The utility of the complete plastid Genomes as barcodes to identify the cultivars of Patchouli (*Pogostemom cablin*)

Cai-Yun Zhang[1], Hui-Run Huang[2], Hai-Fei Yan[2], Xue-Jun Ge[2]

[1]Guangdong Food and Drug Vocational College, Guangzhou 510520, China
[2]Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Abstract

Patchouli plant (*Pogostemon cablin* (Blanco) Benth.) has been grown for medicinal use for more than 1000 years in China. Here we compared plastid genomes for five accessions of Patchouli, in order to barcode the two chemotypes: patchoulol-type and pogostome-type. Although only a 1bp indel was detected, it could be successfully used as a barcode to distinguish the two chemotypes, as verified by PCR and Sanger sequencing. Our findings support the utility of cp genomes as DNA barcode to distinguishing plant cultivars.

Introduction

Pogostemon cablin (Blanco) Benth. (Lamiaceae), known as Patchouli, is of important economic value for its use in medicines and fragrant products. As a well-known Chinese materia medica, it is widely used in gastrointestinal disease and exterior syndromes (Chen *et al*., 2013). Patchouli essential oil, extracted from the dried leaves and young twigs of *P cablin*, is mainly composed of patchouli alcohol and pogostone. Based on their concentrations, *P. cablin* is divided into two chemotypes: pogostone-type, which has a high content of pogostone and a low content of patchouli alcohol, and patchoulol-type, which has a high content of patchouli alcohol and a low content of pogostone (Luo *et al*., 2003). The pogostone-type Patchouli is considered as the best resource for Chinese medicinal uses (Luo *et al*., 2003), however the production of its effective constituent for medicines, pogostone, is extremely low, as this chemotype can be only cultivated in the suburb of Gaoyao and Guangzhou city, and that its cultivated areas are only 0.67 ha and 0.067 ha, respectively (Wu *et al*., 2010). The potchoulol-type Patchouli is more commonly used in medicines, due to its wider cultivated areas and higher production. An easy way to distinguish the two chemotypes is of crucial. Complete plastid genomes have been recently used as plant barcodes, but its usage in identifying plant cultivars has not been well tested. In this study, we compared the chloroplast genome of five *P. cablin* accessions from China. We also verified the chemotype-specific variation by PCR and Sanger sequencing. The objectives of our study are to: (i) characterize gene content of the Patchouli chloroplast genome; (ii) barcode the two chemotypes of this species.

Material and methods

- Genomes were sequenced for 5 Patchouli accessions, of which 2 were patchoulol-type and 3 were pogostone type.
- Reads of an accession SP were initially assembled using SPAdes v3.10.1. Contigs were aligned to the reference plastome of *Pogostemon yatabeanus* (Makino) Press using BLAST, for gene annotations and determination of the order and direction of the contigs.
- The remaining sequencing libraries were aligned to SP, in order to detect genetic variation among the accessions.
- The detected variation was verified by PCR and Sanger sequencing for 29 accessions, of which 16 are patchoulol-type and 13 are pogostone-type.

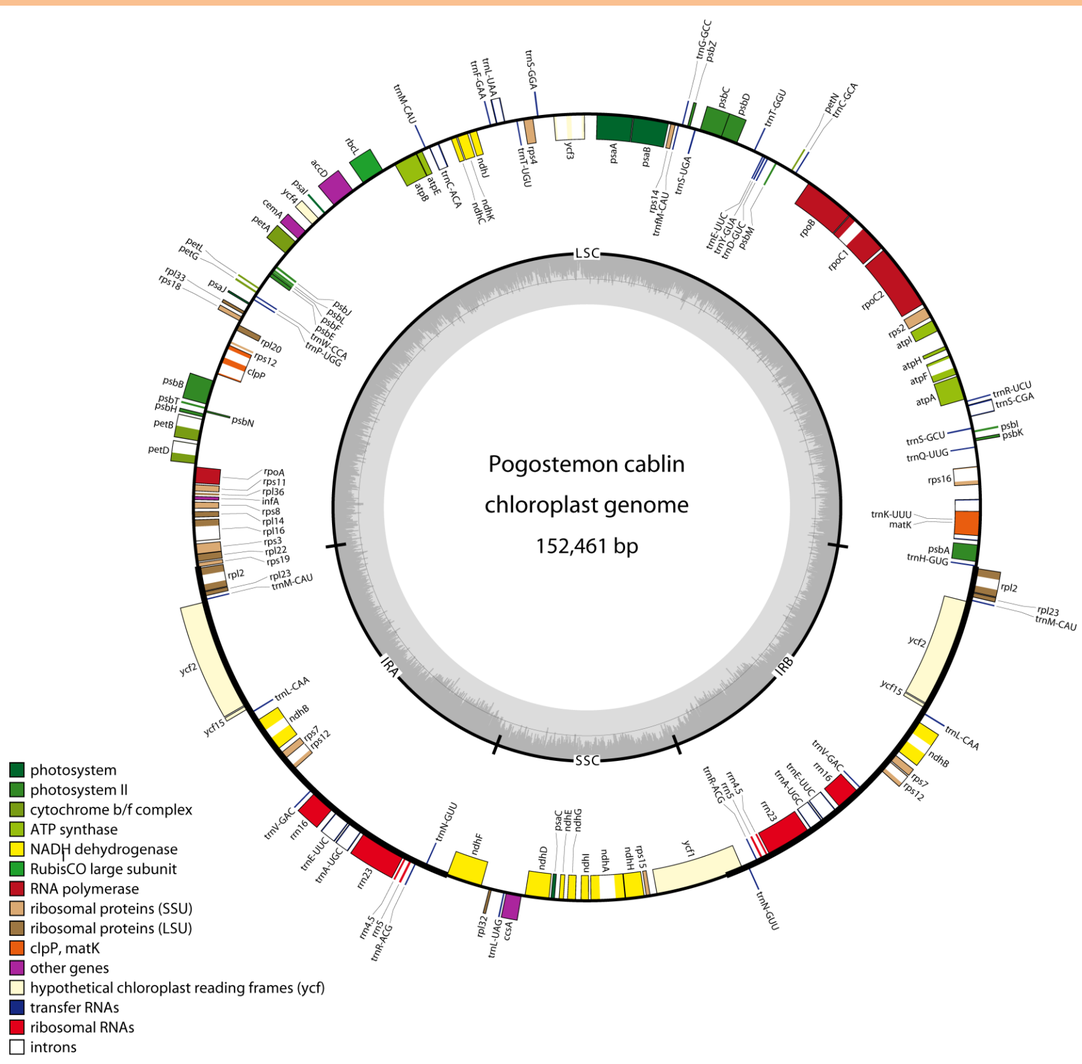

Fig. 1: Chloroplast genome map of *Pogostemon cablin*. Genes on the outside of the outer circle indicate the clockwise direction of transcription; those on the inside indicate the counterclockwise direction. The bar graphs on the inner circle reveal GC content in dark grey with the 50% threshold line.

Table 1: The genotypes of two chemotypes in *Pogostemon cablin*, verified by PCR and Sanger sequencing.

Population	No.	Chemotype	Barcode
SH	11	potchoulol-type	GAA
KK	2	potchoulol-type	GAA
YC	2	potchoulol-type	GAA
GY	1	potchoulol-type	GAA
GY	1	pogostone-type	GA
LT	11	pogostone-type	GA
SP	1	pogostone-type	GA

No.: number of studied accessions for each population.

Results

- The complete chloroplast genome (plastome) of *P. cablin* is 152,461 bp in length and comprises a pair of 25,662 bp inverted repeat regions (IRs) separated by one small and one large single copy region (SSC and LSC) of 17,584 and 83,553 bp, respectively (Fig. 1).
- The plastome contains 132 genes, including 87 protein coding genes (PCGs), 37 tRNA genes and eight ribosomal RNA genes. Of these, seven PCGs, seven tRNA and four rRNA occur in double copies.
- The overall GC content of the *P. cablin* plastome is 38.2%, while the corresponding values of the LSC, SSC and IR regions are 36.3%, 32.1% and 43.4%, respectively.
- Based on the alignment of plastome sequences of 5 Patchouli accessions, the two chemotypes are distinguished by a 1bp indel.
- Through verification by Sanger sequencing, 16 patchoulol-type accessions and 13 pogostone-type accessions can be separated by the only barcode revealed by plastome sequence (Table 1).

Conclusions

Patchouli plants seldom flower, and they are generally propagated by stem cutting, leading to a rapid decline of the genetic diversity of this species (Paul *et al*., 2010; Li *et al*., 2011). Consistent with the low diversity (Huang *et al*., 2016), only a 1bp indel was detected from the chloroplast genomes of different cultivars, but it was successfully to be used as a barcode to distinguish the patchoulol-type and pogostone-type accessions. Our finding supports the utility of plastome to obtain DNA barcode for different plant cultivars.

References

Chen M, Zhang J, Lai Y, Wang S, Li P, Xiao J, Fu C, Hu H, Wang Y. 2013. Analysis of *Pogostemon cablin* from pharmaceutical research to market performances. *Expert Opinion on Investigational Drugs* 22: 245-257.

Huang HR, Wu W, Zhang JX, Wang LJ, Yuan YM, Ge XJ. 2016. A genetic delineation of Patchouli (*Pogostemon cablin*) revealed by specific-locus amplified fragment sequencing. *Journal of Systematics and Evolution* 54: 491-501.

Li CG, Wu YG, Guo QS. 2011. Floral and pollen morphology of *Pogostemon cablin* (Lamiaceae) from different habitats and its taxonomic significance. *Procedia Engineering* 18: 295–300.

Luo JP, Liu YP, Feng YF, Guo XL, Cao H. 2003. Two chemotypes of *Pogostemon cablin* and influence of region of cultivation and harvesting time on volatile oil composition. *Acta Pharmaceutica Sinica* 38: 307-310.

References continued

Paul A, Thapa G, Basu A, Mazumdar P, Kalita MC, Sahoo L. 2010. Rapid plant regeneration, analysis of genetic fidelity and essential aromatic oil content of micropropagated plants of Patchouli, *Pogostemon cablin* (Blanco) Benth. -An industrially important aromatic plant. *Industrial Crops and Products* 32: 366–374.

Wu YG, Guo QS, He JC, Lin YF, Luo LJ, Liu GD. 2010. Genetic diversity analysis among and within populations of *Pogostemon cablin* from China with ISSR and SRAP markers. *Biochemical Systematics and Ecology* 38: 63-72.

Acknowledgements

This work was supported by Natural Science Foundation of Guangdong Food and Drug Vocational College, Guangdong, China (2016YZ007), and Science and Technology Planning Project of Guangzhou, Guangdong, China (201604020041).