https://doi.org/10.17221/71/2022-CJGPB

Complete chloroplast genome sequence and characteristics analysis of Qingda no.1 alfalfa (*Medicago sativa* L. cv. Qingda no.1)

*Yuling Ren[1], Yajun Ma[2], Xue Li[1], Xiaoan Li[1], Guozhu Yang[3], Ping Li[1,4]**

[1]*College of Eco-Environmental Engineering, Qinghai University, Xining, Qinghai, P.R. China*
[2]*College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai, P.R. China*
[3]*Ecological Environment Engineering Research Center, Qinghai University, Xining, Qinghai, P.R. China*
[4]*State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, Qinghai, P.R. China*

**Corresponding author: liping051126@163.com*

Yuling Ren and Yajun Ma contributed equally to this work.

Citation: Ren Y.L., Ma Y.J., Li X., Li X.A., Yang G.Z., Li P. (2023): Complete chloroplast genome sequence and characteristics analysis of Qingda no.1 alfalfa (*Medicago sativa* L. cv. Qingda no.1). Czech J. Genet. Plant Breed., 59: 160–168.

Abstract: *Medicago sativa* is the most widely cultivated forage legume and one of the most economically valuable crops throughout the world. Qingda no.1 (*Medicago sativa* L. cv. Qingda no.1) is an excellent alfalfa local variety with strong cold, drought and salt resistance in the three rivers source area of Qinghai. In this study, the whole chloroplast (cp) genome of Qingda no.1 was sequenced, assembled and its structure was analysed by the Illumina high-throughput sequencing technology. The results showed that the chloroplast genome of Qingda no.1 exhibits no obvious typical quadripartite structure; the total length of the chloroplast genome is 125 637 bp; the chloroplast genome contained 111 genes, including 77 protein-coding genes, 30 tRNA genes, and 4 rRNA genes, with an overall GC content of 38.33%. The relative synonymous codon usage showed that 68.67% of the codons RSCU > 1 in Qingda no.1, with the preference ending with A and T. The simple sequence repeat (SSR) analysis identified 62 SSR loci. The phylogenetic analysis of the cp genome, Qingda no.1 clustered closely with *Medicago sativa* KU321683 (*Medicago sativa* L. cv. KU321683). These results are helpful for the further study of the Qingda no.1 adaptation mechanism to high altitude stress environments.

Keywords: chloroplast genome sequencing; *Medicago sativa* L. cv. Qingda no.1; phylogenetic analysis, Qinghai--Tibet Plateau

Medicago sativa, is an excellent legume forage with a high protein content while having high cellulose characteristics. Its hay crude protein content reaches more than 21%. It is an important high-quality forage for cows and other herbivores and is known as the "the king of grass". As it has the advantages of easy maintenance, strong resistance to stress, and long flowering period, it can be developed and used as a ground cover plant for garden landscapes and slope protection plants for ecological orchards and

Co-supported by the National Natural Science Foundation [31660063], Qinghai Science and Technology Department Project [2022-ZJ-714], Forestry Reform and Development Fund Forestry Science and Technology Extension Demonstration Subsidy Project [Qing (2020) TG11] and Sanjiangyuan Ecology First-class Discipline Postgraduates Science and Technology Innovation Project [2022-stxy-B55, 2022-stxy-B57, 2022-stxy-B58].

https://doi.org/10.17221/71/2022-CJGPB

tea gardens. The dominant production areas of alfalfa in China are concentrated in the north, especially in the Qinghai-Tibet Plateau. These planting areas are faced with severe winters, heavy metal pollution, drought, and high altitude, which accelerate the process of plant senescence. Since alfalfa is a tetraploid plant with a complicated genetic background, chloroplast genetic engineering has the advantages of the site-specific integration of foreign genes, a large number of gene copies, and maternal inheritance compared with nuclear genetic engineering (Jin & Daniell 2015). Therefore, the use of chloroplast genetic engineering to introduce disease resistance, insect resistance, herbicide resistance and other genes into alfalfa to improve its genetic traits will be of great significance to the sustainable development of animal husbandry. Qingda no.1, a new cold-resistant alfalfa strain, was first mentioned by professor Guozhu Yang in 1984, selected by Qinghai University in 1984 and has been growing for more than 20 years in the high altitudes of the Qinghai-Tibet Plateau (Wang et al. 2012; Yang et al. 2013, 2017; Li et al. 2018). This cultivar has obvious adaptability in the high-altitude area of Qinghai Province (Wang et al. 2012; Yang et al. 2013, 2017; Li et al. 2018). In this paper, we reported the complete chloroplast genome of Qingda no.1 alfalfa as well as the phylogenetic analyses.

MATERIAL AND METHODS

Fresh and cleaned Qingda no.1 leaf materials were collected from our team's test base in Geermu, Qinghai, China (33°43'~35°16'N, 98°~100°56'E, 3 920 m a.s.l.). The Qingda no.1 specimens were stored at the College of Eco-Environmental Engineering, Qinghai University (https://www.qhu.edu.cn/, which is in charge by Ping Li, whose email is liping051126@163.com), and the specimen accession number is No. TMSGS21008. DNA was extracted from the collected Qingda no.1 leaves using the modified cetyltrimethylammonium bromide (CTAB) method (Yang et al. 2014). The high-quality DNA obtained was used for the library construction and sequencing at Beijing Biomarker Technologies Co. Ltd. using an Illumina Hiseq X Ten, which was followed by the *de novo* assembling of the genome and the annotation of the obtained data using SPAdes (Bankevich et al. 2012) and CpGAVAS combined with the UGENE ORF Finder (Rombel et al. 2002; Liu et al. 2012). The assembled complete cp genome of Qingda no.1 was deposited to the National Center for Biotechnology Information (NCBI) (accession No. MZ983396). The CodonW1.4.2 (Sharpl & Li 1987) (http://mobyle.pasteur/fr/cgi-bin/portal.py? from=codonw) was used to analyse the relative synonymous codon usage (RSCU) of the Qingda no.1 chloroplast genome. The Qingda no.1 chloroplast genome sequence number and Fasta file were submitted to SSR Hunter (Ver. 1.3) (Li & Wan 2005) and MISA-web (Ver. 2.1) (Beier et al. 2017) to identify the simple sequence repeat (SSR) sites. Parameter setting: The minimum repetition times of the single nucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide and hexanucleotide were 10, 5, 4, 3, 3 and 3, respectively. The minimum distance between two SSRs was set as 100 bp. If the distance was zero, a compound SSR was formed. If the distance is less than 100 bp, an interval SSR was formed. The chloroplast genome sequences of alfalfa were downloaded from the NCBI database, and the chloroplast genome sequences of all the tested species were aligned on MAFFT (Katoh & Standley 2013). The maximum likelihood (ML) method was used to construct the phylogenetic trees using MEGA (Ver. 7.0) and IQtree software (Kumar et al. 2016).

RESULTS

Basic characteristics of the chloroplast genome of Qingda no.1 *Medicago sativa*. The annotated chloroplast genome was submitted to the GenBank with accession number MZ983396. The complete Qingda no.1 chloroplast genome is a circular DNA molecule 125 637 bp in length, and the guanine-cytosine (GC) content was 33.82%, without the obvious characteristic of four zones (Figure 1). The Qingda no.1 chloroplast genome was composed of 111 genes, among which, 77 genes encoded proteins, 30 genes were related to the tRNA, and 4 genes were related to the rRNA (Table 1). The comparison of the three alfalfa cultivars showed that there were 30 tRNAs in all of the cultivars. In addition to the 29 tRNAs in common, tRNAG-GCC existed in Qingda no.1 and Deqin (*Medicago sativa* L. cv. Deqin), tRNAP-GGG existed in KU321683 (Table 1). *Ycf4* is present in Qingda no.1 and Deqin, but absent in KU321683 (Table 1). Similarly, there is an internal stop codon in the *ndhB* gene, which is consistent with the chloroplast genomes of other legumes (Jansen et al. 2008; Tao et al. 2017; Sun et al. 2022), and, in addition, the *rps8* gene has no stop codon (Jansen et al. 2008). Seventeen genes include one or two introns, with eleven be-

https://doi.org/10.17221/71/2022-CJGPB

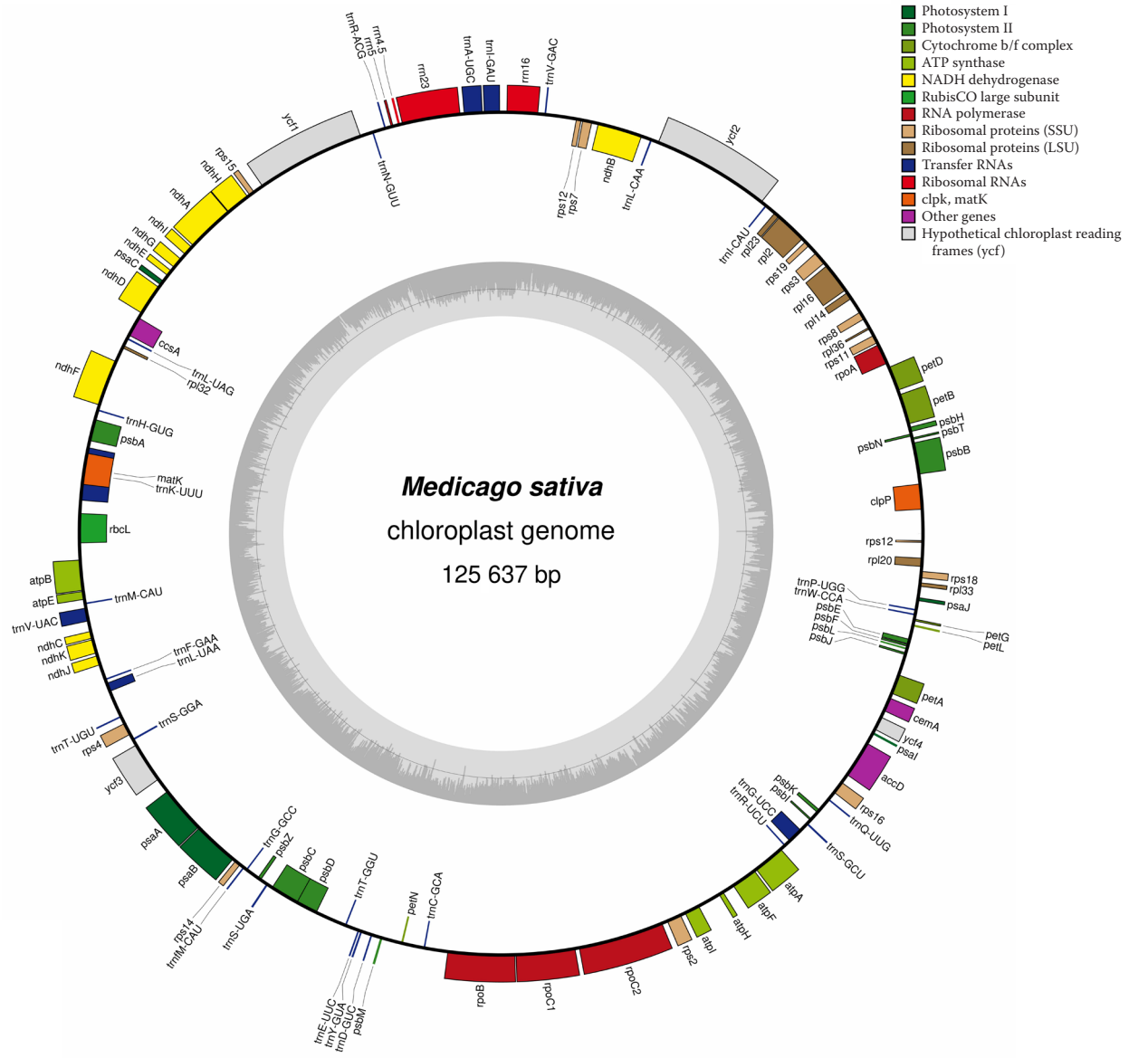

Figure 1. Gene map of the Qingda no.1 chloroplast genome; genes, shown on the outside of the circle, are transcribed in the clockwise direction, and those on the inside of the circle are transcribed in the counter-clockwise direction (GenBank accession number MZ983396)

ing in the protein-coding genes and six are in the *tRNA* genes (Table 2). As a protein-coding gene, *rpl2* contains a 696 bp intron in Qingda no.1, but the length of this intron in KU321683 is 693 bp (Tao et al. 2017). Similarly, trnK-UUU has the biggest intron (2 481 bp), trnL-UAA possess the smallest intron (353 bp), and *ycf3* gene owns two introns of 712 bp and 744 bp (Tao et al. 2017).

Relative synonymous codon usage of the chloroplast genome in Qingda no.1. As shown in Table 3, the relative synonymous codon usage (RSCU) was concluded according to the sequences of the Qingda no.1 chloroplast genome. Moreover, the 77 protein coding genes comprised 66 693 bp coding for 22 231 codons. Among these codons, 2 358 (10.61%) encode leucine and 237 (1.07%) encode cysteine, which are the most and the least prevalent amino acids, respectively. The highest codon usage was observed for AUU, isoleucine (Ile). High codon usage was also observed for leucine (Leu) and isoleucine (Ile), which is different from Tao et al. (2017). The start codon, ATG, is authenticated 505 times (2.27%). All three stop codons are present with UAA being the most frequently used (UAA 58.4%, UGA 24.7%

https://doi.org/10.17221/71/2022-CJGPB

Table 1. Comparison of the types and numbers of genes in three *Medicago sativa* subsp. *sativa*

Group of genes	Types of genes	Qingda no.1	Deqin	KU321683
Transfer RNA genes	*trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-UCC, trnH-GUG, trnI-CAU, trnI-GAU, trnK-UUU, trnM-CAU, trnfM-CAU, trnL-CAA, trnL-UAA, trnL-UAG, trnN-GUU, trnP-UGG, trnQ-UUG, trnR-ACG, trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC, trnV-UAC, trnW-CCA, trnY-GUA*	√	√	√
	trnG-GCC	√	√	×
	trnP-GGG	×	×	√
Ribosomal RNA genes	*rrn23, rrn16, rrn5, rrn4.5*	√	√	√
Small subunit of ribosome	*rps2, rps3, rps4, rps7, rps8, rps11, rps12, rps14, rps15, rps16, rps18, rps19*	√	√	√
Large subunit of ribosome	*rpl2, rpl14, rpl16, rpl20, rpl23, rpl32, rpl33, rpl36*	√	√	√
DNA dependent RNA polymerase	*rpoA, rpoB, rpoC1, rpoC2*	√	√	√
Subunits of photosystem I	*psaA, psaB, psaC, psaI, psaJ*	√	√	√
Subunits of photosystem II	*psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ*	√	√	√
Subunits of cytochrome	*petA, petB, petD, petG, petL, petN*	√	√	√
Subunits of ATP synthase	*atpA, atpB, atpE, atpF, atpH, atpI*	√	√	√
ATP-dependent protease subunits P gene	*clpP*	√	√	√
Large subunits of RuBisCO	*rbcL*	√	√	√
Subunits of NADH dehydrogenase	*ndhA, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK*	√	√	√
Maturase	*matK*	√	√	√
Envelope membrane protein	*cemA*	√	√	√
Subunits of acetyl-CoA-carboxylase	*accD*	√	√	√
C-type cytochrome synthesis gene	*ccsA*	√	√	√
Conserved open reading frames	*ycf1, ycf2, ycf3*	√	√	√
	ycf4	√	√	×

Table 2. The genes with introns in the Qingda no.1 chloroplast genome and the length of the exons and introns

Gene	Intron I	Exon II	Intron II	Exon III	Gene	Intron I	Exon II	Intron II	Exon III
	(bp)					(bp)			
clpP	647	223	–	–	*trnK-UUU*	2 481	35	–	–
petB	830	642	–	–	*trnV-UAC*	579	35	–	–
petD	711	475	–	–	*trnL-UAA*	353	50	–	–
rpl16	1 077	399	–	–	*ycf3*	712	230	744	153
rpl2	696	434	–	–	*rpoC1*	790	1 625	–	–
ndhB	688	758	–	–	*atpF*	693	410	–	–
trnI-GAU	689	35	–	–	*trnG-UCC*	684	48	–	–
trnA-UGC	814	35	–	–	*rps16*	465	30	–	–
ndhA	1 225	539	–	–					

https://doi.org/10.17221/71/2022-CJGPB

and UAG 16.9%). There were 68.67% codons with RSCU > 1 in Qingda no.1, and frequently ended with A and T, which has common ground with KU321683 in this respect (Tao et al. 2017).

Analysis of the chloroplast simple repeats (cpSSRs) in Qingda no.1. Table 4 shows the types and numbers of the SSRs identified in the chloroplast genome of Qingda no.1, as well as the comparison with the types and numbers of SSRs in the chloroplast genome of Deqin and KU321683, which is similar to previous reports (Huotari & Korpelainen 2012; Do et al. 2013; Tao et al. 2017; Sun et al. 2022). A total of 114 SSR loci were identified in the chloroplast genome of Qingda no.1, which could be divided into five categories according to their length. The number of SSR loci was 79, 21, 8, 4 and 2 for the single nucleotide, dinucleotide, trinucleotide, tetranucleotide and pentanucleotide, respectively. Among them, A9, A18, T9, T16, T20, AG4, AT4, AT8, TA4, ATA3, TAA3, TGA4 and TTA3 were identified only in Qingda no.1. A15, A19, T14, T17, T21, AG5, AT6, AT9, TA7, ATA4, TGA6 and TTA4 were not identi-

Table 3. Codon usage and codon-anticodon recognition pattern for the tRNA in the Qingda no.1 chloroplast genome

Amino acid	Codon	No. of codon	RSCU	tRNA	Amino acid	Codon	No. of codon	RSCU	tRNA
Phe/F	UUU	930	1.42	trnF-GAA	Ile/I	AUU	1008	1.53	trnI-GAU
	UUC	377	0.58			AUC	330	0.5	
Tyr/Y	UAU	700	1.66	trnY-GUA		AUA	643	0.97	trnI-CAU
	UAC	143	0.34		Thr/T	ACU	476	1.7	
Cys/C	UGU	183	1.54	trnC-GCA		ACC	183	0.66	trnT-GGU
	UGC	54	0.46			ACA	348	1.25	trnT-UGU
His/H	CAU	393	1.56	trnH-GUG		ACG	110	0.39	
	CAC	111	0.44		Asn/N	AAU	843	1.58	trnN-GUU
Ser/S	UCU	496	1.8	trnS-GGA		AAC	227	0.42	
	UCC	238	0.86		Asp/D	GAU	745	1.61	trnD-GUC
	UCA	323	1.17	trnS-UGA		GAC	182	0.39	
	UCG	146	0.53		Lys/K	AAA	962	1.58	trnK-UUU
	AGU	362	1.32			AAG	256	0.42	
	AGC	86	0.31	trnS-GCU	Val/V	GUU	475	1.54	trnV-GAC
Leu/L	UUA	814	2.07	trnL-UAA		GUC	131	0.42	
	UUG	484	1.23	trnL-CAA		GUA	472	1.53	trnV-UAC
	CUU	478	1.22	trnL-UAG		GUG	155	0.5	
	CUC	121	0.31		Ala/A	GCU	573	1.88	trnA-UGC
	CUA	327	0.83			GCC	167	0.55	
	CUG	134	0.34			GCA	350	1.15	
Pro/P	CCU	374	1.65	trnP-UGG		GCG	130	0.43	
	CCC	156	0.69		Gly/G	GGU	534	1.42	trnG-GCC
	CCA	272	1.2			GGC	127	0.34	
	CCG	105	0.46			GGA	625	1.66	trnG-UCC
Arg/R	CGU	294	1.42	trnR-ACG		GGG	217	0.58	
	CGC	84	0.41		Glu/E	GAA	920	1.56	trnE-UUC
	CGA	286	1.38			GAG	261	0.44	
	CGG	77	0.37		Trp/W	UGG	382	1	trnW-CCA
	AGA	363	1.75	TrnR-UCU	Met/M	AUG	505	1	trnfM-CAU
	AGG	140	0.68		TER/	UAA	45	1.75	
Gln/Q	CAA	621	1.62	trnQ-UUG		UAG	13	0.51	
	CAG	145	0.38			UGA	19	0.74	

RSCU – relative synonymous codon usage

https://doi.org/10.17221/71/2022-CJGPB

Table 4. Comparison of the types and numbers of the simple sequence repeats (SSRs) in three *Medicago sativa* subsp. *sativa* cultivars

SSR type and length/bp	Qingda no.1	Deqin	KU321683
A9	19	–	–
A10	10	18	19
A11	8	11	10
A12	6	8	7
A13	3	6	7
A14	3	3	3
A15	–	3	3
A18	2	–	–
A19	–	2	2
T9	7	–	–
T10	8	7	7
T11	5	7	8
T12	5	6	5
T13	1	5	5
T14	–	1	1
T16	1	–	–
T17	–	1	1
T20	1	–	–
T21	–	1	1
AG4	1	–	–
AG5	–	1	1
AT4	5	–	–
AT5	3	5	5
AT6	–	3	3
AT8	1	–	–
AT9	–	1	1
TA4	8	–	–
TA5	2	8	8
TA6	1	2	2
TA7	–	1	1
ATA3	3	–	–
ATA4	–	3	3
TAA3	2	–	–
TAA4	–	2	2
TGA4	1	–	–
TGA5	1	1	1
TGA6	–	1	1
TTA3	1	–	–
TTA4	–	1	1
ATAA3	1	1	1
TATT3	2	2	2
TTAT3	–	1	–
TTCA3	1	1	1
ATAAG3	1	1	1
ATTAA3	1	1	1
Total	114	115	114

fied in Qingda no.1 but were found in the other two alfalfa species and their amounts were comparable. A10, A11, A12, A13, T11, T12, T13, AT5, TA5 and TA6 were detected less in Qingda no.1 than in the other two kinds of alfalfa. The amounts of A14, TGA5, ATAA3, TATT3, TTCA3, ATAAG3 and ATTAA3 were comparable among the three alfalfa species. T10 was detected more in Qingda no.1. TTAT3 was detected only in Deqin. This suggests that there are differences between the three types of alfalfa.

Phylogenetic analysis. The whole chloroplast genome sequences of 28 species of *Medicago* were downloaded from the NCBI database for the phylogenetic analysis, and the sequences were compared using the default setting of MAFFT V.7.149 (Katoh & Standley 2013). The phylogenetic analysis showed that Qingda no.1 clustered together with *Medicago hybrida* and KU321683 (Figure 2). In this analysis, Qingda no.1 and KU321683 are the most closely related. The complete chloroplast genome of alfalfa provides a reference for further studies on the phylogeny and species evolution of alfalfa and its related genera, as well as a resource for conservation and utilisation.

DISCUSSION

In this study, the whole Qingda no.1 chloroplast genome was constructed. The complete Qingda no.1 chloroplast genome is a circular DNA molecule 125 637 bp in length, without the obvious characteristic of four zones (Figure 1). It is unlike most of the representative land plant chloroplast genomes which have two copies of the IR region. The comparison of the three alfalfa cultivars showed that there were 30 tRNAs in all of them. In addition to the 29 tRNAs in common, tRNAG-GCC existed in Qingda no.1 and Deqin, tRNAP-GGG existed in KU321683. *Ycf4* presented in Qingda no.1 and Deqin, but was absent in KU321683. Qingda no.1 is a long-term domesticated strain in the high altitude area of Qinghai Province in China. KU321683 is a suitable cultivar for cultivation and promotion in the low altitude area of northern China, while Deqin is mainly cultivated in arid and tropical areas (Jiang et al. 2012; Wang et al. 2012; Yang et al. 2013, 2017; Zhao et al. 2015; Li et al. 2018). The differences in the number and species of genes in the chloroplast genomes of the three alfalfa species may be related to the homology and regional adaptability of the three alfalfa species. After long-term evolution, certain species have a preference for codons to adapt to their own

https://doi.org/10.17221/71/2022-CJGPB

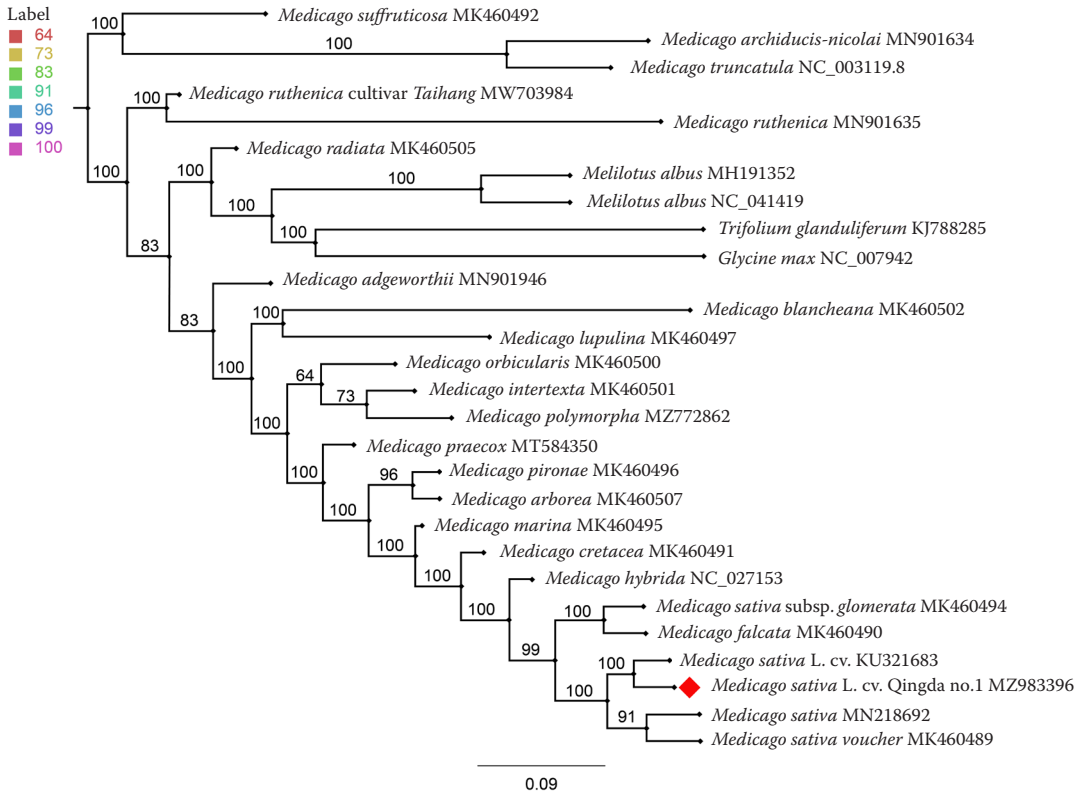

Figure 2. Cluster analysis of 28 species of *Medicago* using the complete chloroplast genome sequence by the maximum likelihood method

genomic environment (Sharp et al. 2010; Rao et al. 2011; Hanson & Coller 2018; Paul et al. 2018). Studies have shown that natural selection, mutation, genome size, gene expression level, tRNA abundance, protein secondary structure, gene density, and gene sequence length all play a role in the species codon bias (Sharp et al. 2010; Rao et al. 2011; Hanson & Coller 2018; Paul et al. 2018). Compared with the first two bases forming the codon, the third base is often under greater selection pressure, so the type of the third base in the codon plays an important role in the use of the codon (Yuan et al. 2021). In addition, dicots and monocots have different preference for codons (Plotkin & Kudla 2011; Quax et al. 2015). In this study, about 68.67% of the chloroplast codons of Qingda no.1 have RSCU > 1, which ends in A/T with a high frequency similar to dicots, but is obviously different from the monocots in their preference for G/C. This is similar to the codon preference of chloroplast genomes of plants, such as Tribulus alfalfa (Yang et al. 2015), KU321683 (Tao et al. 2017), and Deqin (Sun et al. 2022), indicating that the chloroplasts of these plants have high similarity and conservation in the codon preference. Chloroplast simple sequence repeats (cpSSRs) are very effective molecular markers, which are widely used for species identification and the analysis of the genetic differences at the population and individual levels based on their tag number, recessive inheritance and single inheritance characteristics (Ebert & Peakall 2009). The DNA sequences of the chloroplast genomes can provide resources for marker selection in upcoming alfalfa studies. With the rapid development of cpSSRs, studies of important plants and their relatives are becoming more common; therefore, the potential of cpSSRs to provide special perspectives on ecological and evolutionary processes in wild plants remains to be investigated (Ebert & Peakall 2009). There were 114 SSR loci in the chloroplast genome of Qingda no.1. The type and number of SSRs were significantly different from those of Deqin and KU321683. This suggests that there are differences between the three types of alfalfa. In the phylogenetic analysis of the chloroplast genome, Qingda no.1 is closely related to KU321683 (Figure 2). These results are helpful to further study the adaptation mechanism of alfalfa to high-altitude

https://doi.org/10.17221/71/2022-CJGPB

stress environment, and provide a solid theoretical basis for the molecular biology, genetic breeding and molecular evolution of alfalfa.

CONCLUSION

In this study, the complete chloroplast genome of Qingda no.1 was assembled. The total length of the chloroplast genome is 125 637 bp. The genome exhibits no obvious typical quadripartite structure. The chloroplast genome contained 111 genes, including 77 protein-coding genes, 30 tRNA genes, and 4 rRNA genes. In the phylogenetic analysis of the cp genome, Qingda no.1 clustered closely with KU321683.

Acknowledgement. I would like to thank Professor Guozhu Yang for providing the experimental materials, Professor Ping Li for critically revising the paper, and I would also like to thank the members of the research group for helping each other in the joint research. Thanks is also given to several fund projects for their strong support of this research.

REFERENCES

Bankevich A., Nurk S., Antipov D., Gurevich A.A., Dvorki M., Kulikov A.S., Lesin V.M. (2012): A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology, 19: 455–477.

Beier S., Thiel T., Münch T., Scholz U., Mascher M. (2017): A web server for microsatellite predicition. Bioinformatics, 33: 2583–2585.

Do H.D.K., Kim J.S., Kim J.H. (2013): Comparative genomics of four Liliales families inferred from the complete chloroplast genome sequence of *Veratrum patulum* O. Loes. Gene, 530: 229–235.

Ebert D., Peakall R.O.D. (2009): Chloroplast simple sequence repeats (cpSSRs) technical resources and recommendations for expanding cpSSR discovery and applications to a wide array of plant species. Molecular Ecology Resources, 9: 673–690.

Hanson G., Coller J. (2018): Codon optimality, bias and usage in translation and mRNA decay. Nature Reviews Molecular Cell Biology, 19: 20–30.

Huotari T., Korpelainen H. (2012): Complete chloroplast genome sequence of *Elodea canadensis* and comparative analyses with other monocot plastid genomes. Gene, 508: 96–105.

Jansen R.K., Wojciechowski M.F., Wojciechowski, Sanniyasi E., Seung B.L., Henry D. (2008): Complete plastid genome sequence of the chickpea (*Cicer arietinum*) and the phylogenetic distribution of rps12 and clpP intron losses among legumes (Leguminosae). Molecular Phylogenetics and Evolution, 48: 1204–1217.

Jiang H., Bi Y.F., Chen L.X., Shan G. (2012): Physiological characteristics of alfalfa under dry-farming condition. Acta Agrestia Sinica, 20: 1077–1080.

Jin S., Daniell H. (2015): The engineered chloroplast genome just got smarter. Trends in Plant Science, 20: 622–640.

Katoh K., Standley D. (2013): MAFFT multiple sequence alignment software version 7 improvements in performance and usability. Molecular Biology and Evolution, 30: 772–780.

Kumar S., Stecher G., Tamura K. (2016): Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870–1874.

Li Q., Wan J.M. (2005): Development of a local searching software for SSR sites. Hereditas, 27: 808–810.

Li X.A., Yang G.Z., Tian H.N., Yan E., Yin W., Li P., Shang Y.F., Xiao F., Zhang Z.G., Yang F., Zhao H.K., He S.P., Xiong L.L., Yan X.X., Li C.D., Ma R.R., Zhu X.W., Qi X.Q. (2018): Study on physiological adaptation mechanism of Qingda No.1 new alfalfa strain in Shangmei Village around lake. Xining, Qinghai University.

Liu C., Shi L., Zhu Y., Zhu Y.J., Chen H.M., Zhang J.H., Lin X.H., Guan X.J. (2012): CpGAVAS, an integrated web server for the annotation, visualization, analysis, and GenBank submission of completely sequenced chloroplast genome sequences. BMC Genomics, 13: 715.

Paul P., Malakar A.K., Chakraborty S. (2018): Codon usage and amino acids usage influence genes expression level. Genetica, 146: 53–63.

Plotkin J.B, Kudla G. (2011): Synonymous but not the same: The causes and consequences of codon bias. Nature Reviews Genetics, 12: 32–42.

Quax T.E.F., Claassens N.J., Soll D., John V.D.O. (2015): Codon Bias as a means to fine-tune gene expression. Molecular Cell, 59: 149–161.

Rao Y.S., Wu G.Z., Wang Z.F., Wang Z.F., Chai X.W., Nie Q.H., Zhang X.Q. (2011): Mutation bias is the driving force of codon usage in the *Gallus gallus* genome. DNA Research, 18: 499–512.

Rombel I.T., Sykes K.F., Rayner S., Johnston S.A. (2002): A vector for high-throughput gene identification. Gene, 282: 33–41.

Sharp P.M., Emery L.R., Zeng K. (2010): Forces that influence the evolution of codon bias. Philosophical Transactions of the Royal Society B, 365: 1203–1212.

Sharpl P.M., Li W.H. (1987): The codon adaptation index-a measure of directional synonymous codon usage bias, and its potential applications. Nucleic Acids Research, 15: 1281–1295.

Sun Z.X., Ao P.X., Bi Y.F., Shi Q.X.,Yu M. (2022): Complete chloroplast genome sequence and characteristics

https://doi.org/10.17221/71/2022-CJGPB

analysis of Medicago sativa 'Deqin'. Acta Agrestia Sinica, 30: 320–328.

Tao X.L., Ma L.C., Zhang Z.S, Liu W.X., Liu Z.P. (2017): Characterization of the complete chloroplast genome of alfalfa (*Medicago sativa*) (Leguminosae). Gene Reports, 6: 67–73.

Wang X.Y., Chang X.Y., Peng Q.F., Yang G.Z. (2012): Research on development and degeneration of alfalfa in region of Qinghai Lak. Prataculture & Animal Husbandry, 10: 1–5.

Yang G.F, Su K., Zhao Y.R., Song Z.B., SUN J. (2015): Analysis of codon usage in the chloroplast genome of *Medicago truncatula*. Acta Prataculturae Sinica, 24: 171–179.

Yang G.Z., Zou H., Zhou P., Yang Y.H., Tian H.N., Nan M.J., Su L.P. (2013): Breeding of a new cold resistant alfalfa strain "Qingda No.1" in alpine pastoral area. Xining, Qinghai University.

Yang G.Z., Yang Y.H., Zou H., Yin W., Zhang Z.G., Tian H.N., Li X.A., Li P., He S.P., Xiong L.L., Yan X.X., Yang F., Chen Y.S., Ma R.R., Li C.D., Zhang X.C., Wang Z.L., Tian Y.Z., Cao G.B., Liu X.M., Zhu X.W., Qi X.Q. (2017): Study on propagation and seed production technology of Qingda No.1 new alfalfa strain. Xining, Qinghai University.

Yang J.B., Li D.Z., Li H.T. (2014): Highly effective sequencing whole chloroplast genomes of angiosperms by nine novel universal primer pairs. Molecular Ecology Resources, 14: 1024–1031.

Yuan X.L., Li Y.Q., Zhang J.F, Wang Y. (2021): Analysis of codon usage bias in the chloroplast genome of *Dalbergia odorifera*. Guihaia, 41: 622–630.

Zhao Y., Che W.G., Bi Y.F. (2015): Analysis of APX activity and transcription level in *Medicago sativa* L. 'Deqin' under heat stress. Molecular Plant Breeding, 13: 1611–1615.

Received: September 5, 2022
Accepted: February 6, 2023
Published online: April 14, 2023