

Codon Usage Study on Chloroplast Genome in Medicinal Plant *Panax Ginseng*

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Abstract: *Panax ginseng* is usually used as a famous plant medicine in many countries. In order to study the codon usage in whole chloroplast genome in *Panax Ginseng*, whole chloroplast genome sequence is analyzed via codonW software and some results such as relationships between the codon adaptation index, frequency of optimal codons, codon bias index, effective number of codons, especially the relative synonymous codon usage are all discussed. Furthermore, PR2-bias plot, histogram GC content, Codon Bias Index are also analyzed for exploring the sequence characteristics of chloroplast genome in *Panax Ginseng*. These results showed that codon usage bias in the chloroplast genome from *Panax Ginseng* is weak, and the relative results could lay a foundation for other further study on the medicinal plant *Panax Ginseng*.

Keywords: Codon Usage Pattern, *Panax Ginseng*, Chloroplast Genome, Codon Bias Index, Effective Number of Codons

1. Introduction

Panax ginseng is a commonly used medicinal plant in Traditional Chinese Medicine [1]. Meanwhile, modern pharmacology studies also reveal that the *Panax ginseng* has many good functions for human body [2, 3], such as anticancer [4, 5], obesity inhibited for mice and human [6, 7] via regulating thermogenesis [8], antioxidant [9], radiation protection [10], anti-inflammatory [11, 12], anti-chronic liver disease [13], antipigmentation and antiaging [14]. Other studies also found that the *Panax ginseng* exerts have antiproliferative effect for rat hepatocarcinogenesis [15], heat processing may be a useful method to enhance the anticancer effect [16]. Many scientists studied the *Panax ginseng* via the bioinformatics perspective [17, 18], such as Yu-Jin Kim, et al explored that the related genes of *Panax ginseng* and their expression against environmental stresses [19]. Konstantin V. Kiselev, et al, tended to think that mutation of *Panax ginseng* genes may be happened during long-term cultivation [20]. Wan-jing Liu, et al, studied the HMGS and HMGR Genes from *Panax notoginseng* via bioinformatics tools [21]. Hongtao Wang, et al, explored the methods of authenticating the *Panax ginseng* and ginseng products via robust SNP

markers [22]. Erin M. Schlag, et al, put forward a method for studying the relationship between genetic and chemotypic diversity in *ginseng* [23]. When discrimination of *ginseng* is concerned, Guisheng Li, et al. analyzed the differences related mitochondrial from Russian wild *ginseng* [24], Jong-Hak Kim tended to think that the auxin repressed protein gene could be used to discriminate of *Panax ginseng* Meyer cultivar Chunpoong and American *ginseng* [25], Min-Jeong Lee, et al, studied the variation in the ginsenoside profiles of cultivated *Panax ginseng* C.A. Meyer landraces in Korea [26], et al. [27].

Synonymous codon usage may be in all organisms, many scientists studied the issue from different perspective [28], and however, some codons are used more frequently used, which is called codon usage bias. Many factors may affect the codon usage characteristics, such as gene length [29], mutation pressure [30], natural selection [31] and RNA structure et al [32]. In this study, based on the previous studies, a genome comprehensive analysis of codon usage on whole chloroplast genome in medicinal plant *Panax Ginseng* is studied.

2. Materials and Methods

Whole chloroplast genome in medicinal plant *Panax Ginseng* is downloaded from GenBank of NCBI, which

accession number is KF431956.1 [33]. Sequences with more than 300 nucleotides are selected for further study.

CodonW [34] is used to calculate the Codon Adaptation Index (CAI), Frequency of Optimal Codons (Fop), Codon Bias Index (CBI), Effective Number of Codons (ENC), and so on, the calculated results are further processed for getting the Relative Synonymous Codon Usage (RSCU).

Then, the Codon Bias Index vs. Number of coding amino acids, the PR2-bias plot analysis: $A_3 / (A_3 + T_3)$ vs. $G_3 / (G_3 + C_3)$, the histogram GC content, Codon Bias Index, et al are

all analyzed.

3. Results and Discussion

Codon usage characteristics including the T3s, Fop, ENC, C3s, A3s, G3s and GC3s, et al, of whole chloroplast genome in medicinal plant *Panax Ginseng* are calculated. Their relationships are calculated via the correlation analysis method, and the results are shown in Table 1.

Table 1. Correlation analysis for prediction results.

Title	T3s	C3s	A3s	G3s	CAI	CBI	Fop	ENC	GC3s
C3s	-0.538								
A3s	-0.208	-0.425							
G3s	-0.612	0.396	-0.357						
CAI	0.55	-0.324	-0.303	-0.226					
CBI	-0.368	0.379	-0.209	0.131	0.211				
Fop	-0.202	0.573	-0.212	-0.118	0.186	0.796			
ENC	-0.686	0.606	0.037	0.525	-0.484	0.162	0.239		
GC3s	-0.709	0.793	-0.502	0.856	-0.302	0.354	0.256	0.632	
GC	-0.598	0.54	-0.285	0.375	-0.217	0.362	0.366	0.492	0.616

The effective codon number (ENC)-plot with ENC values against GC3s values is plotted and the results is shown in figure.1. The Figure 1 shows that most of the genes from whole chloroplast genome in medicinal plant *Panax Ginseng* are under the expected line. There is only three dots larger than the expected value from the Figure 1.

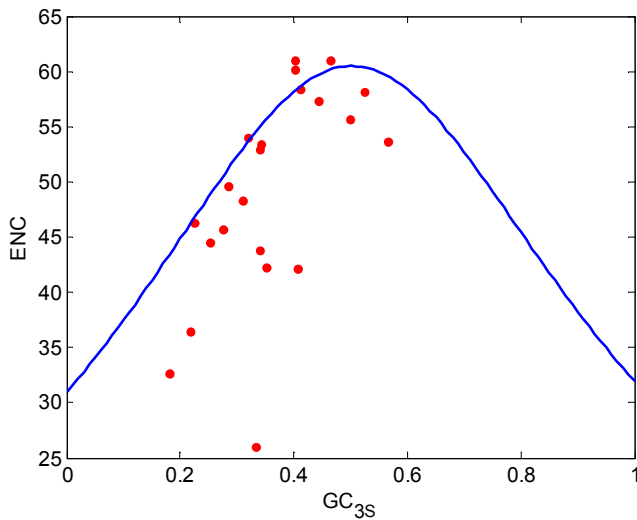


Figure 1. Analysis of ENC and GC₃s relationship.

PR2-bias plot analysis which reflects the relationship $A_3 / (A_3 + T_3)$ vs. $G_3 / (G_3 + C_3)$ is also called the parity rule. If there is no mutation in the two complementary strands of DNA, the base content should obey the law $A = T$ and $G = C$. The distance between the center (0.5, 0.5) and the plot dot represents the degree and direction of the PR2 bias. The prediction result of whole chloroplast genome in *Panax*

Ginseng is shown in the Figure 2. G3 content is overall larger than the value of C3.

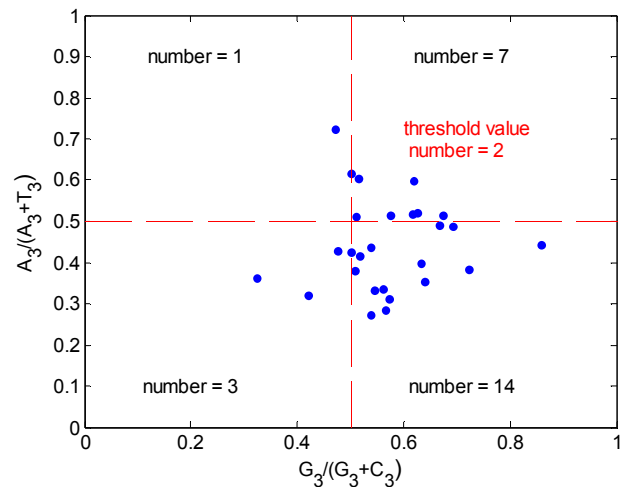


Figure 2. PR2-bias plot of whole chloroplast genome in medicinal plant *Panax Ginseng*.

The relationships between CBI and gene number, the relationships between Fop and gene number, relationships between overall GC content and gene number, et al, in whole chloroplast genome in medicinal plant *Panax Ginseng* is also counted via the prediction results by codonW software. The result is shown in the figure 3.

Based on the figure 3, further examination on the number of amino acids varies with the ENC, CBI, overall GC content, et al, and the result is shown in the figure 4. From the figure 4, all the lengths of the number of amino acids are all less than 500. From the Figure 4, it can conclude that there is no obvious pattern for distribution characterizes.

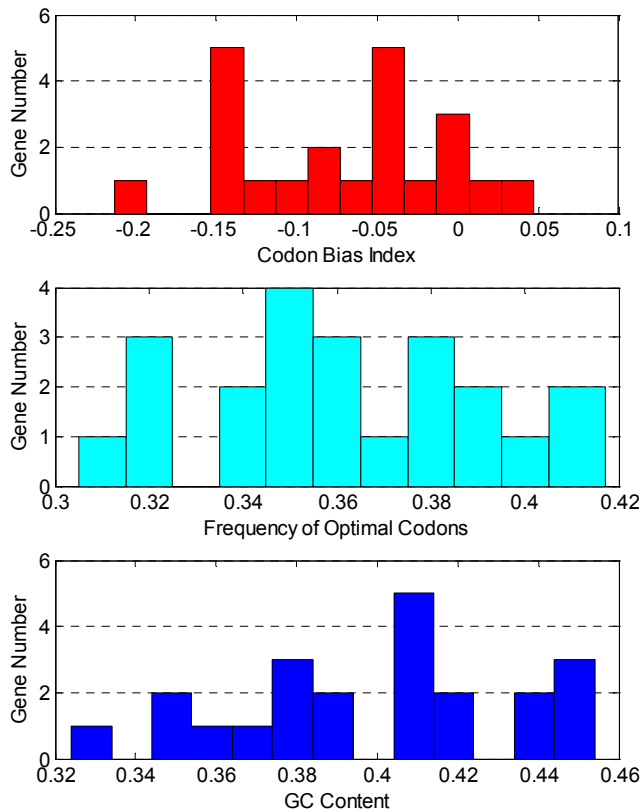


Figure 3. Statistics of CBI, Fop and over GC content.

The neutrality plot was usually used for analysis the directional mutation pressure vs. natural selection that shapes a certain codon usage. The neutrality plot of whole chloroplast genome in *Panax Ginseng* is as shown in the Figure 5. In neutrality plotting, GC₁₂ is as the ordinate, and GC₃ is as the abscissa, each point in the figure represents one coding gene of whole chloroplast genome from *Panax Ginseng*. Most values are distributed diagonally in the vicinity. These regions may reveal that base mutation is a major factor influencing codon preference for chloroplast genome from *Panax Ginseng*.

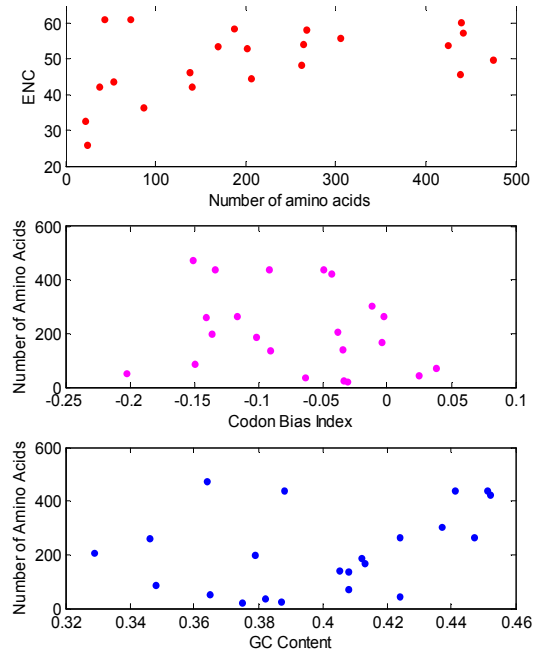


Figure 4. Relationships between number of amino acids and ENC, CBI, GC content.

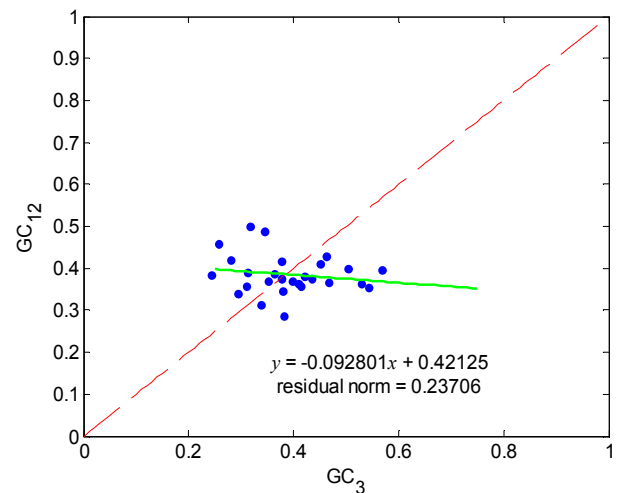


Figure 5. Neutrality plot of whole chloroplast genome in *Panax Ginseng*.

Table 2. RSCU value of whole chloroplast genome in *Panax Ginseng*.

Amino acid	Codon	Num	RSCU	Amino acid	Codon	Num	RSCU	Amino acid	Codon	Num	RSCU	Amino acid	Codon	Num	RSCU
Phe	UUU	266	1.149	Tyr	UAU	218	1.444	Ser	UCU	172	1.550	Cys	UGU	93	1.208
	UUC	197	0.851		UAC	84	0.556		UCC	126	1.135		UGC	61	0.792
	UUA	165	1.157		UAA	78	0.955		UCA	110	0.991		UGA	81	0.992
	UUG	199	1.395		UAG	86	1.053		UCG	89	0.802		UGG	150	1.000
Leu	CUU	182	1.276	His	CAU	118	1.430	Pro	CCU	80	1.131	Arg	CGU	94	1.245
	CUC	86	0.603		CAC	47	0.570		CCC	64	0.905		CGC	26	0.344
	CUA	130	0.911		CAA	153	1.234		CCA	74	1.046		CGA	72	0.954
	CUG	94	0.659		CAG	95	0.766		CCG	65	0.919		CGG	53	0.702
Ile	AUU	248	1.406	Asn	AAU	244	1.452	Thr	ACU	83	1.137	Ser	AGU	123	1.108
	AUC	131	0.743		AAC	92	0.548		ACC	67	0.918		AGC	46	0.414
	AUA	150	0.851		AAA	235	1.237		ACA	88	1.205		AGA	124	1.642
	AUG	151	1.000		AAG	145	0.763		ACG	54	0.740		AGG	84	1.113
Met	GUU	106	1.205	Asp	GAU	202	1.485	Ala	GCU	86	1.522	Gly	GGU	120	1.159
	GUC	60	0.682		GAC	70	0.515		GCC	51	0.903		GGC	47	0.454
	GUA	104	1.182		GAA	195	1.318		GCA	62	1.097		GGA	132	1.275
	GUG	82	0.932		GAG	101	0.682		GCG	27	0.478		GGG	115	1.111

The RSCU value can estimate the preference of synonymous codon used, which is defined as the ratio of the observed value of the number of synonymous codons used to the expected value of occurrences of the codon. The overall RSCU value for each chloroplast gene in *Panax Ginseng* codon is shown in the table 2.

From the result, the codon usage preference of UCU (1.550), GCU (1.522) and AGA (1.642) are larger than 1.5, which can be regarded as the preferred codons in chloroplast genome from *Panax Ginseng*. There are four relatively small values for RSCU, GCG (0.478), CGC (0.344), AGC (0.414) and GGC (0.454).

Codon preference can not only reflect the evolution of biological groups, but also from the molecular level it can explain the basic phenomenon of biology. But most previous studies emphasized on the medicinal usages of the *Panax Ginseng* [35], randomly concerned its bioinformatics characteristics of the whole chloroplast genome in *Panax Ginseng* although its genome sequences is very important for explaining its functions [36, 37]. For instance, Galina N. Chelomina tended to think that differences between cultivated oriental ginseng and wild *ginseng* could be explored via examining their 18S ribosomal DNA sequences in leaf cells [38].

4. Conclusion

The phenomenon of unequal use of synonymous codons is common. There are usually significant differences of codon usage bias in different organisms. In this paper, the preference of whole chloroplast genome in *Panax Ginseng*, such as the relationship between the A3 / (A3 + T3) and G3 / (G3 + C3), ENC vs. the number of amino acids, the relationship between GC12 and GC3, et al. are all examined and discussed. All results may be benefit for further understanding the medicinal function of the *Panax Ginseng*.

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