc to obtain the high-quality clean sequence data by removing adaptor sequences. The high-quality filtered reads were then assembled using spades (Bankevich et al., 2012). The assembled cp genome was annotated using default parameters (Tillich et al., 2017; Stothard, 2000) of GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq.html), the NCBI GenBank sequence of *Larrea tridentata* (DC.) Coville (family Zygophyllaceae) (GenBank accession number NC_028023.1) was used as a reference for annotation and for further comparison with the closely related *L. tridentata* using the mVISTA program (http://
Figure 3. Comparison of the border positions of SSC, LSC, and IR regions between the chloroplast (cp) genome of the *Larrea tridentata* and *Fagonia indica*. The selected genes or portions of genes have been indicated by the boxes above the genome. SSC: small single copy; LSC: large small copy; IR: reverse complementary repeat region; bp: base pairs.

Table 1

| Features | *F. indica* | *L. tridentata* |
|----------|-------------|-----------------|
| Length   | 128,379     | 136,194         |
| %GC      | 34.02372    | 35.092589       |
| Gene     | 115         | 126             |
| CDS      | 80          | 75              |
| rRNA     | 4           | 4               |
| tRNA     | 31          | 28              |

Figure 4. Percent identity plot of the comparison of the chloroplast (cp) genome of the *Fagonia indica* with *Larrea tridentata*. 
In the genom.lbl.gov/vista/mvista/submit.shtml in Shuffle-LAGAN mode (Brudno et al., 2003).

2.3. Repeat structure and small inversion

The tandem repeats were analyzed using the ‘Tandem Repeat Finder’ (https://tandem.bu.edu/trf/trf.html) (Benson 1999), (Timme et al., 2007), and REPuter (https://bibiserv.cebitec.uni-bielefeld.de/reputer/) (Castro et al., 2013) was used to identify and locate dispersed repeats including the direct (forward) and inverted (palindrome) repeats. The tandem repeats less than 15 bp in length and the REPuter redundant results were removed manually, and then the candidate small inversions (SIs) were identified when the repeats’ distance was less than 50 bp (Yang et al., 2010), and the likely secondary structures of the SIs were evaluated using MFOLD (version 3.2) (http://unafold.rna.albany.edu/q=mfold). The potential microsatellite regions were tracked by looking for five or more repeats of the nucleotides A and T using MISA (http://misaweb.ipk-gatersleben.de/) (Beier et al., 2017).

2.4. Phylogenetic analysis

A total number of 48 chloroplast genes (Supplementary Table S1) present in the cp genomes belonging to 49 taxa (Supplementary Table S2) from 49 different orders and the three outgroup sequences belonging to Gymnosperm clade were retrieved from the GenBank, and aligned using ClustalX (Thompson et al., 1997). The maximum likelihood (ML) analyses was performed using MEGAX software (Kumar et al., 2018).

Fig. 5. Repeat structure analysis in the chloroplast (cp) genome. The cutoff value was 15 bp for a tandem repeat and 30 bp for a dispersed repeat. (A) frequency of repeats by length, (B) repeat type, (C) location distribution of repeats. (CDS: coding sequence).

Fig. 6. Repeat structures in Larrea tridentata and Fagonia indica (Zygophyllaceae).
Fig. 7. Folded stem-loop structures in the three small inversions (Sis) of Fagonia indica.

Table 2
Simple sequence repeat (SSR) loci in the chloroplast (cp) genome of Fagonia indica.

| Position | Repeat | Repeat length of consensus | Locus Region |
|----------|--------|----------------------------|--------------|
| 285      | A      | 10                         | trnH-GUG-psbA intergenic |
| 2380     | T      | 11                         | matK CDS     |
| 17,585   | A      | 14                         | trnS-GGA-ycf3 intergenic |
| 17,960   | A      | 10                         | ycf3 intron |
| 18,892   | A      | 12                         | ycf3 intron |
| 26,911   | T      | 12                         | psbZ-trnS-UGA intergenic |
| 31,068   | AT     | 6                          | psbD-trnT-GGU intergenic |
| 31,686   | T      | 12                         | trnT-GGU-trnK-UUC intergenic |
| 32,350   | A      | 11                         | trnV-GUA-trnD-GUC intergenic |
| 36,011   | A      | 10                         | trnC-GCA-rpoB intragenic |
| 36,458   | A      | 10                         | trnC-GCA-rpoB intragenic |
| 36,548   | A      | 10                         | rpoC1 intron |
| 40,452   | A      | 10                         | rpoC2 CDS    |
| 44,927   | A      | 13                         | rpoC2 CDS    |
| 46,844   | A      | 11                         | psbC-psbl    |
| 49,022   | A      | 11                         | psbC-psbl    |
| 52,145   | T      | 10                         | accD-psal    |
| 52,145   | T      | 10                         | accD-psal    |
| 52,145   | T      | 10                         | psal-ycf4    |
| 58,939   | T      | 11                         | psal-ycf4    |
| 59,528   | T      | 10                         | psal-ycf4    |
| 60,008   | A      | 10                         | psbC-psbl    |
| 64,326   | AT     | 6                          | psbC-psbl    |
| 66,729   | A      | 11                         | trnP-UGG-psalintergenic |
| 68,792   | T      | 11                         | rps18-rpl20  |
| 69,919   | TA     | 6                          | rpl20-clpP   |
| 70,918   | T      | 10                         | clpP intron  |
| 76,067   | T      | 11                         | petB-petD    |
| 81,388   | A      | 13                         | rpl14-rpl16  |
| 110,755  | T      | 10                         | ycf1 CDS     |
| 110,875  | A      | 10                         | ycf1 CDS     |
| 111,474  | A      | 10                         | ycf1 CDS     |
| 113,104  | A      | 11                         | ycf1 CDS     |
| 114,482  | TA     | 10                         | ycf1-rps15   |
| 114,945  | A      | 11                         | rps15 CDS    |
| 119,431  | AT     | 6                          | ndhF-ndhG    |
| 123,288  | TA     | 7                          | ndhD-ccsA    |
| 125,155  | T      | 11                         | rpl32 CDS    |
| 125,883  | T      | 10                         | rpl32-ndhF   |
| 127,343  | A      | 10                         | ndhF CDS     |
3. Results

3.1. Content and organization of plastome

The mapping of the assembled cp genome resulted into circular molecule (Fig. 2) with a total number of 115 unique genes [represents 1,28,379 base pair (bp) nucleotides (nt)] which includes 80 CDS (represents 80,200 bp nucleotides coding for 42,793 codons), 31 tRNA genes and four rRNA genes. The assembled cp genome sequence was submitted to the NCBI (GenBank accession number MN521457). The cp genome size of *F. indica* was approximately 128 kb, which was smaller than that of *L. tridentata* (Fig. 3, Table 1).
The coding regions were less divergent than the non-coding regions (Fig. 4).

A total of 13 genes, including seven protein-coding genes and six tRNA genes, contained one or two introns (Supplementary Table S3). Among the intron-containing genes, trnK-UUU had the largest intron (2511 bp) that includes the matK gene, and trnL- UAA had the smallest intron (551 bp). The ycf3 gene had two introns of 722 and 758 bp. The sequence analysis indicates that 58.49%, 6.87%, and 3.53% of the genome sequence encode proteins, tRNAs, and rRNAs, respectively, whereas 41.50% of the genome sequence is a non-coding sequence filled with introns, intergenic spacers, and pseudogenes. Based on the sequences of protein-coding and tRNA genes within the cp genome, Phe (0.05%) and Arg (0.0038%) were the most and least used amino acids, respectively (Supplementary Table S4).

The tandem and dispersed repeats were analyzed in the cp genome of F. indica. Forty-one tandem repeats were identified, of which 23 were 15–20 bp, 14 were 21–30 bp, two were 31–40 bp, one was 41–50 bp, and another one was 81–90 bp in size. Similarly, 43 dispersed repeats were identified, of which one was 21–30 bp, 22 were 31–40 bp, 11 were 41–50 bp, four were 51–60 bp, one was 61–70 bp, another one was 81–90 bp, and three were more than 91 bp in size. In total, 84 repeats were identified, of which 87% were in the intergenic spacer regions, 6% in introns, and 7% in the CDS regions, respectively (Fig. 5). The repeat structures in other members of Zygophyllaceae (L. tridentata) were also analyzed using Reputer (Fig. 6). The forward and inverted repeats were common in L. tridentata and F. indica. In addition, in the same Zygophyllaceae family, different repeat structures were found between F. indica and L. tridentata. Of the two Zygophyllaceae cp genomes studied, F. indica contained the highest total number of repeats that were 75 bp or greater in length and SI5 ranging from 11 to 24 bp in size. The folded stem-loop structures of the three SI5 of F. indica are shown in Fig. 7.

Within the cp genome of F. indica, 37 different SSR loci were repeated more than five times (Table 2). Of these, 31 loci were homopolymers and six were di-polymers. All homopolymeric loci contained multiple A or T nucleotides, whereas all di-polymeric loci contained multiple AT or TA nucleotides. These SSR loci contribute to the A-T richness of the cp genome of F. indica.

The present maximum likelihood (ML) bootstrap analysis revealed two major clades—monocots and eudicots. In the eudicots clade, F. indica clades with L. tridentata and T. mongolica (family Zygophyllaceae, order Zygophyllales) nested within the clade fabids. The maximum likelihood tree (MLT) also revealed two distinct clades of Krameriaceae and Zygophyllaceae (Fig. 8).

4. Discussion

In the present study, the mapping of the assembled cp genome was found similar to the angiosperm (Raubeson et al., 2007), except for the loss of one copy of the IR as similar to majority of papilionoid (Doyle et al., 1996; Kato et al., 2000; Sasaki et al., 2005; Guo et al., 2007). The rps16 gene was found in the cp genomes of most angiosperms, including the representatives of the early-branching lineages (Goremykin et al., 2003; Raubeson et al. 2007; Hansen et al., 2007); however, it was not found in the F. indica.

F. indica had a single copy of inverted repeat resulted into the inverted gene order compared to its taxonomically close relative L. tridentata. The lengths of the cp genomes of angiosperms remain variable primarily because of nucleotide substitutions, gene/intron losses, and expansion and contraction of the inverted repeat IR region (Jansen et al., 2007). It was noted that the coding regions were less divergent than the non-coding regions (Fig. 4); however, further analysis showed that clpP and accD were the most divergent coding regions (Supplementary Table S5). Photosynthesis is the ultimate source of biomass production (Beadle and Long, 1985). The PAR (photosynthetically active radiation) intensity is an important factor that determines the rate of photosynthesis (Wimalasekera, 2019). The intensity of light varies in different major habitats (Warrant and Johnsen, 2013). The comparative cp genome analysis of F. indica as a representative from hot sand desert with the representatives of flowering plants occurring in different major habitats further supports the conservative pattern of the cp genome and suggests that the genes contained in the cp genome might not have roles solely in organism yield, rarity, or abundance and biomass, and in encountering stress (Elshikh et al., 2020).

The knowledge of phylogeny is used in almost every branch of biology (Yang and Rannala, 2012) including taxonomy (Philippe et al., 2005; APG IV, 2016), evolution (Edwards, 2009; Soltis et al., 2019) and comparative biology (Eisen, 1998; Maser et al., 2001; Kellis et al., 2003; Pedersen et al., 2006; Lindblad et al., 2011), medicine (Marra et al., 2003; Grenfell et al., 2004; Salipante and Horwitz, 2006), and genomics (Paten et al., 2008; Green et al., 2010; Cronau et al., 2011; Li and durbin, 2011; Ma, 2011). Moreover, the family Zygophyllaceae has previously been treated as being related either to Geraniaceae (Geraniales) or to Sapindales/Rutales or Linales/Malpighiales (Sheahan and Chase, 1996). Secondly, the phylogenetic relationships of the two sister families, e.g., Zygophyllaceae and Krameriacae (Soltis et al., 1998; Savolainen et al., 2000; Wang et al., 2009; Tao et al., 2018) under the order Zygophyllales, have often been controversial APG IV, 2016. The wood anatomy supports the separation of Krameriacae from the Zygophyllaceae (Carliquist, 2005). Granot and Grafi (2014) argued, based on epigenetic studies, that the placement of the families Krameriacae and Zygophyllaceae under the order Zygophyllales should be re-examined. The present maximum likelihood (ML) bootstrap analysis revealed two major clades—monocots and eudicots. In the eudicots clade, F. indica clades with L. tridentata and T. mongolica (family Zygophyllaceae, order Zygophyllales) nested within the clade fabids. The maximum likelihood tree (MLT) also revealed two distinct clades of Krameriacae and Zygophyllaceae.

5. Conclusions

The analyses of de novo genome sequence of F. indica (family Zygophyllaceae) have added the evidence of the loss of a copy of the IR in the cp genome of the taxa capable to grow in the hot sand desert. The maximum likelihood analysis revealed two distinct sub-clades i.e. Krameriacae and Zygophyllaceae of the order Zygophyllales.

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.

Acknowledgements

The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSP-2021/306), King Saud University, Riyadh, Saudi Arabia. This research was supported by the grant from the KIRIBB Initiative Program of the Republic of Korea.
Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbs.2021.11.011.

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