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RESEARCH ARTICLE

Analysis of codon usage bias of chloroplast genomes in *Gynostemma* species

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Abstract *Gynostemma* plants are important Chinese medicinal material and economic crops. Codon usage analysis is a good way to understand organism evolution and phylogeny. There is no report yet about analysis of codon usage bias of chloroplast genomes in *Gynostemma* species. In this study, the chloroplast genomes in nine *Gynostemma* species were analyzed systematically to explore the factors affecting the formation of codon usage bias. The codon usage indicators were analyzed. Multivariate statistical analysis including analysis of neutrality plot, effective number of codons plot, parity rule 2 plot and correspondence were performed. Composition analysis of codons showed that the frequency of GC in chloroplast genes of all nine *Gynostemma* species was less than 50%, and the protein-coding sequences of chloroplast genes preferred to end with A/T at the third codon position. The chloroplast genes had an overall weak codon usage bias. A total of 29 high frequency codons and 12 optimal codons were identified. These could provide useful information in optimizing and modifying codons thus improving the gene expression of *Gynostemma* species. The results of multivariate analysis showed that the codon usage patterns were not only affected by single one factor but multiple factors. Mutation pressure, natural selection and base composition might have an influence on the codon usage patterns while natural selection might be the main determinant. The study could provide a reference for organism evolution and phylogeny of *Gynostemma* species and help to understand the patterns of codons in chloroplast genomes in other plant species.

Keywords Codon usage bias · Chloroplast genomes · Optimal codon · *Gynostemma* species

Introduction

Chloroplasts are essential organelles and unique energy converters, which regulate photosynthesis to provide the required energy for higher plants and some algae (Zhang et al. 2012). The chloroplast genome has a simple and relatively conservative molecular structure, which is favorable for the study of plant barcoding (Galtier and Lobry 1997). Because of the advantages of easy sequence acquisition, high expression efficiency of exogenous genes, effectively control of the spread of transformed genes, and hereditary stability, chloroplast genome is widely used in the research of phylogeny, molecular evolution, and genetic expression (Kwak et al. 2019; Ruf et al. 2019).

Codons are the sequence units for the transmission of genetic information in organisms. Amino acids can be encoded by one to six codons, which is called codon degeneracy (Mcclellan 2000). The codons encoding the same amino acid are synonymous codons (Prabha et al. 2012). Synonymous codons in most organisms are not used uniformly, but some specific ones are preferentially used, which is called codon usage bias (Jia and Xue 2009). Researches on codon usage bias showed the profound impact of diverse factors, such as selection pressure, mutation pressure, the phylogenetic relationship of certain species, and several other genomic attributes (Das et al. 2006; Yadav and Swati 2012; Zhao et al. 2016). Studies on

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codon usage patterns can determine the optimal codons, which helps design gene expression vectors to increase the expression of the target gene (Qi et al. 2015). Furthermore, it can be used to judge the expression of unknown genes or to predict some unknown functional genes based on their association with a certain function degree (Tang et al. 2000). It can help to study the molecular mechanism of organisms adapting to the external environment to explore the evolutionary relationship between species (Singh et al. 2005). Moreover, it also has important value in the improvement of varieties.

The genus *Gynostemma* (family Cucurbitaceae) contains about 17 species, mainly distributed in tropical Asia to East Asia, from the Himalayas to Japan, Malaysia, and New Guinea. In China, there are 14 species, while nine of them are endemic (Flora of China 2011). *Gynostemma* plants are important Chinese medicinal material and economic crops, which are called "Southern Ginseng". Researchers have found that it had a variety of pharmacological activities in *Gynostemma* plants, such as reducing the levels of blood glucose and blood lipid (Lu et al. 2018; Huang et al. 2013), anti-tumor (Xing et al. 2019), protection of liver and blood vessels (Li et al. 2017), anti-oxidation (Du et al. 2018). In addition, *Gynostemma* plants have been developed into tea and health products, which have produced considerable economic benefits. However, the taste of most *Gynostemma* plants is bitter, and many scientists have worked to improve the taste of *Gynostemma* plants so that *Gynostemma* products can be more popular. It has been reported that the chloroplast genomes of nine *Gynostemma* species have been sequenced (Zhang et al, 2017; Wang et al. 2020a), but the codon usage bias has not been analyzed in detail. In the present study, we systematically analyzed the codon usage patterns of chloroplast genomes in nine *Gynostemma* species and evaluated the influence factors on codon usage. This study provided information on factors that influenced the codon usage patterns of chloroplast genomes in *Gynostemma* species during evolution and it could provide a reference for organism evolution and phylogeny of *Gynostemma* species.

Materials and methods

Genomes and coding sequences

The chloroplast genomes of nine *Gynostemma* species (*G. longipes, G. pubescens, G. burmanicum, G. cardiospermum, G. laxiflorum, G. caulopterum, G. pentagynum, G. yixingense*) were downloaded from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov). The accession numbers of nine *Gynostemma* species were shown in Table 1. All protein-coding sequences (CDS) of the chloroplast genomes were filtered following the rules (Sharp and Cowe 1991; Wang et al. 2020b): (1) each CDS begins with exact initiation codon (ATG) and termination codons (TAG, TGA and TAA); (2) the number of bases is multiple of three; (3) the length of sequences should be longer than 300 bp; (4) sequences with an intermediate stop codon are excluded. The CDSs were processed by BioEdit v 7.0.9.0 software.

Table 1 Species of nine *Gynostemma* plants and their accession numbers

No	Species	Accession numbers
1	*Gynostemma pentaphyllum*	KX852298.1
2	*Gynostemma longipes*	MF152730.1
3	*Gynostemma pubescens*	MF152732.1
4	*Gynostemma burmanicum*	MF152731.1
5	*Gynostemma cardiospermum*	KX852299.1
6	*Gynostemma laxiflorum*	MF136486.1
7	*Gynostemma caulopterum*	MF136487
8	*Gynostemma pentagynum*	KY670737.1
9	*Gynostemma yixingense*	MT028489.1

Indices of codon usage

The codon usage indicators are listed below, including: (1) relative synonymous codon usage (RSCU); (2) effective number of codons (ENC); (3) GC content including the overall GC content (GC), the GC content at the first (GC1), second (GC2) and third codon position (GC3), the GC content at the third position of the synonymous codons (GC3s); (4) the overall nucleotide composition (A, T, G, C) and its composition at third codon position (A3, T3, G3, C3); (5) codon adaptation index (CAI); (6) the total number of amino acids (L_aa). MEGA-X software was used for the analysis of GC content (GC, GC1, GC2, GC3) and nucleotide composition (A, T, G, C, A3, T3, G3, C3). The other parameters were analyzed by Codon W 1.4.2 software (http://codonw.sourceforge.net/).

High frequency and optimal codons

RSCU is the ratio of observed frequency to the expected frequency of a certain codon when it is used without bias. It is an important indicator of codon usage of chloroplast genomes (Sharp and Li 1986). If the RSCU value is greater than one, strong positive codon usage bias can be observed (Sharp and Li 1987). The codon with RSCU value greater than one was defined as high frequency codon (Liu et al. 2020b). The 10% of all the filtered chloroplast genes with the highest and lowest ENC values were selected and considered as the high and low expression genes datasets, respectively. The RSCU values of the codons in two datasets were calculated and compared by ΔRSCU. The codons with ΔRSCU $>$ 0.08 and RSCU $>$ 1 were determined as the optimal codons (Liu et al. 2020a).

Neutrality plot analysis

In neutrality plot analysis, we used GC12 and GC3 to make a scatter plot to study the correlation among bases at three codon positions, thus analyzing the effect of mutation pressure and natural selection (Sueoka 1988). GC12 is the average of GC1 and GC2. If it is significantly correlative between GC12 and GC3, namely, the regression coefficient is almost near or equal to one, it indicates that mutation pressure is the determinant in codon usage patterns. Contrastingly, if the correlation is not significant, then the regression coefficient is close to zero, it indicates that the codon preference is dominated by natural selection (Sueoka 1988, 1999b).

ENC-plot analysis

ENC value reflects the degree of deviation of codons from random selection, and it is an important indicator reflecting the degree of imbalanced usage of synonymous codons (Wright 1990). The value of ENC ranges from 20 to 61. The larger the ENC value, the lower the codon usage bias, and vice versa (Wu et al. 2018). Usually, 35 is used as the threshold for judging the strength of the codon preference (Wright 1990; Jiang et al. 2008). GC3s is the GC content at the third position of the synonymous codons. We calculated the value of GC3s excluding methionine (Met) and tryptophan (Trp), because the codons encoding Met and Trp have no synonymous codon. ENC-plot was compiled on the value of ENC against GC3s, which was mainly to reveal the influence of base composition and to further determine whether there were other factors on codon usage bias. The standard curve was calculated according to the following formula: $ENC = 2 + \mathrm{GC3s} + \frac{29}{GC3s^2+(1-GC3s)^2}$ (Wright 1990). The points distributing along or around the standard curve reveal that mutation pressure plays an important role in the codon usage. While the points distribute far below the standard curve, natural selection and other factors may be the major influence factor. The ENC radio was calculated according to the formula below: $\mathrm{ENCradio} = \frac{(ENCexp-ENCobs)}{\mathrm{ENCexp}}$, which showed the difference between actual and expected ENC values (Kawabe and Miyashita 2003; Zhang et al. 2008).

Parity rule 2 (PR2) plot analysis

Researchers have shown that the composition of four bases at the third position of the codon is closely related to the formation of codon usage patterns (Wan et al. 2004). PR2-plot was analyzed with A3/(A3 + T3) as ordinate and G3/(G3 + C3) as abscissa in a graph (Sueoka 1999a). In theory, when single mutation pressure influences on codons of the chloroplast genes, the proportion of A/T and C/G is balanced, that is, the center points of both coordinates are equal to 0.5 (A = T and G = C). Otherwise, codon usage may be affected by natural selection and other factors (Xiang et al. 2015).

Correspondence analysis (COA)

COA is a multivariate statistical analysis method (James and McCulloch 1990), which is widely used for the analysis of codon usage patterns (Sharp and Devine 1989; Shields and Sharp 1987). COA was performed based on RSCU values of codons using Codon W 1.4.2 software. In this study, each CDS was distributed in a 59-dimensional (59 synonymous codons devoid of the codons encoding Met, Trp and the three stop codons) vector space, where each point represented a synonymous codon. The first axis (axis 1) was the one that captured most of the variation in codon usage followed by each subsequent axis explaining a diminishing amount of the variance (Zhou et al. 2008b). Correlation analysis between axis1 and GC3s, ENC, CAI and L_aa were performed using the statistical software SPSS v22.0.

Results

Codon usage patterns

Composition analysis of codon

In order to analyze the codon preference accurately, indicators of codon usage were calculated and analyzed. The frequency of GC of chloroplast genes in all the nine *Gynostemma* species was less than 50% (Fig. 1). The content of GC1 was higher than the content of GC2 and GC3, and the content of GC3 was the lowest in nine *Gynostemma* species. The nucleotide composition analysis indicated that nucleotides A, T, G, and C were distributed unequally, while nucleotide T showed the highest usage percentage followed by A, G, and C in nine *Gynostemma* species (Fig. 2).

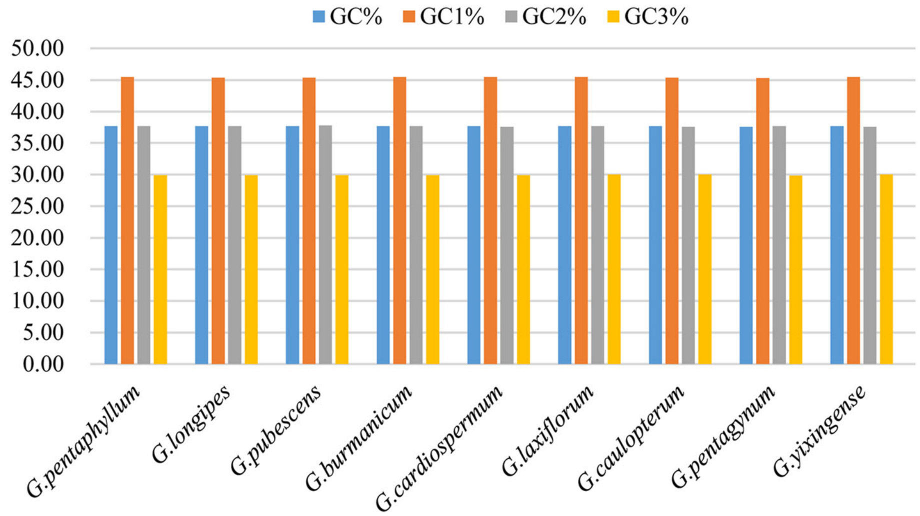


Fig. 1 Distribution of overall GC content, GC1, GC2, and GC3 of chloroplast genes in nine *Gynostemma* species

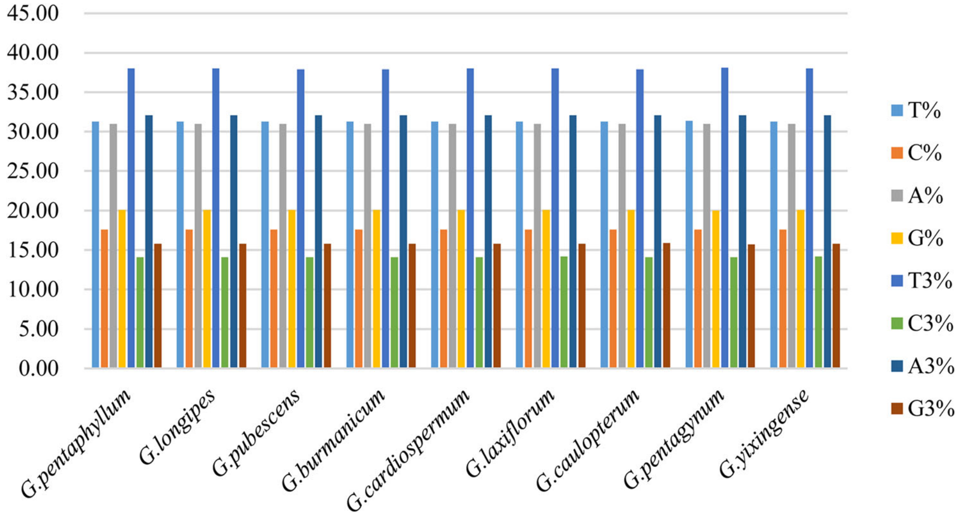


Fig. 2 Distribution of nucleotides for chloroplast genes in nine *Gynostemma* species

High frequency codons and optimal codons

The results of frequency analysis of synonymous codons for the CDSs of chloroplast genes showed that nine *Gynostemma* species had a high similar preference for codon usage (Table S1). There were 29 high frequency codons with RSCU > 1 while 28 codons of them ended with A/T accounting for 96.55%. The number of codons with RSCU < 1 was 30 with 28 codons ending with C/G that accounted for 93.33%. This indicated that high frequency codons (RSCU > 1) tended to be A/T ending while the codons with negative bias (RSCU < 1) were prone to end with G/C. The nine *Gynostemma* species possessed 29 identical high frequency codons of chloroplast genes including GCT, GCA, TGT, GAT, GAA, TTT, GGA, GGT, CAT, ATT, AAA, TTA, CTT, TTG, AAT, CCT, CCA, CAA, AGA, CGA, CGT, TCT, TCA, AGT, ACT, ACA, GTA, GTT and TAT.

The ENC values of chloroplast genes were calculated in nine *Gynostemma* species. The results showed that a majority of chloroplast genes had ENC values greater than 35, except rps8 in *G. pentaphyllum* and *G. longipes*, indicating an overall weak codon usage bias. Based on the ENC values and the ΔRSCU method (Liu et al. 2020a), the optimal codons were determined (Table S1). The results showed that nine *Gynostemma* species contained eight identical optimal codons including TCA, ACA, TAT, CAT, AAT, GAT, AGA, GGA. However, there were one more optimal codon (TTG) in *G. cardiospermum* and four more optimal codons (TTG, CTT, GCA, CGA) in *G. pentagynum* than the other seven *Gynostemma* species respectively. In a total of 12 optimal codons, 11 codons ended with T (5/12) or A (6/12), while only one codon ended with G (1/12). The results above indicated that the high frequency and optimal codons of chloroplast genes in *Gynostemma* species preferred A/T ending.

Multivariate statistical analysis

Neutrality plot analysis

The neutrality plots were performed for the chloroplast genes in nine *Gynostemma* species (Fig. 3). There was no significant correlation between GC12 and GC3 (r1 = 0.0414, r2 = 0.0403, r3 = 0.0192, r4 = 0.0358, r5 = 0.0100, r6 = 0.0109, r7 = 0.0165, r8 = 0.0654, r9 = − 0.0048). The slope of regression line was 0.0581, 0.0565, 0.0272, 0.0507, 0.0145, 0.0156, 0.0024, 0.0931, 0.0067, respectively, indicating mutation pressure effect accounted only 0.24–5.81%. Those above indicated that codon usage bias was affected slightly by the mutation pressure, but the natural selection and other factors seemed to make more contributions.

ENC-plot analysis

In the ENC-plot, we observed that the distributions of ENC and GC3s of nine *Gynostemma* species were similar (Fig. 4). As shown in Fig. 4, only a few points approached the standard curve, which revealed that GC3s was not the main factor affecting the codon bias. And for most of the points distributed in a discrete distribution, it implied that there might exist other factors influencing the codon usage patterns. In order to further reflect the difference, we analyzed the ENC frequency distribution of chloroplast genes in nine *Gynostemma* species (Fig. 5). The ENC ratio was between − 0.25 and 0.25. Of these, 36–39 (63.16–68.42%) chloroplast genes distributed in the range of − 0.05–0.05.

PR2-plot analysis

The PR2-plot analysis reflected the degree of deviation of four bases of chloroplast genes. We observed that the genes were unevenly distributed in four areas of the PR2-plane (Fig. 6). Most of the points are distributed at the bottom right of the plane, and a few points are distributed close to the center. The AT-bias is 0.470, 0.470, 0.470, 0.470, 0.470, 0.470, 0.470, 0.469, 0.470, while the GC-bias is 0.529, 0.529, 0.528, 0.528, 0.529, 0.528, 0.530, 0.529 and 0.527 respectively. It showed that the usage frequency of T at the third position of codons in chloroplast genes was higher than A, so as G was higher than C, which indicated the A/T preference in codon usage. Unbalanced usage of bases suggested that not only mutation pressure but also

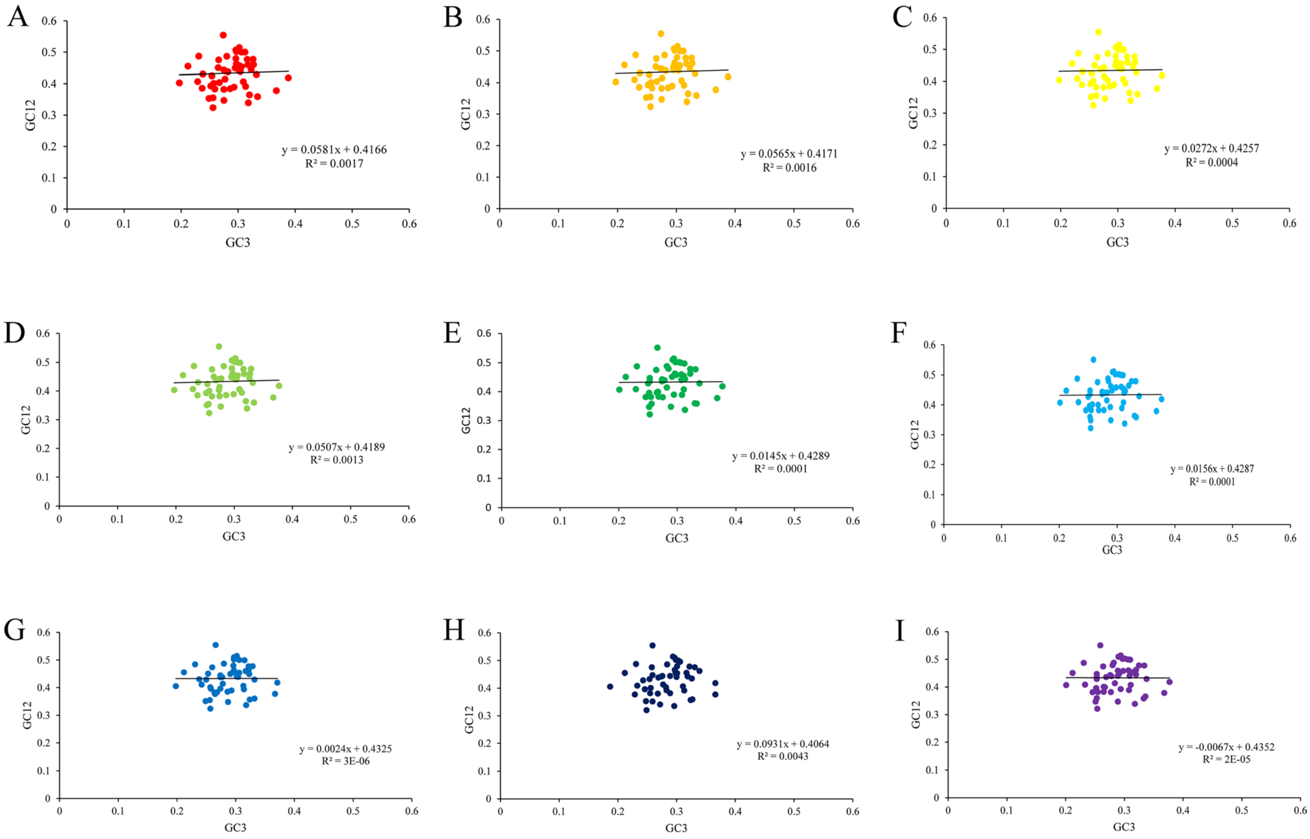


Fig. 3 Neutrality plot of chloroplast genomes of nine *Gynostemma* species. (**A**) *G. pentaphyllum*, (**B**) *G. longipes* (**C**), *G. pubescens* (**D**), *G. burmanicum*, (**E**) *G. cardiospermum*, (**F**) *G. laxiflorum*, (**G**) *G. caulopterum*, (**H**) *G.pentagynum*, (**I**) *G. yixingense*

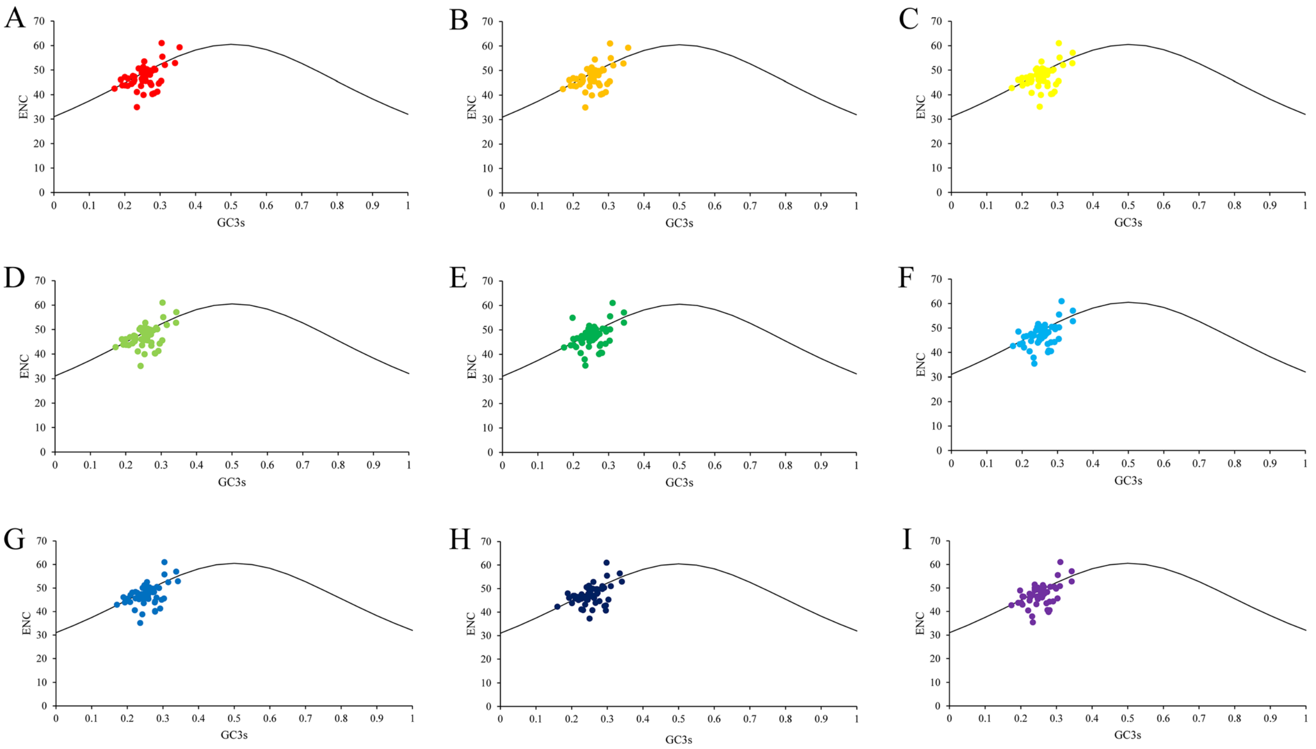
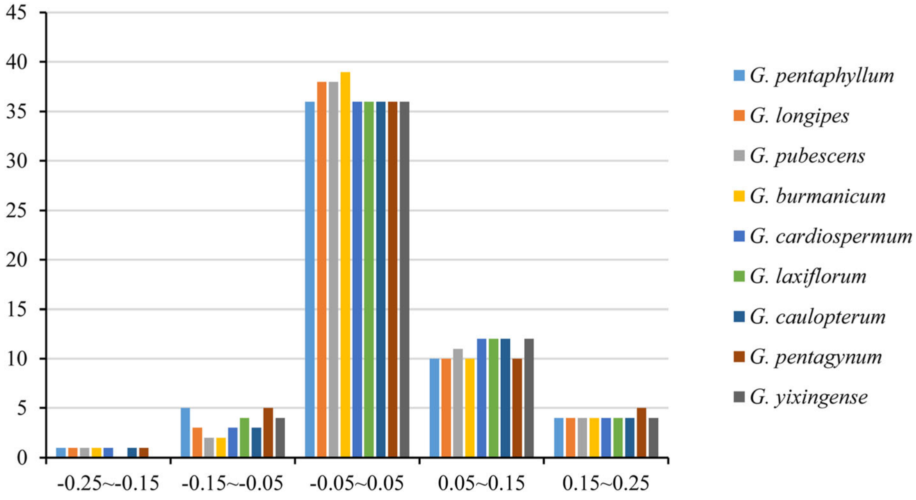


Fig. 4 ENC-plot of chloroplast genomes of nine *Gynostemma* species. (**A**) *G. pentaphyllum*, (**B**) *G. longipes*, (**C**) *G. pubescens*, (**D**) *G. burmanicum*, (**E**) *G. cardiospermum*, (**F**) *G. laxiflorum*, (**G**) *G. caulopterum*, (**H**) *G.pentagynum*, (**I**) *G. yixingense*

Fig. 5 Distribution of ENC frequency of chloroplast genomes in nine *Gynostemma* species



natural selection might have an influence on the codon usage patterns in *Gynostemma* species.

Correspondence analysis (COA)

COA based on RSCU values of synonymous codons was performed to understand the relationship of chloroplast genes thus revealing the possible factors that affect the codon usage bias. The first four axes accounted for 34.41%, 34.40%, 34.50%, 34.43%, 34.80%, 35.00%, 34.67%, 34.21% and 34.90% of the total variation in nine *Gynostemma* species, respectively. The first axis, which being responsible for about 10% of the total variation, was the main factor of variation, while each subsequent axis explained a decreasing amount of variation. The first two axes of the COA were shown in Fig. 7. It could be observed that most of the genes were distributed around zero, while some genes had a high degree of dispersion, indicating that codon usage might be affected by different factors. Although the nine *Gynostemma* species had similar

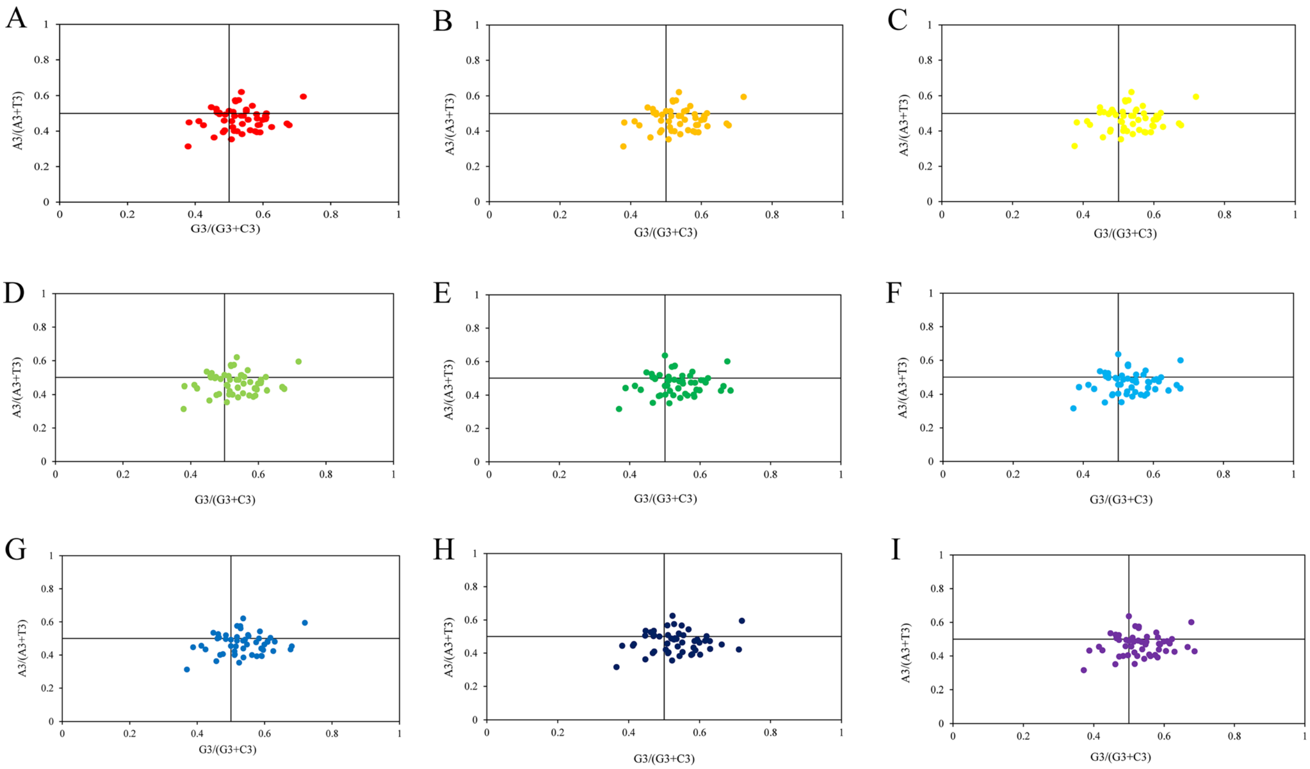


Fig. 6 PR2-plot of chloroplast genomes of nine *Gynostemma* species. (**A**) *G. pentaphyllum*, (**B**) *G. longipes*, (**C**) *G. pubescens*, (**D**) *G. burmanicum*, (**E**) *G. cardiospermum*, (**F**) *G. laxiflorum*, (**G**) *G. caulopterum*, (**H**) *G.pentagynum*, (**I**) *G. yixingense*

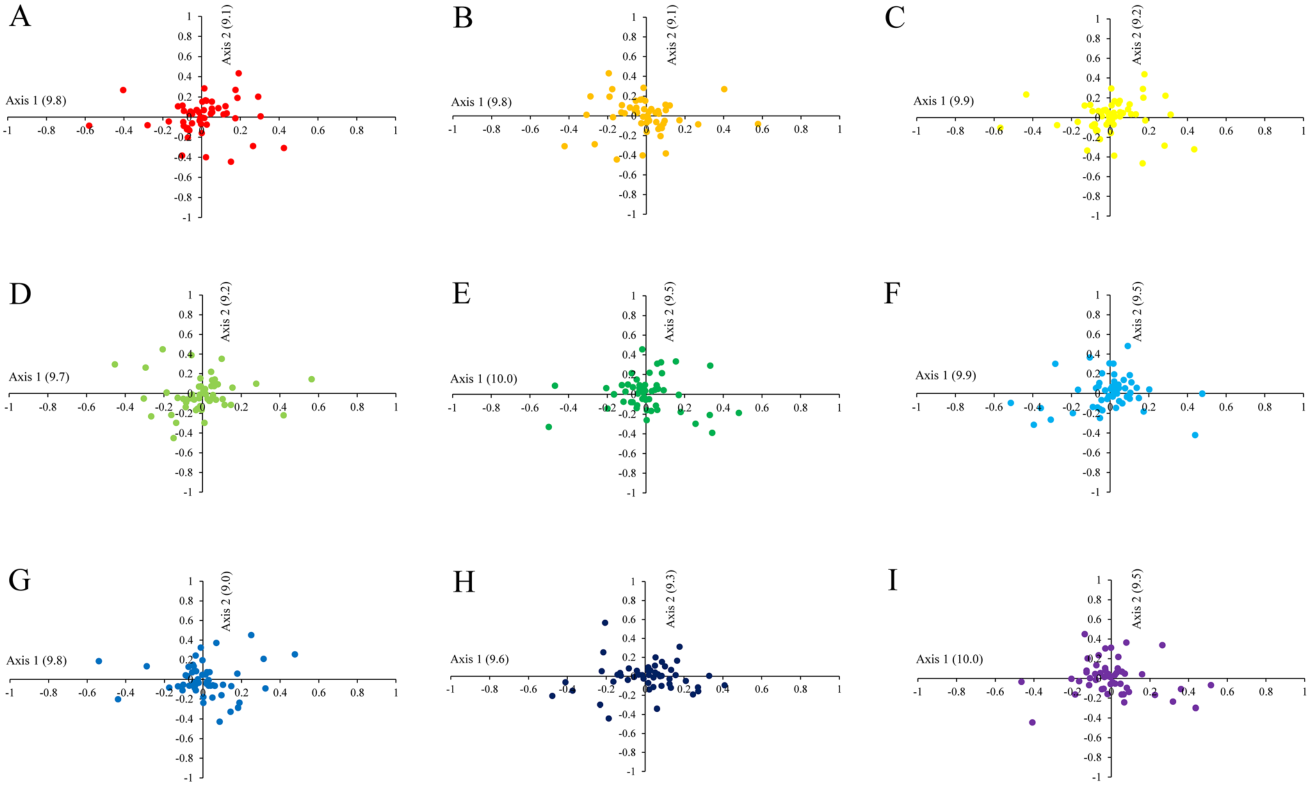


Fig. 7 Correspondence analysis of chloroplast genomes of nine *Gynostemma* species. (**A**) *G. pentaphyllum*, (**B**) *G. longipes*, (**C**) *G. pubescens*, (**D**) *G. burmanicum*, (**E**) *G. cardiospermum*, (**F**) *G. laxiflorum*, (**G**) *G. caulopterum*, (**H**) *G.pentagynum*, (**I**) *G. yixingense*

overall codon usage patterns, genes in different species still had their special evolutionary characteristics in codon usage.

Furthermore, we calculated Karl Pearson's correlation coefficients between axis 1 and different indices of codon usage including GC3s, ENC, CAI, and L_aa (Table 2). It was found that axis 1 had a significant correlation with GC3s ($p \leq 0.01$) in *G. pubescens*, while *G. pentaphyllum*, *G. longipes*, *G. burmanicum*, *G. cardiospermum* and *G. caulopterum* had a correlation with GC3s ($p \leq 0.05$). These results indicated that base composition might play a role in shaping codon usage bias.

Discussion

Codon usage bias is a widespread phenomenon in many organisms from prokaryotes to eukaryotes (Sharp et al. 1988; Nie et al. 2014). Extensive researches revealed that codon usage bias was affected by many biological factors, such as mutation bias in nucleotide composition and GC composition (Li et al. 2002; Sueoka and Kawanishi 2000), natural selection at the level of gene expression (Blake et al. 2003), gene length (Duret and Mouchiroud 1999), tRNA abundance (Duret and Mouchiroud 1999; Rao et al. 2011), secondary structure in mRNA (Gu et al. 2003). However, directional mutation pressure and natural selection are known as the major factors (Sharp et al. 2010; Sueoka 1999b).

Researches on codon usage bias have been carried out in many species, such as *Oryza* species (Chakraborty et al. 2020), *Euphorbiaceae* plants (Wang et al. 2020b), *Hemiptelea davidii* (Liu et al. 2020a). However, this is the first research on the systematic analysis of the codon usage of *Gynostemma* species. In the present study, we analyzed the chloroplast genomes of nine *Gynostemma* species to explore codon usage patterns and evolutionary forces which influenced the codon usage bias. There existed the same pattern in *Gynostemma* species, that was, the overall percentage of GC was less than 50%, and the content of GC1, GC2, and GC3 decreased in turn. The results of base composition analysis were consistent with PR2 analysis, which revealed that codons of chloroplast genomes in *Gynostemma* species tended to end with A and T, especially with T being the most preferred nucleotide. Some scholars had shown that codon nucleotide composition was highly conserved, while the third nucleotide position of codons was AT-rich in the eudicot genomes, but GC-rich in the monocot genomes (Wang and Roossinck 2006). It was reported that codons of chloroplast genomes preferred to end with A or T at the third position in *Populus alba* (Zhou et al. 2008a), Poaceae family (Zhang et al. 2012), and *Oryza* species (Chakraborty et al. 2020), which was in line with the present work.

The nine *Gynostemma* species contained 29 identical high frequency codons and eight identical optimal codons, but one more optimal codon in *G. cardiospermum* and four more optimal codons in *G. pentagynum*. Most of the high frequency codons (28/29) and optimal codons (11/12) ended with A or T, which was consistent with previous studies in *Hemiptelea davidii* (Liu et al. 2020a) and *Porphyra umbilicalis* (Li et al. 2019). It helps improve the gene expression of *Gynostemma* species through codon optimization.

It was suggested that synonymous codons usage bias was caused because of the preference for GC or AT at the third codon position. If a mutation occured on the third codon position neutrally, a synonymous codon would be chosen randomly (Zhang et al. 2008). The unequal usage of nucleotides revealed that not only mutation pressure but also other factors such as natural selection had an influence on the codon usage. Similar studies have also been reported for the codon usage of chloroplast genomes of *Asteraceae* (Nie et al. 2014) and *Euphorbiaceae* species (Wang et al. 2020b).

The neutral theory of molecular evolution holds that the effect of base mutation and natural selection on the third position of codons is neutral or close to neutral (Sharp et al. 1993). In the present study, there was no significant correlation between GC12 and GC3, and the slope of the

Table 2 Correlation coefficients between axis 1 and indices of codon usage of chloroplast genomes in nine *Gynostemma* species

	G. pentaphyllum	*G. longipes*	*G. pubescens*	*G. burmanicum*	*G. cardiospermum*	*G. laxiflorum*	*G. caulopterum*	*G. pentagynum*	*G. yixingense*
GC3s	− 0.305*	0.311*	− 0.339**	0.314*	− 0.261*	0.253	− 0.316*	0.034	− 0.256
ENC	− 0.013	0.016	− 0.006	0.047	− 0.082	0.113	− 0.062	0.232	− 0.157
CAI	0.172	− 0.172	0.201	− 0.210	0.108	− 0.113	0.187	0.007	0.116
L_aa	− 0.040	0.043	− 0.035	0.057	− 0.072	0.080	− 0.056	0.072	− 0.092

*Significant at $p \leq 0.05$ level (two-tailed)

**Significant at $p \leq 0.01$level (two-tailed) regression line was close to zero. Combined with analysis of neutrality plot and ENC-plot, it revealed that mutation pressure made few contributions in framing the codon usage bias of chloroplast genomes in *Gynostemma* species, while natural selection might be the main determinant. Our study supported the previous work on chloroplast genes of *Pisum* species (Bhattacharyya et al. 2019) and *Oryza* species (Chakraborty et al. 2020). Tang and his team reported that chloroplast genes might be affected by natural selection and mutation pressure (Tang et al. 2021). Previous researches showed that natural selection played a significant role in the codon usage of chloroplast genes, but the strength of the effect was different among different populations (Morton 1998).

Analysis of COA showed that base composition made a contribution to a certain extent in the shaping codon usage bias, which was consistent with the report of researcher Wang in chloroplast genes of six *Euphorbiaceae* species (Wang et al. 2020b). Some scientists had shown that the influence factors of chloroplast codon usage bias were more complex (Morton 2003). The other factors which were related to codon usage bias, such as gene expression length, RNA structure needed to be further explored.

Conclusions

In general, the codon usage patterns of chloroplast genomes were similar but not the same among *Gynostemma* species. Furthermore, genes in different species had their special evolutionary characteristics in codon usage. The codon usage bias was low, and the CDSs of chloroplast genes preferred to end with A/T. A total of 29 high frequency codons (GCT, GCA, TGT, GAT, GAA, TTT, GGA, GGT, CAT, ATT, AAA, TTA, CTT, TTG, AAT, CCT, CCA, CAA, AGA, CGA, CGT, TCT, TCA, AGT, ACT, ACA, GTA, GTT and TAT) were identified. Eight identical optimal codons (TCA, ACA, TAT, CAT, AAT, GAT, AGA, GGA) were filtered out in nine *Gynostemma* species, but one more optimal codon (TTG) in *G. cardiospermum* and four more optimal codons (TTG, CTT, GCA, CGA) in *G. pentagynum* than other seven *Gynostemma* species respectively. These results could provide useful information in optimizing and modifying codons thus improving the gene expression of *Gynostemma* species. The results of multivariate analysis showed that the formation of codon usage bias might be affected by multiple factors but mainly mutation pressure and natural selection, while natural selection played a major role and mutation pressure played a minor role. Correspondence analysis revealed that base composition partly contributed in shaping codon usage bias. But the degree of influences on chloroplast genomes varied in different *Gynostemma* species. The study could provide a reference for organism evolution and phylogeny of *Gynostemma* species and help to understand the patterns of codons in chloroplast genomes in other plant species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12298-021-01105-z.

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Author contributions Peipei Zhang collected, processed and analyzed data, produced charts and figures, authored and reviewed the draft manuscript, approved the final manuscript; Wenbo Xu analyzed and processed data, reviewed the manuscript, approved the final manuscript; Xu Lu conceived the project, reviewed the draft manuscript, approved the final manuscript; Long Wang conceived and designed the experiment, provided materials and analysis tools, guided the writing of the manuscript, reviewed the draft manuscript, approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest, financial or other aspects.

Ethical approval Not applicable.

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