The cp genome characterization of Adenium obesum: Gene content, repeat organization and phylogeny

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Abstract
Adenium obesum (Forssk.) Roem. & Schult. belonging to the family Apocynaceae, is remarkable for its horticultural and ornamental values, poisonous nature, and medicinal uses. In order to have understanding of cp genome characterization of highly valued medicinal plant, and the evolutionary and systematic relationships, the complete plastome / chloroplast (cp) genome of A. obesum was sequenced. The assembled cp genome of A. obesum was found to be 154,437 bp, with an overall GC content of 38.1%. A total of 127 unique coding genes were annotated including 96 protein-coding genes, 28 tRNA genes, and 3 rRNA genes. The repeat structures were found to comprise of only mononucleotide repeats. The SSR loci are composed of only A/T bases. The phylogenetic analysis of cp genomes revealed its proximity with Nerium oleander.

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1. Introduction
Adenium obesum (Forssk.) Roem. & Schult. (family Apocynaceae), the ‘Desert Rose’ is a poisonous, medicinal plant, distributed from Africa to Arabia, and is used traditionally in the treatment of various ailments e.g. skin diseases, wounds, muscle pain, joint pain, venereal diseases, tooth decay, septic wounds, and nasal infections (Dimmitt and Hanson, 2002; Mouza and Hossain, 2015; Hossain et al., 2017). It is also used as a pesticide (Versiani et al., 2014), arrow poison for hunting in Africa (Oyen, 2008) and fish toxin (Wiseman, 2009). The A. obesum plant extract reported to possess cytotoxic (Almehdar et al., 2012), antimicrobial (Hossain et al., 2017) and anti-influenza (Kiyohara et al., 2012) activities. The phytochemical compounds identified from A. obesum include cardiac glycosides (cardenolides), pregnanes, triterpenes, flavonoids, and acetyldigitoxigenin (Versiani et al., 2014). The molecular docking of acetyldigitoxigenin elucidates the plausible mechanisms underlying the anticancer properties (Gurung et al., 2020).

The recent development in plastome or chloroplast (cp) genomics due to massive progress in the next-generation sequencing (NGS) platforms (Eid et al., 2009; Rothberg et al., 2011; Pattnaik et al., 2014; Jain et al., 2016; Shendure et al., 2017) and bioinformatics tools (Mavromatis et al., 2007; Knudsen et al., 2010; Huang et al., 2012; McElroy et al., 2012; Shendure and Aiden, 2012; Yang and Rannala, 2012; Caboche et al., 2014; Shcherbina 2014; Kwon et al., 2015; Langmead and Nellore, 2018) have greatly impact on biotechnology application (Spök et al., 2008; Zhang et al., 2015; Daniell et al., 2016). We herein for the first time report the cp genome characterization of highly valued medicinal plant A.
obesum, and discuss its structure including gene content, repeat organization, and phylogeny.

2. Materials and methods

2.1. DNA sequencing, assembly and annotation

The fresh leaves of A. obesum were collected from the wild condition of desert habitat near to Riyadh, Saudi Arabia. The total genomic DNA was isolated using QIAGEN DNeasy DNA extraction kit. The de novo sequencing base calling was performed using the Illumina Pipeline 1.3.2 (Nie et al., 2012). The raw reads were filtered using FastQC to obtain the high-quality clean data by removing adaptor sequences using trimmomatic and low-quality reads with Q-value < 20. The filtered reads were assembled using Spades (Bankevich et al., 2012), and annotated using GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq.html) (Tillich et al., 2017; Hansel et al., 2007). Further downstream analysis from the assembled cp genome included the repeat structure (Benson 1999; Timme et al., 2007) and small inversion (Nagano et al., 1991; Yang et al., 2010; Doorduin et al., 2011; Castro et al., 2013; Beier et al., 2017).

2.2. Comparison of cp genome and phylogenetic analysis

The cp genome of A. obesum were plotted using the mVISTA (http://genome.lbl.gov/vista/mvista/submit.shtml) program with a total number of nine complete cp genomes of Apocynaceae [i.e. (1) Asclepias nivea Forssk., (2) Carissa macrocarpa (Eckl.) A. DC., (3) Catharanthus roseus (L.) G. Don, (4) Cynanchum auriculatum Buch.-Ham. ex Wight, (5) Echites umbellatus Jacq., (6) Nerium oleander L., (7) Oncinotis tenuiloba Stapf, (8) Pentalinon luteum (L.) B.F. Hansen & Wunderlin, and (9) Rhazya stricta Decc.).

The cp sequences of 48 genes [e.g. ATP synthase genes (atpA, atpB, atpE, atpF, atpH, and atpI), c-type cytochrome synthesis gene (ccsA), envelope membrane protein gene (cemA), Maturase gene (matK), cytochrome b6/f genes (petA, petB, petD, petG, and petN), Photosystem I genes (psaA, psaB, psaC, and psaL), Photosystem II genes (psbA, psbB, psbE, psbH, psbI, psbJ, psbK, and psbT), Rubisco gene (rbcL), Large-subunit ribosomal protein genes (rpl14, rpl2, rpl20, rpl32, rpl33, and rpl36), RNA polymerase subunit genes (rpoB, rpoC1, and rpoC2), Small-subunit ribosomal protein genes (rps14, rps15, rps18, rps19, rps2, rps3, rps4, and rps7), Genes of unknown function (ycf3, and ycf4)] were retrieved from 19 ingroup taxa comprising 10 species of the family Apocynaceae, the representative of the family Apiaceae, Aquifoliaceae, Apocynaceae, Aredoxaceae, Eucommiaceae, Gentianaceae, Icacinaceae, Lamiaceae, Solanaceae, and the outgroup from the family Cornaceae (Table 1), and aligned using Clustal X (Thompson et al., 1994), and the molecular phylogenetic analysis was performed by Maximum Evolution method (Rzhetsky and Nei, 1992) using in MEGA X (Kumar et al., 2018).

3. Results and discussion

The present study reports assembly of the complete cp genome map as a conserved circular structure comprising a total length of 154,437 bp (including LSC, SSC, IRA, and IRB), with an overall GC content of 38.1% (Fig. 1). The results revealed the gene contents, orientation, and the conservation as well as polymorphisms were found in the chloroplast genome as similar to those of other cp genome of angiosperms (Daniell et al., 2016). A total number of 127 genes were annotated including 96 protein-coding genes, 28 tRNA genes, and 3 rRNA genes (NCBI GenBank accession number: MN765097).

The sequence identity of A. obesum plotted with the nine different complete cp genomes from the family Apocynaceae e.g. A. nivea, C. macrocarpa, C. roseus, C. auriculatum, E. luteola, O. tenuiloba, P. luteum and R. stricta using the mVISTA revealed high similarities amongst them with few regions where the identities was below 90% (Fig. 2).

Moreover, the present study depicted the distribution and location of repeated structures and microsatellites in the cp genome. The microsatellites or simple sequence repeats (SSRs) are tandem repeats which ranges from 1 to 6 bp and are present commonly in cp genomes (Meng et al., 2018). SSRs have been served as an important marker for molecular characterization of plant species. A total of 40 SSRs were predicted in A. obesum (Table 2) which were composed of a length of at least 10 bp, all of which were found to be homopolymers containing multiple A or T nucleotides at each locus. These reveal that SSR loci are rich in A-T content in the A. obesum cp genome which supports previous chloroplast SSRS

| Sl. No. | Taxon | Order | Family | Subfamily | Tribe | Subtribe | GenBank |
|--------|-------|-------|--------|-----------|-------|----------|---------|
| 1.     | Adenium obesum (Forssk.) Roem. & Schult. | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | MN765097* |
| 2.     | Asclepias nivea Forssk. | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_022431.1* |
| 3.     | Cynanchum auriculatum Buch.-Ham. ex Wight | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_025460.1 |
| 4.     | Carissa macrocarpa (Eckl.) A. DC. | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_033534.1 |
| 5.     | Catharanthus roseus (L.) G. Don | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_021423.1* |
| 6.     | Rhazya stricta Decc. | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_024292.1* |
| 7.     | Echites umbellatus Jacq. | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_025555.1 |
| 8.     | Pentalinon luteum (L.) B.F. Hansen & Wunderlin | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_025658.1 |
| 9.     | Nerium oleander L. | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_025656.1* |
| 10.    | Oncinotis tenuiloba Stapf | Gentianales | Apocynaceae | Apocynoideae | Nerieae | Nerinae | NC_025656.1 |
| 11.    | Anethum graveolens L. | Apiaceae | Apioideae | Apioideae | Apioideae | Apioideae | NC_029470.1 |
| 12.    | Ilex delavayi Franch. | Aquifoliaceae | Aquifoliaceae | Aquifoliaceae | Aquifoliaceae | Aquifoliaceae | KX426470.1 |
| 13.    | Helianthus annuus L. | Asterales | Asterales | Asterales | Asterales | Asterales | NC_007977.1 |
| 14.    | Viburum betulifolium Batalin | Dipsacales | Dipsacales | Dipsacales | Dipsacales | Dipsacales | NC_037951.1 |
| 15.    | Eucommia ulmoides Oliv. | Garryales | Eucommiaceae | Eucommiaceae | Eucommiaceae | Eucommiaceae | NC_025319.1 |
| 16.    | Gentiana tibetica King ex Hook. f. | Gentianales | Gentianaceae | Gentianaceae | Gentianaceae | Gentianaceae | NC_025319.1 |
| 17.    | Iodes cirrhosa Turcz. | Icacinaceae | Icacinaceae | Icacinaceae | Icacinaceae | Icacinaceae | NC_036254.1 |
| 18.    | Premna microphylla Turcz. | Lamiaceae | Lamiaceae | Lamiaceae | Lamiaceae | Lamiaceae | NC_026291.1 |
| 19.    | Tochona australie Griseb. | Solanaceae | Solanaceae | Solanaceae | Solanaceae | Solanaceae | NC_028333.1 |

**Ingroup**

**Outgroup**

The ingroup and outgroup taxon with their classification and GenBank accession number included in the phylogenetic analyses. The GenBank accession number marked with * were included in the mVISTA alignment.
reports (Li et al., 2017). Among these SSRs, four SSRs were situated in coding regions and 31 were located in the intergenic regions (Table 2). A total number of 19 genes including 11 protein-coding genes and 8 tRNA genes contained one or two introns (Table 3). Furthermore, five SSRs were found in intronic regions. Thus, most of the repeats were situated in the intergenic region. Tandem and dispersed repeats were analyzed for A. obesum cp genomes and a total of 25 tandem and 19 dispersed repeats were observed (Fig. 3).

The phylogenetic relationships of a total number of 48 cp genes from the 19 cp genomes including the family Apocynaceae and the representative members of the family Apiaceae (Apiales), Aquifoliaceae (Aquifoliales), Adoxaceae (Dipscales), Eucommiaceae (Garryales), Gentianaceae (Gentianales), Icacinaceae (Icinales), Lamiaeeae (Lamiiales), Solanaceae (Solanales), and the outgroup at the family Cornaceae (Cornales) revealed the proximity of A. obesum (Subfamily Apocynoideae, Tribe Nerieae, Subtribe Neriinae) with Nerium oleander (Subfamily Apocynoideae, Tribe Ner-
Fig. 2. The percent identity plot for comparison of cp genome of *A. obesum* with the other Apocynaceae genomes. Lane from up to down: *A. nivea*, *C. macrocarpa*, *C. roseus*, *C. auriculatum*, *E. umbellatus*, *N. oleander*, *O. temuloba*, *P. luteum*, and *R. stricta*. 
The family Apocynaceae is one of the 10 largest angiosperm families with c. 4,500 species under c. 370 genera globally with the greatest diversity in the tropics and subtropics (Stevens, 2001; Endress et al., 2014; APG, 2016). Apart from the large number of molecular phylogenetic studies on the family Apocynaceae (e.g. Liede and Täuber, 2000, 2002; Liede, 2001; Liede and Meve, 2001, 2002; Meve and Liede, 2001, 2002, 2004a,b; Potgieter and Albert, 2001; Liede and Kunze, 2002; Khalid Mashay Alanazi, Mohammad Ajmal Ali, Soo-Yong Kim et al. Saudi Journal of Biological Sciences 28 (2021) 3768–3775

Table 2
The SSR loci in the cp genome of Adenium obesum.

| Start | End  | Repeat | Repeat length of consensus | Locus Region |
|-------|------|--------|----------------------------|--------------|
| 2109  | 2189 | (A)10  | 81                         | ycf1         |
| 2914  | 2925 | (A)12  | 12                         | ycf1         |
| 9557  | 9566 | (T)10  | 10                         | ndhF-ndhG    |
| 13,831| 13,840| (A)10 | 10                         | ccsA-trnL-UAG|
| 15,378| 15,388| (T)11 | 11                         | rpl22-ndhF   |
| 15,614| 15,624| (A)11 | 11                         | rns23-trnA-UGC|
| 23,933| 23,950| (T)18 | 18                         | trnM-GCC     |
| 43,878| 43,887| (A)10 | 10                         | rps16        |
| 49,254| 49,266| (T)13 | 13                         | atpF         |
| 52,132| 52,141| (A)10 | 10                         | psbI-trnS-GCU|
| 53,347| 53,356| (T)10 | 10                         | ndhI-ndhG    |
| 53,607| 53,620| (T)14 | 14                         | trnG-GCC     |
| 53,763| 53,775| (T)13 | 13                         | trnR-UUC-trnA|
| 55,426| 55,435| (A)11 | 11                         | rpl22         |
| 55,657| 55,667| (T)10 | 10                         | rpl32         |
| 56,129| 56,139| (T)11 | 11                         | rps16        |
| 57,865| 57,874| (T)10 | 10                         | psbA-trnS-GCU|
| 58,082| 58,093| (T)12 | 12                         | ndhI-ndhG    |
| 60,138| 60,148| (A)10 | 10                         | ycf1         |
| 62,367| 62,377| (T)11 | 11                         | rps16        |
| 72,576| 72,585| (T)10 | 10                         | ycf1         |
| 79,736| 79,747| (T)12 | 12                         | ycf1         |
| 88,261| 88,272| (T)12 | 12                         | ycf1         |
| 95,456| 95,465| (T)10 | 10                         | ndhI-ndhG    |
| 96,134| 96,144| (T)10 | 10                         | ycf1         |
| 97,206| 97,257| (T)12 | 12                         | ycf1         |
| 104,329| 104,341| (T)13 | 13                         | ycf1         |
| 105,304| 105,315| (T)12 | 12                         | ycf1         |
| 105,629| 105,642| (T)14 | 14                         | ycf1         |
| 109,940| 109,960| (T)10 | 10                         | ycf1         |
| 111,353| 111,366| (T)14 | 14                         | ycf1         |
| 113,000| 113,009| (A)10 | 10                         | ycf1         |
| 115,212| 115,222| (T)11 | 11                         | ycf1         |
| 120,988| 120,997| (A)10 | 10                         | trnK-trnL    |
| 122,446| 122,455| (A)10 | 10                         | ycf1         |
| 125,125| 125,136| (T)12 | 12                         | ycf1         |
| 125,645| 125,710| (A)10 | 10                         | ycf1         |
| 128,414| 128,424| (T)11 | 11                         | ycf1         |
| 128,795| 128,804| (T)10 | 10                         | ycf1         |
| 148,732| 148,749| (A)18 | 18                         | ycf1         |

Table 3
The intron containing genes in the cp genome of Adenium obesum.

| Gene   | Location | Exon I bp | Intron I bp | Exon II bp | Intron II bp | Exon III bp |
|--------|----------|-----------|-------------|------------|--------------|-------------|
| trnA-UCC| IR       | 35        | 818         | 38         |              |             |
| trnL-CAU| IR       | 35        | 943         | 42         |              |             |
| rps12* | LSC-IR   | 234       | 536         | 25         |              | 114         |
| ndhB   | IR       | 777       | 684         | 756        |              |             |
| rpl2   | IR       | 391       | 649         | 434        |              |             |
| trnK-UUU| LSC      | 35        | 2476        | 37         |              |             |
| rps16  | LSC      | 226       | 843         | 41         |              |             |
| trnG-GCC| LSC      | 23        | 691         | 37         |              |             |
| atpF   | LSC      | 411       | 706         | 144        |              |             |
| rpoC1  | LSC      | 1613      | 749         | 451        |              |             |
| ycf1   | LSC      | 155       | 773         | 228        |              |             |
| trnL-UAA| LSC     | 37        | 491         | 50         |              |             |
| trnV-UAC| LSC     | 37        | 586         | 38         |              |             |
| ctpP   | LSC      | 229       | 657         | 291        |              | 746         |
| rpl2   | IR       | 434       | 649         | 391        |              | 71          |
| ndhB   | IR       | 756       | 684         | 777        |              |             |
| rps12  | IR       | 25        | 536         | 234        |              |             |
| trnL-CAU| IR       | 42        | 943         | 35         |              |             |
| trnA-UCC| IR      | 38        | 818         | 35         |              |             |

*rps12 is trans-spliced gene with 5’ end exon located in the LSC region and the duplicated 3’ end exon located in IR regions.

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ieae, Subtribe Neriinae) (Fig. 4). The family Apocynaceae is one of the 10 largest angiosperm families with c. 4,500 species under c. 370 genera globally with the greatest diversity in the tropics and subtropics (Stevens, 2001; Endress et al., 2014; APG, 2016). Apart from the large number of molecular phylogenetic studies on the family Apocynaceae (e.g. Liede and Täuber, 2000, 2002; Liede, 2001; Liede and Meve, 2001, 2002; Meve and Liede, 2001, 2002, 2004a,b; Potgieter and Albert, 2001; Liede and Kunze, 2002;
Liede et al., 2002a,b; Verhoeven et al., 2003; Rapini et al., 2003, 2004, 2006, 2007; Simões et al., 2004, 2006, 2007; Liede-Schumann et al., 2005; Venter et al., 2006; Endress et al., 2007; Goyder et al., 2007; Ionta and Judd, 2007; Lahaye et al., 2007; Livshultz et al., 2007; Meve and Liede-Schumann, 2007; Wanntorp and Forster, 2007), the family has also been intensely studied for their pollination biology, plant–herbivore interactions, and secondary chemistry (Wyatt and Broyles, 1994; Góngora Castillo et al., 2012; Courdavault et al., 2014; Agrawal et al., 2015). The phylogenetic nesting of the family Asclepiadaceae in Apocynaceae s.s. has been demonstrated repeatedly (Wanntorp, 1988; Judd et al., 1994; Sennblad and Bremer, 1996; Potgieter and Albert, 2001). The most recent classification of Apocynaceae (Endress et al., 2014) segregated the family into five subfamilies, two paraphyletic which correspond to the former Apocynaceae s.s. (Rauvolfioideae and Apocynoideae) and three monophyletic that relates to the former Asclepiadaceae (Periplocoideae, Secamonoideae, and Asclepiadoideae).

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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