RNA editing analysis of some chloroplast transcripts and its response to light and salt stress in *Mesona chinensis* Benth

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**Abstract**

To study the effects of light quality and salt stress on RNA editing of *Mesona chinensis* Benth (MCB) chloroplast genome, the RNA editing sites in the MCB chloroplast protein-coding genes were predicted and then partially verified by PCR and RT-PCR. Meanwhile, the RNA editing efficiency and relative expression of accD, ndhB, ndhF, and rpoB under red and blue light and salt stress conditions were analyzed. A total of 45 editing sites were predicted and all the editing sites were C-to-U conversion. 12 predicted editing sites were verified. The expression level of accD was down-regulated under red light compared with the blue light, as well as down-regulated under salt stress compared with the normal condition (CK). Additionally, the editing efficiency of accD-287 was 96.7% under normal condition, higher than that under salt stress (93.3%) but lower than those under blue and red light (both 100%). In ndhB, ndhB-494 was partially edited under normal growth condition but completely edited under blue and red light and salt stress, and other sites were completely edited under all conditions. It was indicated that the editing frequency was not positively relevant to the transcript level. Besides, accD-287 and ndhB-494 might be involved in response to salt stress.

**1. Introduction**

RNA editing refers to the deletion, substitution or insertion of one or more nucleotide bases in a certain gene and alters its transcript. The first RNA editing event was reported in the *coxII* gene of a trypanosome and it was found that there were 4 nucleotides inserting in the transcript of *coxII* (Benne et al. 1986). In addition to the mRNA, RNA editing can occur in tRNA, rRNA and snRNA. Owing to its genetic information modification mechanism, RNA editing plays significant roles in gene expression regulation, signal transduction, stress response, etc (Huang et al. 2012; Penzo et al. 2016; Cui et al. 2019).

RNA editing can occur in plants, viruses, bacteria, and mammals (Mehedi et al. 2011; Peng et al. 2012; Kumbhar et al. 2018; Nie et al. 2020) according to the organism types and can occur in the chloroplast, mitochondria, and nucleus (Brennicke et al. 1999) according to the organelle types. RNA editing includes many types and the most common types of conversion are cytosine-to-uracil (C-to-U) in plant organelles and A-I in the animal nucleus (Ma YL 2009). In higher plants, most RNA editing events occur in the protein-encoding genes of mitochondria and chloroplast and C-to-U conversion is the most common type (Yan et al. 2018). Chloroplast RNA editing usually occurs in the exon of coding region, rarely in the intron or noncoding region. The majority of editing events are detected at the first or second position of a codon, resulting in the conversion of hydrophilic amino acid to hydrophobic (Shikanai 2006). Previous studies have shown that RNA editing tended to restore the functions of evolutionarily conserved amino acid residues in the chloroplast (Maier et al. 1995; Tsudzuki et al. 2001).

Chloroplast, as the site of photosynthesis, has always been the focus of plant research. However, the molecular mechanism of RNA editing in chloroplast protein-coding genes still remains unclear. It is widely accepted that PPR (pentatricopeptide repeat) proteins act as a key editing factor to bind to the upstream cis-acting element target site, and then regulate the alteration of target RNA molecule (Kotera et al. 2005; Hayes and Mulligan 2011; Takenaka et al. 2013). Additionally, there are other editing factors involved in the chloroplast RNA editing process, such as MORF (multiple organellar RNA editing factors) proteins, ORRM (organelle RNA recognition motif) proteins, OZ (organelle zinc finger) proteins, and so forth (Glass et al. 2015; Sun et al. 2015; Shi et al. 2017). In addition to the molecular factors, chloroplast RNA editing can be affected by darkness, salt, temperature and other external environmental factors (Rodrigues et al. 2017; Cui et al. 2019; Ramadan 2020).

*Mesona chinensis* Benth (MCB) is one of the most important medicinal and edible plants, which is broadly cultivated in tropical and subtropical areas (Tang et al. 2021a, 2022a, 2022b, 2021b). Due to its multiple uses in food, packaging,
and medical industries, MCB has gradually become a hot research plant. However, up to now, very few information is available regarding the molecular genetics of MCB. Our team has completed the sequencing of the chloroplast genome and the analysis of codon usage bias of chloroplast genes in MCB (Tang et al. 2022a, 2021b). In addition, our previous study showed that MCB responded differently to red and blue light. Compared to the blue light condition, the SPAD (soil and plant analyzer development) value was reduced and the chloroplast ultrastructure was changed under the red light condition (Tang et al. 2021a). Moreover, the MCB leaves were severely withered under salt stress (data not shown). Therefore, we aim to study the effects of the light quality and salt stress on RNA editing of MCB chloroplast.

In this study, the RNA editing sites in the MCB chloroplast protein-coding genes were predicted using bioinformatic tools and then were also partially verified by PCR and RT–PCR. The RNA editing patterns of the MCB chloroplast genome were compared with that of the other plants. Furthermore, the effects of the light quality (red and blue) and salt stress (200 mM NaCl) on RNA editing of the accD (acetyl-CoA carboxylase beta subunit) and ndhB (the second subunit of NADH dehydrogenase) genes were also investigated. The current study laid a foundation for further exploring the biological function and mechanism of RNA editing in the MCB chloroplast and provided a reference for the study of chloroplast RNA editing in other medicinal plants.

2. Materials and methods

2.1. Plant materials

The Mesona chinensis Benth (MCB) seedlings were provided by Guangxi Key Laboratory of Medicinal Resources Protection and Genetic Improvement, Nanning, China. For salt treatment, nearly 20-cm cutting seedlings were grown in 1/2 Hoagland solution supplemented with 200 mM NaCl for 24 h. For blue and red light treatment, nearly 10-cm tissue culture seedlings were exposed to blue (200 µmol m\(^{-2}\) s\(^{-1}\)) and red (200 µmol m\(^{-2}\) s\(^{-1}\)) light at a day/night time of 16/8 h for 1 month, respectively. Young leaves were collected and frozen in liquid nitrogen immediately, then stored in a refrigerator at \(-80^\circ\text{C}\).

2.2. Prediction of RNA editing sites in the chloroplast genome of Mesona chinensis Benth

Our team had already completed the high-throughput sequencing of the chloroplast genome in Mesona chinensis Benth (Tang et al. 2022a). Based on this, we submitted the chloroplast protein-coding genes into two online softwares, Prep-Cp (http://prep.unl.edu/cgi-bin/cp-input.pl) and CURE (http://www.Bioinfo.au.tsinghua.edu.cn/pure/), for the RNA editing sites prediction. Parameters were set to default.

2.3. Extraction of total DNA and RNA

Total DNA and RNA were extracted using FastPure Plant DNA Isolation Mini Kit (Vazyme, Nanjing, China) and FastPure Plant Total RNA Isolation Kit (Vazyme, Nanjing, China), respectively. Complementary DNA (cDNA) was synthesized using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, Beijing, China). Experimental operations reference manual.

2.4. Validation of partial RNA editing sites by PCR and RT–PCR

The extracted DNA and synthesized cDNAs were used as PCR templates for the validation of 22 RNA editing sites, selected from four genes according to the prediction, namely accD, ndhB, ndhF and rpoB. Notably, rpoB was divided into two fragments for amplification due to its long fragment. All specific primers were designed with Primer Premier 5.0 (Table 1). The PCR reaction was performed as follows: 95°C for 3 min, 95°C denaturing for 15 s, 55°C annealing for 15 s, and an elongating time for 2 min at 72°C, 35 cycles. The PCR amplification products were ligated into cloning vectors and then introduced into E.coli DH5α. The sequencing was carried out by Sangon Biological Engineering Technology & Services (Shanghai, China). Ten positive clones were selected for editing efficiency analysis.

2.5. RT-qPCR analysis of chloroplast genes under salt stress, blue and red light conditions

The RT-qPCR was performed to explore the transcript levels of accD, ndhB, ndhF and rpoB under salt stress, blue and red light conditions. The reaction was conducted by ABI QuantStudio 3 real-time PCR system (ABI, Waltham, MA, United States) using TransStart Tip Green qPCR SuperMix (TransGen, Beijing, China). The primers of four genes were designed and listed in Supplementary Table 1. The RNA and cDNA used in RT-qPCR were the same as those used for editing efficiency analysis. GAPDH was employed as an internal control and the real-time PCR system and condition were according to Tang et al. (Tang et al. 2021a).

2.6. Protein structure prediction before and after editing

In order to clarify the impact of RNA editing on protein structure, the online tool SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) was utilized to predict the secondary structures before and after editing. Transmembrane domain prediction was carried out by TMHMM - 2.0 (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0). ProtScale (https://web.expasy.org/protscale/) was used to analyze protein hydrophobicity.

Table 1. Primers used for PCR and RT-PCR.

| Primer name | Primer sequence (5’–3’) | Expected size (bp) |
|-------------|-------------------------|-------------------|
| accD | F: AACCTCTAGGAAAAGATGGTGTGTAA | 1236 |
| R: CCCACACGTAGAAAGATAGTAA | |
| ndhB | F: GCCTTTCTTATGCTTCTTCTTATG | 1465 |
| R: GGATCTCTGGAGCGGTAACTTAA | |
| ndhF | F: AAAAAACTGCTGCTGTTGGGCTT | 668 |
| R: TCCCTTCAATAGTCCGGAAGAC | |
| rpoB-1 | F: AAGGAAAGAAATGCTGTGTATGAAT | 452 |
| R: TTTTCCGTCCTATCATGAGA | |
| rpoB-2 | F: TATGGCCTGGGAGGTTGAGATCTT | 486 |
| R: GCTATTTATGCATTTGATTG | |
2.7. Comparative analysis of chloroplast RNA editing sites in six higher plants

RNA editing sites in four genes, namely accD, ndhB, ndhF and rpoB, were subjected to a comparative analysis with their counterparts in other five species to gain a comprehensive understanding of editing similarities and differences. RNA editing sites in chloroplasts of Nicotiana tabacum (Sasaki et al. 2003), Arabidopsis thaliana (Lutz and Maliga 2001), Gossypium hirsutum (Jiang et al. 2012), Aegilops tauschii (Wang et al. 2016) and Pisum sativum (Inada et al. 2004) have been systematically investigated.

2.8. Statistical analysis

All experiments were performed three times. The statistical analysis of variance was completed by Excel 2007. The statistical difference was clarified through analysis of variance by t-test, with $P < 0.05$ (*) and $P < 0.01$ (**) to be significantly different.

3. Results

3.1. The predicted RNA editing sites in the chloroplast protein-coding genes of Mesona chinensis Benth

In all, 45 editing sites were identified in 20 protein-coding genes in the Mesona chinensis Benth (MCB) chloroplast genome (Table 2), and all of the editing sites exhibited single-nucleotide editing, which were C→U conversion. Among them, 9 out of 45 editing events occurred at the first position of the codon and the rest occurred at the second position of the codon. Additionally, ndhF genes possessed the most abundant editing sites (16), which made up 35.6% of the total editing sites, followed by rpo genes (11), accD gene (5), atp genes (3), rps genes (3), matK gene (2), petB gene (2), rpl genes (2) and ccsA gene (1).

Notably, all of the editing sites were predicted to be non-silent, which lead to the alterations of the corresponding amino acids. Among them, 15 editing events converted Serine to Leucine, followed by Proline to Leucine (7), Alanine to Valine (5), Serine to Phenylalanine (4), Histidine to Tyrosine (4), Threonine to Methionine (3), Arginine to Tryptophan (2), Threonine to Isoleucine (2), Leucine to Phenylalanine (1), Proline to Serine (1) and Arginine to Cysteine(1). Furthermore, 27 out of the 45 editing sites switched amino acids from hydrophilic to hydrophobic, meanwhile 17 editing sites maintained their hydrophobic properties. Only 1 editing site transformed its hydrophobic property to hydrophilicity.

3.2. Partial predicted RNA editing sites validated by PCR and RT–PCR

In order to verify the accuracy of the RNA editing prediction, four genes (accD, ndhB, ndhF, and rpoB) with 22 predicted RNA editing sites were selected for verification by PCR and RT–PCR (Figure 1). Totally, there were 12 editing sites validated in the four genes (Table 2 and Figure 2). The sequencing analysis indicated that only one site accD-287 underwent editing in accD. Similarly, simply ndhF-97 was validated in ndhF. On the contrary, 8 out of 9 predicted sites in ndhB underwent editing except ndhB-431. Two sites rpoB-667 and rpoB-809 were detected in rpoB, not including rpoB-113, rpoB-473, rpoB-184 and rpoB-189. Totally, there were 12 editing sites validated in four genes. Further analysis indicated that most of the validated sites either altered hydrophilic amino acids to hydrophobic or maintained the hydrophobicity.

3.3. Impacts on protein structures before and after editing

Further to study the effects of RNA editing on protein structures, the online tool SOPMA was utilized to estimate the impacts on protein structures before and after editing (Figure 3). The result showed that there were four kinds of secondary structures within these proteins, namely alpha helix, extended strand, beta turn and random coil (Table 3). Among them, alpha helix and random coil made up more than 70% of the protein structure, followed by extended strand. Beta turn made up the least proportion. The statistics revealed that slight impacts were imposed on the protein structures after editing. To be specific, the alteration in the proportion of each secondary structure was less than 3%. Despite eight editing sites in ndhB, the maximum alteration was less than 0.6%.

| Table 2. The predicted RNA editing sites in chloroplast genes of Mesona chinensis Benth. |
|------------------|------------------|------------------|------------------|------------------|
| Gene            | Nucleotide position | Amino acid position | Codon conversion | Amino acid conversion | Validation |
| accD            | 86               | aCg→aUg           | T→M             | -                |           |
|                 | 134              | aCg→aUg           | T→M             | -                |           |
|                 | 70               | Ctc→Uct           | P→L             | -                |           |
|                 | 860              | tcg→tgl            | S→L             | +                |           |
|                 | 1082             | gcA→gluA         | A→V             | -                |           |
| atpA            | 791              | ccc→cUc           | P→L             | -                |           |
| atpF            | 92               | cca→cUa           | P→L             | -                |           |
| atpl            | 629              | tca→tUa           | S→L             | -                |           |
| ccsA            | 559              | Ctc→Utc           | L→F             | -                |           |
| matK            | 460             | Cac→Uac           | H→Y             | -                |           |
| ndhA            | 341             | tca→tUa           | S→L             | -                |           |
| ndhB            | 149             | tca→tUa           | S→L             | +                |           |
|                 | 467             | cca→cUa           | P→L             | -                |           |
|                 | 586             | Cac→Uac           | H→Y             | +                |           |
|                 | 737             | cca→cUa           | P→L             | +                |           |
|                 | 746             | tct→tUt           | S→F             | +                |           |
|                 | 830             | tct→tUt           | S→F             | +                |           |
|                 | 836             | tca→tUa           | S→L             | +                |           |
|                 | 1292            | tcc→tUc           | S→F             | -                |           |
|                 | 1481            | 4ccA→cUa          | P→L             | -                |           |
| ndhD            | 2               | aCg→aUg           | T→M             | -                |           |
|                 | 32              | gcA→gluA         | A→V             | -                |           |
|                 | 878             | tca→tUa           | S→L             | -                |           |
| ndhF            | 205             | 69               | Cac→Uac           | H→Y             | -                |           |
|                 | 290             | 97               | tca→tUa           | S→L             | +                |           |
| ndhG            | 314             | 105              | acc→ala          | T→L             | -                |           |
| petB            | 418             | 140              | Cgg→Ugg           | R→W             | -                |           |
|                 | 611             | 204              | cca→cUa           | P→L             | -                |           |
| rpl2            | 593             | 198              | gcg→glu           | A→V             | -                |           |
| rpl20           | 308             | 103              | tca→tUa           | S→L             | -                |           |
| rpoA            | 251             | 84               | gcg→glu           | A→V             | -                |           |
| rpoB            | 338             | 113              | tct→tUt           | S→F             | -                |           |
|                 | 473             | 158              | tca→tUa           | S→L             | -                |           |
|                 | 551             | 184              | tca→tUa           | S→L             | -                |           |
|                 | 566             | 189              | tca→tUa           | S→L             | -                |           |
|                 | 2009            | 667              | tct→tUt           | S→F             | +                |           |
|                 | 2426            | 809              | tca→tUa           | S→L             | -                |           |
| rpoC1           | 650             | 217              | act→aUt           | T→I             | -                |           |
| rpoC2           | 1444            | 482              | Cgc→Ugc           | R→C             | -                |           |
|                 | 2066            | 689              | gcg→glu           | A→V             | -                |           |
| rps2            | 2287            | 763              | Cgc→Ugc           | A→V             | -                |           |
| rps14           | 248             | 83               | tca→tUa           | S→L             | -                |           |
|                 | 149             | 50               | cca→cUa           | P→L             | -                |           |

*+* indicates editing; *−* indicates no editing.

including rpoB-113, rpoB-473, rpoB-184 and rpoB-189. Totally, there were 12 editing sites validated in four genes. Further analysis indicated that most of the validated sites either altered hydrophilic amino acids to hydrophobic or maintained the hydrophobicity.

3.3. Impacts on protein structures before and after editing

Further to study the effects of RNA editing on protein structures, the online tool SOPMA was utilized to estimate the impacts on protein structures before and after editing (Figure 3). The result showed that there were four kinds of secondary structures within these proteins, namely alpha helix, extended strand, beta turn and random coil (Table 3). Among them, alpha helix and random coil made up more than 70% of the protein structure, followed by extended strand. Beta turn made up the least proportion. The statistics revealed that slight impacts were imposed on the protein structures after editing. To be specific, the alteration in the proportion of each secondary structure was less than 3%. Despite eight editing sites in ndhB, the maximum alteration was less than 0.6%.
Additionally, the results of transmembrane domain analysis indicated that little effects of RNA editing were exerted on the protein sequences of accD, ndhF and rpoB, except ndhB. After editing, the amino acids in these sites, ndhB-156, ndhB-277 and ndhB-279, altered to hydrophobic leucine, creating two added transmembrane domains in codons 150th to 169th and 262th to 284th (Figure 4). Hydrophobicity analysis revealed that all the 12 editing sites tended to increase the hydrophobicities of corresponding proteins, implying that the protein structures were strengthened after editing (Table 4).

3.4. Comparative analysis of chloroplast RNA editing sites in six higher plants

RNA editing sites in MCB and other five higher plants were subjected to comparison (Table 5). The analysis showed that ndhB-50, ndhB-196, ndhB-277, ndhB-279 and ndhB-494 were highly conserved among these plants, followed by ndhB-156, ndhB-246, ndhB-249, ndhF-97 and rpoB-184. No conserved editing site was found in accD. Notably, unlike in most species, four editing sites i.e. ndhB-204, rpoB-113, rpoB-184 and rpoB-189, were absent in MCB. Moreover, there were no unique editing sites in four chloroplast genes of MCB.

Figure 1. Amplification of four genes by PCR and RT-PCR. (M) marker. (1–5) Amplification of ndhB, rpoB-1, rpoB-2, ndhF and accD by PCR, respectively. (6–10) Amplification of ndhB, rpoB-1, rpoB-2, ndhF and accD by RT-PCR, respectively.

Figure 2. Sanger sequencing results of accD, ndhB, ndhF and rpoB.
3.5. The relative expression levels of chloroplast genes under blue and red light and salt stress conditions

To explore the responses of chloroplast genes to blue and red light and salt stress condition, the relative expression levels of the four above-mentioned genes were firstly estimated. The relative expression levels of accD, ndhB, ndhF, and rpoB under red light were decreased to 56%, 99%, 91% and 68% of those under blue light, respectively (Figure 5(A)). Similarly in salt treatment, the relative expression level of accD was significantly decreased to 40% of the control (CK), while those of ndhB, ndhF and rpoB were just slightly decreased to 68%, 99%, and 83% of the control (CK), respectively (Figure 5(B)).

3.6. The editing efficiency of chloroplast genes under blue and red light and salt stress conditions

Because the expression levels of accD and ndhB could be affected by blue and red light and salt stress conditions, we speculated that the editing efficiencies of editing sites in accD and ndhB might also be influenced by these environmental factors. Thus, the editing efficiencies of editing sites in accD and ndhB were further estimated. As shown in Figure 6, under blue and red light conditions, accD-287 was completely edited. However, accD-287 was partially edited under normal growth condition (CK) with a 96.7% editing efficiency and the editing efficiency slightly decreased to 93.3% under salt stress condition. In ndhB, ndhB-494 was partially edited under normal growth condition (CK) with a 96.7% editing efficiency, while completely edited under blue and red light and salt stress condition. Most notably, other seven editing sites (ndhB-50, ndhB-156, ndhB-196, ndhB-246, ndhB-249, ndhB-277 and ndhB-279) were completely edited under all these environmental conditions.

Table 3. Statistics of secondary structures of accD, ndhB, ndhF and rpoB.

| Protein | Alpha helix | Extended strand | Beta turn | Random coil |
|---------|-------------|-----------------|-----------|-------------|
| accD-DNA | 180 (35.09%) | 96 (18.71%) | 37 (7.21%) | 200 (38.99%) |
| accD-cDNA | 193 (37.62%) | 92 (17.93%) | 28 (5.46%) | 200 (38.99%) |
| ndhB-DNA | 263 (51.53%) | 84 (16.47%) | 29 (5.69%) | 134 (26.27%) |
| ndhB-cDNA | 262 (51.37%) | 85 (16.67%) | 26 (5.10%) | 137 (26.86%) |
| ndhF-DNA | 339 (45.50%) | 146 (19.60%) | 41 (5.50%) | 219 (29.40%) |
| ndhF-cDNA | 330 (44.30%) | 154 (20.67%) | 41 (5.50%) | 220 (29.53%) |
| rpoB-DNA | 362 (33.83%) | 207 (19.35%) | 69 (6.45%) | 432 (40.37%) |
| rpoB-cDNA | 371 (34.67%) | 211 (19.72%) | 66 (6.17%) | 422 (39.44%) |

Figure 3. Secondary structures before and after editing. (A-D) The secondary structures of accD, ndhB, ndhF and rpoB before editing, respectively. (a–d) The secondary structures of accD, ndhB, ndhF and rpoB after editing, respectively. Blue represents for alpha helix, green represents for beta turn, red represents for extended strand, purple represents for random coil.
4. Discussion

RNA editing is an essential post-transcriptional biological process that mainly occurs in chloroplast, chondriosome and cell nucleus. Since the first chloroplast RNA editing event was reported by Hoch et al. (Hoch et al. 1991), extensive chloroplast RNA editing sites were detected in higher plants, such as Nicotiana tabacum (Sasaki et al. 2003), Arabidopsis thaliana (Lutz and Maliga 2001), Gossypium hirsutum (Jiang et al. 2012), Triticum monococcum (Kumbhar et al. 2018), etc. However, the RNA editing events in Mesona chinensis Benth (MCB) chloroplast have not been investigated yet.

In this study, RNA editing sites in MCB chloroplast protein-coding genes were predicted using bioinformatics tools and 22 predicted sites (selected from accD, ndhB, ndhF and rpoB) were identified by PCR and RT–PCR, which suggested that 12 sites were experimentally confirmed (Table 2). Thus it was inferred that it was necessary to study the chloroplast RNA editing sites by bioinformatics method accompanied with sanger sequencing. In addition, all the 12 validated editing sites were C-to-U alterations and occurred at the first and second codon position and most of the validated sites (9 out of 12) belong to NAD(P)H dehydrogenase (ndh) coding genes. It was consistent with other higher plants (Tsuzuki et al. 2001).

RNA editing can alter the protein structures for appropriate functions and most of the unedited proteins have lower functional levels than the edited proteins (Sasaki et al. 2001; Hammani et al. 2011). In this study, the secondary structures of four selected genes before and after editing were further analyzed. All the editing events had a slight impact on the secondary structure composition and two transmembrane domains were created in codons 150th to 169th and 262th to 284th of ndhB (Figures 3 and 4). Moreover, all the 12 editing sites tended to increase the hydrophobicities of corresponding proteins, resulting in strengthened structures (Table 4). These suggested that the alterations in the protein structures might facilitate proper folding and thus increase the functional level. Additionally, comparing RNA editing sites in MCB and other 5 higher plants (Table 5), most of the sites were found to be conservative, which indicated that RNA editing cases in MCB primarily restore the conserved amino acid of homologous protein (Maier et al. 1995; Tsuzuki et al. 2001).

A great many RNA editing sites in higher plants have been experimentally characterized. In Arabidopsis thaliana,
editing at accD-265 was proved to be essential for acetyl-CoA carboxylase activity and chloroplast biogenesis (Sasaki and Nagano 2004; Yu et al. 2009). This site in pea also shared similar functions (Sasaki et al. 2001). Unediting at ndhB-494 and ndhF-97 led to lower stability and activity in NAD(P)H dehydrogenase complex (Hammani et al. 2009). Lack-}

ing editing at rpoA transcript and rpoB-113 was confirmed to reduce RPOA stability and RPOB activity, respectively (Chat-}

eigner-Boutin et al. 2008; Zhou et al. 2009). In Zea mays, ndhB-204 was edited in green seedlings but unedited in etio-

lated seedlings (Karcher and Bock 2002). Some of these editing sites are shared in the MCB chloroplast (Table 5),
}suggesting that they may play identical roles in the physiological and biochemical processes of MCB. More noteworthy
}are the absent editing sites in MCB chloroplast, such as accD-474, ndhB-419, ndhF-21, rpoB-113, rpoB-158, etc, they may undergo editing in specific conditions, just like cases in other species (Chat-}

eigner-Boutin and Hanson 2003; Miyata and Sugita 2004; Rodrigues et al. 2017), and their roles are needed to further explore.

RNA editing involved in environmental stimuli response has been extensively studied over years. Substantial numbers of studies demonstrated that editing efficiency changed under the influence of environmental stimuli. For example,

### Table 5. Comparative analysis of Mesona chinensis Benth chloroplast RNA editing sites with other higher plants.

| Gene | Amino acid position | Codon conversion | Mesona chinensis Benth | Arabidopsis thaliana | Nicotiana tabacum | Gossypium hirsutum | Aegilops tauschii | Pisum sativum |
|------|---------------------|------------------|-----------------------|---------------------|------------------|------------------|-----------------|--------------|
| accD | 265/267/271/287     | tCg(S)→tUg(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 474                 | cCt(P)→cUt(L)    |                       | +                   | +                | +                | +               | +            |
|      | 50                  | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 156                 | cCa(P)→cUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 181                 | aCg(T)→aUg(M)    |                       | +                   | +                | +                | +               | +            |
|      | 196                 | Cat(H)→Uat(Y)    |                       | +                   | +                | +                | +               | +            |
|      | 204                 | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 204                 | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 235                 | tCt(S)→tCt(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 246                 | tCa(S)→tCt(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 249                 | tCt(S)→tCt(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 291                 | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 419                 | Cat(H)→Uat(Y)    |                       | +                   | +                | +                | +               | +            |
|      | 494                 | cCa(P)→cUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 50                  | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 97                  | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 113                 | tCt(S)→tCt(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 158                 | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 184                 | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 189                 | tCg(S)→tCg(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 206                 | cCg(P)→cUg(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 667                 | tCt(S)→tCt(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 184                 | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |
|      | 809/811             | tCa(S)→tUa(L)    | +                     | +                   | +                | +                | +               | +            |

Note: ‘+’ indicates editing; The superscript numbers represent for the position of codon.

Figure 5. Transcript levels of four selected genes. (A) Blue and red light conditions. (B) Salt stress. Data represent means ± SE from three replicates. * and ** indicate significant difference at \( P < 0.05 \) and \( P < 0.01 \) probability, respectively.
exposure to light rapidly increased the editing efficiency of ndhD-674 in Arabidopsis thaliana (Tseng et al. 2013). Chu et al. found that the global C-to-U RNA editome has significantly reduced editing efficiency under heat stress when compared to the normal condition by analyzing the transcriptome and translatome data of normal and heat-shocked Arabidopsis thaliana (Chu and Wei 2020). In soybean, an increase in editing efficiency of ndhA, ndhB, rps14 and rps16 was observed in response to salt stress (Rodrigues et al. 2017). A previous study found that partial editing might regulate plastid gene expression by using a different editing frequency in the non-photosynthetic tissues of Arabidopsis (Tseng et al. 2013). It can be hypothesized that there may be a positive correlation between gene expression and editing frequency under environmental stimuli. Especially later He and Rodrigues et al. demonstrated that the expression level and editing frequency of ndhB were up-regulated in soybean under salt stress (He et al. 2015; Rodrigues et al. 2017). Similarly in our present study, the expression level of accD was significantly down-regulated and the editing frequency of accD-287 was found to decreased in response to salt stress (Figure 5(B), Figure 6). However in different light quality, even though the expression level of accD under red light obviously decreased compared with that under blue light (Figure 5(A)), both the editing frequencies of accD-287 under blue and red light conditions were 100%, demonstrating that the occurrence of accD-287 was irrelevant to the transcript level of accD under blue and red light (Figure 6). Similar result was observed in ndhB-494. These results also implied that accD-287 and ndhB-494 might be involved in response to salt stress. Taken together, the editing frequency was not positively relevant to the transcript level and there were different underlying mechanisms of RNA editing in response to diverse environmental stimuli.

5. Conclusions

In this study, RNA editing sites in Mesona chinensis Benth chloroplast protein-coding genes were predicted using bioinformatics tools and 12 sites from accD, ndhB, ndhF and rpoB were experimentally validated. The secondary structure composition slightly changed and the hydrophobicity was improved, implying that alterations in the protein structures facilitated proper folding and thus increased the functional level. The RNA editing patterns of MCB chloroplast genome were compared with those of the other plants, indicating that most of the editing sites were conservative. Two RNA editing sites accD-287 and ndhB-494 might be involved in response to salt stress. The editing frequency was not positively relevant to the transcript level and there might be different underlying mechanisms of RNA editing in response to diverse environmental stimuli in the MCB chloroplast. This study laid a foundation for further exploring the biological function and mechanism of RNA editing in the MCB chloroplast and provided a reference for the study of chloroplast RNA editing in other medicinal plants.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Danfeng Tang, Shiming Chen, and Jianhua Miao conceived and designed the experiments; Changqian Quan, Fan Wei and Suhua Huang performed the experiments and wrote the article; Kunhua Wei provided support and experimental guidance for this study. All authors have read and approved the published version of the manuscript.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author on reasonable request.

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