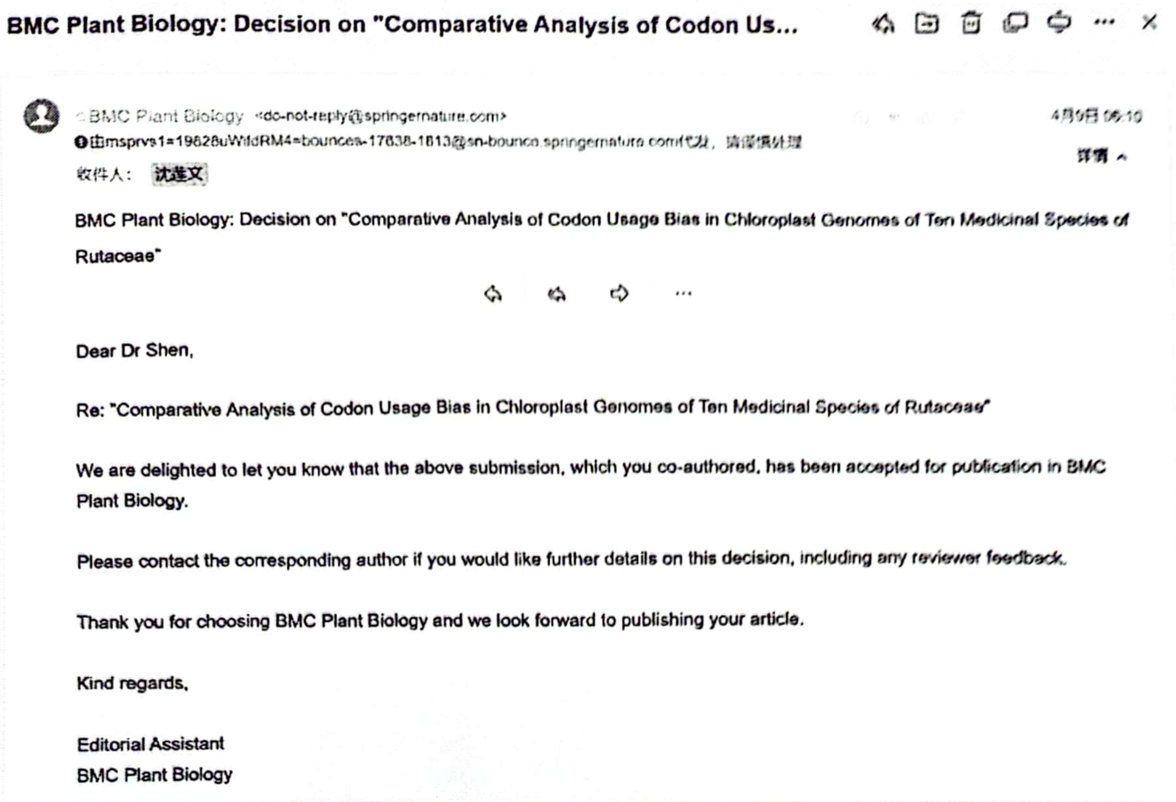

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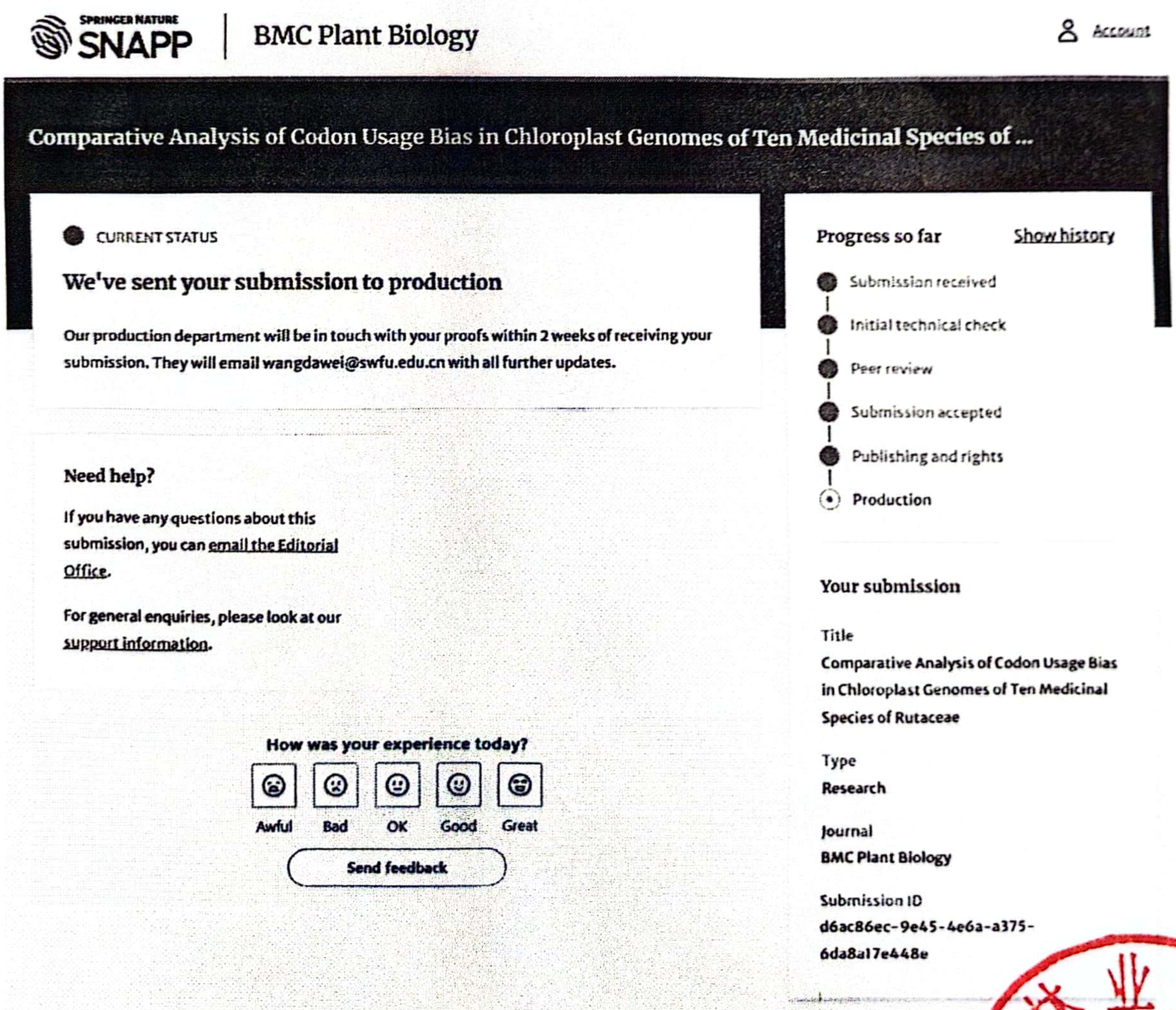

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Comparative analysis of codon usage bias in chloroplast genomes of ten medicinal species of Rutaceae

Lianwen Shen[1,2,3†], Shengqun Chen[3†], Mei Liang[4†], Shang Qu[3], Shijing Feng[5], Dawei Wang[1,2*] and Gang Wang[3*]

Abstract
Rutaceae family comprises economically important plants due to their extensive applications in spices, food, oil, medicine, etc. The Rutaceae plants is able to better utilization through biotechnology. Modern biotechnological approaches primarily rely on the heterologous expression of functional proteins in different vectors. However, several proteins are difficult to express outside their native environment. The expression potential of functional genes in heterologous systems can be maximized by replacing the rare synonymous codons in the vector with preferred optimal codons of functional genes. Codon usage bias plays a critical role in biogenetic engineering-based research and development. In the current study, 727 coding sequences (CDSs) obtained from the chloroplast genomes of ten Rutaceae plant family members were analyzed for codon usage bias. The nucleotide composition analysis of codons showed that these codons were rich in A/T(U) bases and preferred A/T(U) endings. Analyses of neutrality plots, effective number of codons (ENC) plots, and correlations between ENC and codon adaptation index (CAI) were conducted, which revealed that natural selection is a major driving force for the Rutaceae plant family's codon usage bias, followed by base mutation. In the ENC vs. CAI plot, codon usage bias in the Rutaceae family had a negligible relationship with gene expression level. For each sample, we screened 12 codons as preferred and high-frequency codons simultaneously, of which GCU encoding Ala, UUA encoding Leu, and AGA encoding Arg were the most preferred codons. Taken together, our study unraveled the synonymous codon usage pattern in the Rutaceae family, providing valuable information for the genetic engineering of Rutaceae plant species in the future.

Keywords Codon usage bias, Rutaceae family, Natural selection, Optimal codon

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2种玉兰属植物叶绿体基因组密码子偏好性分析

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（1. 西南林业大学云南省高校林木遗传改良与繁育重点实验室，云南 昆明 650233；2. 西南林业大学西南山地森林资源保育与利用教育部重点实验室，云南 昆明 650233）

摘要： 利用Codon W、CUSP及R语言等软件对2种玉兰属植物叶绿体基因组密码子的使用偏好性进行分析。结果表明：两者叶绿体基因组密码子的GC含量均为$GC_1>GC_2>GC_3$，说明密码子第3位碱基A/U含量高。大部分有效密码子数（ENC）都在45以上，CAI平均值为0.167，说明密码子偏好性弱。ENC−plot、PR2−plot及中性绘图分析表明GC_1、GC_2与GC_3之间相关性不明显；且第3位碱基使用频率为T > A，G > C，说明2种玉兰属植物叶绿体基因组密码子的偏好形成受到自然选择和基因突变的共同影响，自然选择为主要影响因素。利用相对使用度(RSCU)、ENC值共筛选出30个最优密码子，大部分以A/U结尾，证明二乔玉兰和日本辛夷2种玉兰属植物叶绿体基因组密码子第3位碱基偏好A/U。

关键词： 二乔玉兰；日本辛夷；叶绿体基因组；密码子偏好性

中图分类号： S685.15　　**文献标志码：** A　　**文章编号：** 2095−1914(2023)02−0044−10

Analysis of Codon Usage Bias(CUB) in the Chloroplast Genomes of 2 *Yulania* species

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Abstract: This paper analyzed the codon usage in chloroplast genome of 2 *Yulania* species by Codon W, CUSP and R software. The results showed that the GC content of chloroplast genomes of 2 *Yulania* species was $GC_1 > GC_2 > GC_3$, which indicated the content of base A/U at codon 3 was high. The effective number of codons (ENC) of CDS sequences exceeding 45 were more than 80 base pair, and the average CAI value was 0.167, which indicated weak codon bias. The results of ENC−plot, PR2−plot and neutral plot analysis showed that the correlation among GC_1, GC_2 and GC_3 was not significant. And the usage frequency of the third base was T > A, G > C. It indicated that the formation of codon bias in chloroplast genome of 2 *Yulania* species was affected by natural selection and gene mutation cooperatively, and natural selection is the main affecting factor. According to the relative synonymous codon usage(RSCU)and ENC values, 30 optimal ciphers ending in A/U were selected. It was demonstrated that the codon 3 position in the chloroplast genome of 2 species of *Yulania soulangeana* and *Yulania kobus* has an A/U preference.

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