Dynamic landscape of mitochondrial Cytidine-to-Uridine RNA editing in tobacco (Nicotiana tabacum) shows its tissue specificity

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Abstract
RNA editing is a prevalent nucleotide modification at the RNA level in higher plants. However, little is known about the dynamic distribution of RNA editing among tissues. In this study, we explored the tissue specificity of mitochondrial RNA editing in tobacco (Nicotiana tabacum) based on publicly available RNA-seq data from four tobacco tissues: root, stem, leaf, and flower. As a result, 473 RNA editing sites involved in 60 mitochondrial genes were identified. The results showed an uneven distribution of editing sites among tobacco tissues, a total of 106 sites and 11 genes were identified as tissue-specific editing in the four tissues, and a total of 11 sites located in six genes were detected differentially edited statistically (p-value < 0.01). The expression of RNA edited genes and RNA editing factors was analyzed, and we observed that most tissue-specific edited genes were expressed at a low level. There were about ~ 20 RNA editing factors that were differentially expressed between different tissues, indicating that the heterogeneity of RNA editing in different tissues might result from the expression regulation of RNA editing factors. Our analyses provide insights into the understanding of landscape, regulation, and function of RNA editing events in higher plants.

Key message
Dynamic landscape of conserved editing sites revealed the tissue specificity of mitochondrial RNA editing in tobacco.

Keywords RNA editing · Mitochondria · PPR · Tissue specificity

Abbreviations
C-to-U Cytidines substituting uridines
PPR Pentatricopeptide repeat
RNA-seq RNA sequencing
SNPs Single nucleotide polymorphisms
WGS Whole genome re-sequencing
cox1 Cytochrome oxidase subunit 1
nad NADH dehydrogenase
MORF Multiple organelle RNA editing factors
rps Ribosomal protein gene
rps14 Ribosomal protein S14
ccmB Cytochrome c maturation gene

Introduction
RNA editing is defined as the insertion, deletion, and replacement of nucleotide bases that occurs after transcription, which usually results in differences between the RNA genetic information and the genome template (Liscovitch-Brauer et al. 2017; Walkley and Li 2017; Zahn 2017; Peng et al. 2018). The specific RNA positions affected by RNA editing and the corresponding DNA positions are called editing sites (Edera et al. 2018a). For instance, the transcript of maize chloroplast gene 50S ribosomal protein L2 (rpl2) can only create the initiation codons after uridine replacement of cytidine (Hoch et al. 1991). In 1986, Benne et al.
biologists discovered an RNA editing event in the mitochondrial gene cytochrome c oxidase subunit II (cox2) of trypanosomes firstly (Benne et al. 1986), they found that the insertion of four uridines caused the cox2 gene to form a continuous open reading frame, resulting in changes in genetic information. In 1989, Hiesel et al. revealed that RNA editing also exists in primrose mitochondria (Hiesel et al. 1989). Subsequently, increasing RNA editing events were confirmed, to date, RNA editing has been found in primitive eukaryotes, vertebrates, plants, fungi and viruses (Palladino et al. 2000; Bahn et al. 2012; Alon et al. 2015; Guo et al. 2015; Riemondy et al. 2018; Zaidan et al. 2018).

Numerous studies have shown that RNA editing alters the genetic information of the genome and enriches the expression products of genes; on the other hand, it provides new genetic structures and functions for the evolution of organisms (Small et al. 2020; Lukes et al. 2021). For mammals, the regulation of RNA editing events has been widely studied. For example, RNA editing in mammals has been proved to be a dynamic landscape across tissues and additional potential editing factors have been discovered to be involved in the editing events (Tan et al. 2017; Blanc et al. 2019). In the case of higher plants, RNA editing occurs mainly in the coding regions of mitochondrial and chloroplast genes, where the most common types of editing is cytidines substituting uridines (C-to-U) (Covello and Gray 1989; Tillich et al. 2006; Takenaka et al. 2013). So far, in higher plants, the total number of RNA editing sites in the chloroplast genome (20–100) is much less than that in the mitochondrial genome (300–600) (Chen et al. 2017; Edera et al. 2018a; Brenner et al. 2019; Nawae et al. 2020).

RNA editing in the plant is mainly mediated by editing complex involving editing factors such as Pentapeptide repeat (PPR) proteins and multiple organellar RNA editing factors (MORF) (Haag et al. 2017; Tian et al. 2019). Editing factors recognize a 20–25 nucleotide sequence upstream of the editing site, and the editing factors required for different RNA editing sites may differ (Yagi et al. 2014; Yan et al. 2018). The PPR protein is the largest class of RNA editing factors in plants, which is encoded by nuclear genes and located in mitochondrion and/or chloroplast, playing a role of site-specific recognition in editing (Yagi et al. 2013, 2014; Ichinose and Sugita 2017). PPR protein has two sub-families, P-type and PL5-type, the P-type consists of the 35-amino acid classic PPR (P) motif, while the PL5-type consists of the classic P-motif and its variants L (35 or 36 amino acids) and S (31 amino acids) (Manna 2015). A plant-specific conserved E domain often exists at the C-terminus of the PL5-type PPR protein. Generally, in plant organelles, it is the PL5-type PPR protein that recognizes the specific editing sites (Shikanai 2015; Yan et al. 2018). MORF is a small protein family, with 10 members in Arabidopsis, and MORF mutants exhibit reduced efficiency at multiple sites (Takenaka et al. 2012). However, the target relationship of editing factors catalyzing RNA editing sites is still unclear.

Previous studies have revealed that RNA editing is specific in terms of tissues and developmental stages, suggesting that RNA editing may function as a regulatory device in plastid gene expression (Bock et al. 1993; Zeltz et al. 1993; Miyata and Sugita 2004). Tissue-specific and development-specific RNA editing were detected in the bryophyte ribosomal protein S14 (rps14) gene in previous studies (Miyata and Sugita 2004). Tseng also observed that the RNA editing efficiency of Arabidopsis plastid mRNA was variable among tissues (Tseng et al. 2013). Actually, earlier researches on RNA editing mainly focused on selected genes based on experimental methods with a disadvantage of low throughput. With the rapid improvement of genomic and transcriptome sequencing technology, a large number of plant genomes have been sequenced and numerous RNA sequencing (RNA-seq) data have also been generated, offering an opportunity to test the function and regulatory mechanism of RNA editing in plant growth and development. In the future, the regulation and function of RNA editing in plants and their effects on traits, especially some essential agronomic traits, will attract more people’s attention (Small et al. 2020).

As a model plant, tobacco (Nicotiana tabacum) plays a key role in plant molecular research and is also an economically important plant worldwide. In this study, to illustrate the tissue distribution characteristics of RNA editing in the higher plant, we identified and analyzed RNA editing sites in four tobacco tissues (root, stem, leaf and flower) based on transcriptome data. And then, the dynamic landscape of RNA editing sites was analyzed, we discovered that editing sites were distributed differently in the four tissues, among which the root had the least editing sites, which might be related to the physiological function requirements for the root. Simultaneously, we also analyzed the expression levels of RNA editing factors in different tissues, its heterogeneity of expression levels might partly explain the varied RNA editing events happening in different tissues. Our findings showed that RNA editing was differentially regulated in various types of tissues, and may contribute to the functional differentiation of tobacco tissues.

Materials and methods

Data collection and pre-treatment

The transcriptome data of four tissues (root, stem, leaf, and flower) and whole genome re-sequencing (WGS) data from Nicotiana tabacum cultivar TN90 (common tobacco) were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA208209), each sample consists of three replicates (Sierro et al. 2014), detailed information
was listed in Table S1. In addition, the genome (assembly: GCF_000715135.1) with mitochondrial sequences (accession number: NC_006581.1) of *Nicotiana tabacum* cultivar Ntab-TN90, as well as their annotation files were also downloaded from NCBI data repository (https://www.ncbi.nlm.nih.gov) (Sugiyama et al. 2005). We utilized the FastQC v0.11.8 tool to check the quality of the transcriptome data firstly (Brown et al. 2017). To increase sequencing depth, we merged the three duplicates from each tissue into one sample. The transcriptome data were mapped to mitochondrial genome reference using HISAT2 v2.1.0 software with default parameters (Kim et al. 2015). Afterward, each SAM file was converted into a BAM file, sorted and duplicates-removed with SAMtools v1.9 (Li et al. 2009; Danecek et al. 2021). The variant calling process was conducted by SAMtools ‘mpileup’ command, the single nucleotide polymorphisms (SNPs) were identified by BCFtools ‘call’ command (Danecek and McCarthy 2017).

### Identification of RNA editing sites

Based on the SNP results and genome annotation files, RNA editing sites were identified by using the REDO tool (Wu et al. 2018). REDO identifies RNA editing events in plant organelles and adopts a series of stringent rule-dependent filters to evaluate the reliability of editing sites (Wu et al. 2018), such as (1) quality control filter: the low-quality sites are filtered based on reads quality; (2) mapping depth filter: sites with total reads depth more than 4 are kept; (3) alt proportion filter: alt_proportion-error_proportion] <0.1; (4) multiple alt filter: only the variant with one alt allele is retained for RNA editing detection; (5) distance filter: given the possible positional interference for RNA editing, the variant sites in short distance (< 3 bp) are filtered out; (6) spliced junction filter: variants within short spliced anchor (< 2) are removed; (7) indel filter: the indel variants are excluded by default; (8) likelihood ratio (LLR) test filter (LLR < 10): LLR test is a probabilistic test incorporating error probability of bases (error probability is obtained using adjacent nonvariant sites in specific window) for detecting RNA editing sites (Chepelev 2012; Sun et al. 2016); (9) Fisher’s exact test filter (p-value < 0.01): the significance of the RNA editing site is evaluated by using Fisher exact test to compare the expected level of itself, and the p value is used for filter; (10) complicated filter model: according to the statistical results of the RNA editing sites that have been experimentally verified and the attributes of the codon table, the complicated filter model consists of five characteristics of RNA editing sites, which are RNA editing types (C→T, A→G, T→C, etc), alt proportion, amino acids change, codon phase, and hydrophobic/hydrophilic change (Wu et al. 2018). Finally, the corresponding annotation information files for all identified RNA editing sites were generated. To improve the accuracy of RNA editing sites filtering, genome SNP-calling result was further used to eliminate genomic variation. Prediction of RNA editing events using by PREPACT (Lenz et al. 2018) was further compared with the above results to test the reliability of the final editing sites.

### Characteristic statistics and comparison of RNA editing sites

For each tissue, the resulted editing sites were used for further characterization, including statistics on the number of editing, editing types, editing efficiency, codon positions, amino acid changes, and involved genes. The nucleotide substitution types of total editing sites were counted. For the triplet codon, we calculated the substitutions of base at each position and counted the editing events separately. In addition, according to codon functional traits, we counted the number of start codons and termination codons and analyzed their changes at the amino acid level. For one site, the value of RNA editing efficiency was expressed as the proportion between edited transcripts and total transcripts, if the site was edited, the C/G base (wild type) should be altered to the T/A base (edited type), since one editing site could be detected hundreds of times via RNA sequencing, the number of wild type (C/G) or edited type (T/A) of bases could then be counted at this particular site, hence, the editing efficiency at one site could then be calculated by the formula: depth of edited bases (T and A)/total read depth of bases. Furthermore, we compared the RNA editing efficiency between every two tissues, RNA editing sites with statistical significance (p-value < 0.01) were identified. Aiming to decipher the tendency of RNA editing efficiency for each tissue, cluster analysis was performed based on the matrix of RNA editing efficiency, which was normalized by subtracting the row-wise mean from the values in each row of data and multiplying all values in each row of data by standard deviation value documented in our previous study (Zhang et al. 2020). ‘Pheatmap’ function in R (http://www.R-project.org/) was used to plot the heatmap of editing efficiency, ‘dist’ function was used to calculate the distance matrix of different samples with the default ‘euclidean’ method, ‘hclust’ function was used to compute the hierarchical clustering.

### Expression analysis

Furthermore, to measure and compare the gene expression level of RNA edited genes and RNA editing factors, we also performed transcriptome analysis as described in the previous study for the RNA-seq data (Pertea et al. 2016). Two main types of RNA editing factors, including PPR proteins and MORF proteins, were chosen to explore their expression...
in RNA editing activity. To obtain all tobacco PPR and MORF genes, two representative protein sequences (UniProtKB ID: Q9SAD9, O49429) from Arabidopsis thaliana were used as queries to search against the tobacco protein databases using BLASTP v2.10.1 (http://blast.ncbi.nlm.nih.gov). All positive hits were retrieved for gene function annotation to blast against the Swiss-Prot protein database.

The clean reads of RNA-seq data from each sample were mapped against the tobacco genome reference with HISAT2 v2.1.0 (Kim et al. 2015), each SAM file was converted into a BAM file, and sorted, duplicates-removed with SAMtools v1.9 (Li et al. 2009; Danecek et al. 2011). Further transcript assembly and quantification of the read alignments were performed using Stringtie v1.3.3b (Pertea et al. 2015). Gene expression levels were measured using FPKM (fragments per kilobase of transcript per million mapped reads), expression values were also normalized by the method mentioned above. EdgeR package (Robinson et al. 2010) was used to determine the differentially expressed genes between every two tissues. A heatmap with all samples was plotted using ‘pheatmap’ function in R (v.3.3.1) environment.

Statistical analysis

A two-tailed Wilcoxon rank-sum test was used to perform the pairwise comparison of RNA editing efficiency between every two tissues. As for the pairwise comparison for each editing site, Fisher’s exact test was used.

Results

Alignment of transcriptome data

A total of 12 RNA-seq extracted from four tobacco tissues (root, stem, leaf, and flower) and one WGS samples from PRJNA208209 were used in our study. There are three replicates for each tissue. We merged the replicates of each tissue and aligned the transcriptome data against the tobacco mitochondrial genome as reference. The tobacco mitochondrial genome used in our study is 430,597 bp in size and has 116 Open Reading Frames (ORFs). To avoid data bias in sequencing depth that might affect the comparability among four tissues, we unified the number of reads in four tissues RNA-seq data, then a total of 130,415,792 reads were selected randomly for transcriptome alignment. At last, there were 56,774, 81,378, 125,616 and 92,165 reads in the root, stem, leaf, and flower tissues mapped onto the tobacco mitochondrial genome, with corresponding overall alignment rate was about 0.04%, 0.06%, 0.09% and 0.07% respectively (Table 1). The alignment results of transcriptome data revealed that roots have a lower mapping rate, one reason is that genes were expressed at a lower level in root, the other reason is that variation in tissue composition, like the mitochondrial density, may affect the RNA source of different tissue sample. We observed that the average mapping depths were around 20× that meets the minimum requirement of identification of editing sites.

Characteristic statistics of tobacco mitochondrial RNA editing

We used REDO tool that is an automated approach based on multiple layers of filtering to detect RNA editing sites, therefore, for merged RNA-seq data from four tobacco tissues, a total of 487 raw editing sites were detected. To improve the accuracy of RNA editing sites filtering, genome SNP-calling result was further used to eliminate genomic variation, 14 sites were both detected in RNA and genome level with the same substitution type, we excluded them as genomic variations. After that, we also inspected the remaining editing sites manually to ensure the reliability of results. Finally, a total 473 editing mitochondrial RNA editing sites were predicted that located in 60 mitochondrial genes in four tobacco tissues, However, we only detected RNA editing sites that occurring within mitochondria genes, and excluded the sites occurring within non-coding regions, hence, the number of RNA overall editing sites is lower than that of a previous work (Grimes et al. 2014). We also compared our results with that predicted by PREPACT method (http://www.prepact.de/prepact-main.php) (Lenz and Knoop 2013), the resulting number and distribution agrees with that of our results, see Table S2, hence, we deemed that the identification results from the REDO tool in our study were reliable.

The statistics of RNA editing types revealed that C-to-U is the most common editing type, accounting for more than

![Table 1 The SRA accession numbers for RNA-seq data from four tissues of Nicotiana tabacum](image-url)
There were also other substitution types, such as U-to-C, which was recently assumed to be RNA editing type in *Arabidopsis thaliana* (Ruchika et al. 2021), other mismatches might be the result of sequencing errors and double transcription of nuclear genes and mitochondrial genes (Edera et al. 2018a). Detailed information on editing sites is listed in Table S3. The average numbers of editing sites varied widely among genes. On average, *NADH dehydrogenase subunit 7* (*nad7*) gene had the largest number of editing sites, approximately 17, followed by 16 editing sites found in *cytochrome oxidase subunit 1* (*cox1*) gene. Out of the 473 identified editing sites, 143 (30.23%), 281 (59.41%), and 48 (10.15%) were located in first, second, and third codon positions respectively. It means that RNA editing events occurred preferentially on the second codon and followed by the first codon (Figure 1B). The statistics of editing type showed that there were far more editing events of non-synonymous mutations than synonymous mutations, with non-synonymous editing events up to 88%, as shown in Table S3. In addition, the editing level of non-synonymous mutations was significantly higher than that of synonymous mutations (Figure 1C), indicating that non-synonymous C-to-U editing was favored by natural selection. In addition, two sites located in *cox1* and *ribosomal protein S10* (*rps10*) gene were detected to produce functional start codon, another five sites were found to produce premature

**Fig. 1** RNA editing in the tobacco (*Nicotiana tabacum*) mitochondrial genome. **A** The nucleotide substitution types in tobacco mitochondrion. The C-to-U RNA editing sites account for more than 95% of all sites. **B** Distribution of mitochondrial RNA editing sites in codon phases. **C** Mitochondrial RNA editing efficiency of non-synonymous (419) and synonymous (54) editing events. Discrete points are indicated in red. Silent, synonymous editing; Non-Silent, non-synonymous editing. **D** The changes of amino acid types in *Nicotiana tabacum*. 101 (21.35%) out of 473 diversifications were Ser-to-Leu changes. (Color figure online)
termination codon, see Table 2. Edits at \textit{cox1}_2 and \textit{rps10}_2 were found in all tissues, and the average editing efficiency was 87.2% and 94.2%, respectively. Sugiyama et al. similarly reported that the genomic ACG codon in the \textit{rps10} gene was changed to the start ATG codon by RNA editing, but the same change was not reported in \textit{cox1} gene (Sugiyama et al. 2005). The edited positions of five premature termination codons were all located at the first position of codon, the editing efficiency ranges from 17.3% to 97%, four out of five codons were translated into glutamine before editing. Most of the amino acid changes tended to be hydrophobic, the most amino acid changes were Ser-to-Leu (21.35%) and Pro-to-Leu (18%), see Figure 1D, these changes of hydrophobicity could greatly restore the conservation of proteins and were essential for maintaining normal protein function.

The proportion of hydrophobic amino acids in the root, stem, leaf, and flower issues were 40.98%, 37.38%, 41.67%, and 81.97 %, 80.84%, 81.67%, and 81.9% respectively (Supplementary Figure S1). For RNA editing efficiency, the average is 80%, ranging from 10 to 100% (see Figure S2).

**Tissue-specific analysis of tobacco mitochondrial RNA editing**

For the four tissues (root, stem, leaf, and flower), the number of RNA editing sites identified in mitochondrial genome corresponded to 204, 294, 234, and 343, involving 43, 45, 45 and 54 genes respectively, as shown in Table S3, more editing sites were detected in flower, whereas root had the least number. As for RNA editing efficiency, the RNA editing efficiency of root, stem, leaf, and flower corresponds to 85.6%, 88.5%, 89% and 86.6% respectively (Table S1), leaf had the highest editing efficiency, followed by stem, flower, and root. Clustering and heatmap plotting was also performed based on the normalized matrix of RNA editing efficiency, as is shown in Figure 2, root clustered far away from other tissues. These results demonstrated an uneven distribution and dynamic landscape among different tissues, for example, compared with other tissues, root had not only the least number of editing sites but also the lowest editing efficiency. The venn diagram analysis (Figure 3) showed that there were 131 common editing sites and 34 edited genes shared in all four tissues, considerable editing sites were only detected in certain tissues, the tissue-specific editing sites in root, stem, leaf, and flower correspond to 16, 18, 13, 59 respectively, more sites were specifically edited in flower. Detailed information on tissue-specific editing sites is listed in Table S5. For example (Figure 4), RNA editing in \textit{cob}_114 (genome position: 41934) occurred in all the tissues except root; \textit{matR}_326 (genome position: 5687) was only edited in leaf tissue. As for tissue-specific edited genes, a total of 11 genes, containing 14 editing sites, were specifically edited in certain one tissue, including one gene (\textit{orf105}) in root, two genes (\textit{rrn26} (tRNA), \textit{orf306}) in stem, one gene (\textit{succinate dehydrogenase subunit 3 (sdh3)}) in leaf, and seven genes (\textit{30S ribosomal gene (rps14), orf121a, orf158, orf101a, orf274, cytochrome c maturation genes (ccmB)}) in flower, which owned more tissue-specific edited genes (Table 3).

We found that all these genes contained only one or two editing sites, nearly all the sites were located in the second position of codons except two sites (\textit{orf306}_242, \textit{orf121a}_327). In the root, both editing sites of \textit{orf105} genes did not change the amino acid type, however, there were still more sites that altered the amino acid type through RNA editing, such as \textit{orf306}_242, \textit{rrn26}_305, \textit{ccmB}_204, and so on. In addition, these editing sites demonstrated a high editing efficiency except for two sites (\textit{orf306}_242, \textit{rrn26}_295). The above results revealed that the editing events are tissue-specific, it seems editing is more active in flower and weaker in the root, however, the vast majority of genes were conservative and edited in at least two tissues, tissue-specific edited genes were mostly \textit{orf} genes whose functions remain unclear.

**Changes in RNA editing efficiency among different tobacco tissues**

Despite tissue-specific editing, we also observed that a batch of sites demonstrated varied editing efficiency among different tissues, some of them were remarkably reduced or increased in certain tissues. Pairwise comparisons of editing efficiency between any two tissues

| Name  | Genome_pos | Gene_pos | AA_pos | Phase | Ref→Alt | RefCodon→AltCodon | RefAA→AltAA | AltRatio (%) |
|-------|-------------|----------|--------|-------|---------|-------------------|-------------|--------------|
| \textit{cox1} | 130,286 | 2 | 1 | 2 | C→T | ACG→ATG | T→M | 87.2 |
| \textit{rps10} | 131,586 | 2 | 1 | 2 | C→T | ACG→ATG | T→M | 94.2 |
| \textit{rpl16} | 155,606 | 37 | 13 | 1 | C→T | CAG→TAG | Q→* | 84.7 |
| \textit{atp9} | 145,769 | 223 | 75 | 1 | C→T | CAA→TAA | Q→* | 89.8 |
| \textit{rrn26} | 130,483 | 331 | 111 | 1 | C→T | CGA→TGA | R→* | 97 |
| \textit{matR} | 5613 | 400 | 134 | 1 | C→T | CAG→TAG | Q→* | 36.2 |
| \textit{nad5} | 193,902 | 910 | 304 | 1 | C→T | CAG→TAG | Q→* | 17.3 |
were conducted, a total of 11 sites located in six genes were detected differentially edited statistically ($p$-value < 0.01), see Table 4. The six genes consisted of three ribosomal genes ($\text{rps10}$, $\text{rpl16}$, $\text{rps4}$), one hypothetical gene ($\text{orf125e}$), and two other functional genes ($\text{atp9}$, $\text{nad5}$). One site of ribosomal protein S10 ($\text{rps10}_2$) was nearly completely edited in stem, leaf and flower, but reduced to 69.2% in root, in addition, another site in ribosomal protein L16 ($\text{rpl16}_488$) was completely edited in the stem, but reduced to 54.5%, 77.3%, and 82% in leaf, root, flower respectively; another one site of $\text{ATP synthase F0 subunit 9}$ ($\text{atp9}_223$) in root and flower corresponds to

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**Fig. 2** RNA editing efficiency in four tissues of *Nicotiana tabacum* mitochondrial genome. A Heatmap of RNA editing efficiency in four tissues of *Nicotiana tabacum* mitochondrial genome. The $x$ axis represents different samples, and the $y$ axis represents RNA editing sites.

**Fig. 3** Venn diagram demonstration of tissue specificity of C-to-U RNA editing sites and genes in *Nicotiana tabacum* mitochondrial. A Venn diagram demonstration of tissues specificity of C-to-U RNA editing sites. B Venn diagram demonstration of genes with tissue-specific RNA editing sites.
97.5% and 100%, but reduced to 78.9% and 69.2% in leaf and stem respectively. Remarkably, two sites in \textit{orf125e} (\textit{orf125e}\textsubscript{153}, \textit{orf125e}\textsubscript{192}) demonstrated an opposite trend of change, compared with the other three tissues, \textit{orf125e}\textsubscript{153} was up-edited in leaf, whereas \textit{orf125e}\textsubscript{192} was down-edited, indicating that the heterogeneity and complexity of editing not only occurred in different tissues, but also in different sites of the same gene. Detailed information on these editing sites can be found in Table S6.

\textbf{Fig. 4} Snapshot of alignment of reads from four tissues against mitochondrial genome for two editing sites in \textit{Nicotiana tabacum}. The emphasized sites were indicated by a black dotted box. A Snapshot of editing site \textit{cob}\textsubscript{114} (genome position: 41934) in four tissues of \textit{Nicotiana tabacum}, demonstrating that this site was edited in all the four tissue except root. B Snapshot of editing site \textit{matR}\textsubscript{326} (genome position: 5687) in four tissues of \textit{Nicotiana tabacum}, demonstrating that this site was only edited in leaf tissue.
Expression analysis of RNA edited genes in different tissues

To investigate the reason for RNA editing variation in different tissues, we examined the expression of RNA edited genes and RNA editing factors. For the 60 RNA edited genes, we observed that most of the RNA edited genes (54 out of 60) were expressed in all tissues with no bias, however, the overall expression level was highest in stem, but lowest in root (Figure 5A). There were only six genes that were not expressed in certain tissues, including four orf genes (orf132, orf158, orf190, orf111), one tRNA gene (trnD(guc)), and cytochrome c maturation gene (ccmFc). In terms of the 11 tissue-specific edited genes, we observed that they were nearly expressed in all tissues at a low level except rrn26 and rps14 (Figure 5B), although some genes were expressed at a low level compared with that of other tissues, they were still only edited in this tissue.

### Table 3 Information of 11 tissue-specific edited genes in tobacco mitochondrion

| Tissue | Gene  | Genome_pos | Gene_pos | AA_pos | Phase | RefAA→AltAA | AltRatio (%) | Gene function |
|--------|-------|-------------|----------|--------|-------|-------------|--------------|--------------|
| Stem   | orf105| 373,240     | 184      | 62     | 1     | L→L        | 100          | Hypothetical protein |
| Stem   | orf105| 373,242     | 186      | 62     | 3     | L→L        | 100          | Hypothetical protein |
| Leaf   | orf306| 215,608     | 242      | 81     | 2     | S→L        | 51.6         | Hypothetical protein |
| Leaf   | rrn26 | 69,809      | 2957     | /      | /     | /           | 50           | rRNA |
| Leaf   | sdh3  | 77,451      | 74       | 25     | 2     | S→F        | 100          | Succinate dehydrogenase subunit 3 |
| Flower | rps14 | 43,397      | 305      | 102    | 2     | T→I        | 92.3         | Ribosomal protein S14 |
| Flower | orf121a| 43,749    | 327      | 109    | 3     | V→V       | 75           | Hypothetical protein |
| Flower | orf158| 139,624     | 208      | 70     | 1     | R→C       | 100          | Hypothetical protein |
| Flower | orf158| 139,649     | 233      | 78     | 2     | S→F       | 100          | Hypothetical protein |
| Flower | orf111| 150,632     | 86       | 29     | 2     | D→V       | 85.7         | Hypothetical protein |
| Flower | orf111| 150,706     | 160      | 54     | 1     | V→I       | 83.3         | Hypothetical protein |
| Flower | orf101a| 150,706   | 121      | 41     | 1     | P→S       | 83.3         | Hypothetical protein |
| Flower | orf1274| 260,636   | 226      | 76     | 1     | T→A       | 100          | Hypothetical protein |
| Flower | ccmB  | 359,983     | 611      | 204    | 2     | S→L       | 90.9         | Cytochrome c maturation protein CcmB |

### Table 4 Differentially RNA editing sites between each two tissue in tobacco mitochondrion

| Gene  | Tissues     | Proportion_1 (%) | Proportion_2 (%) | Fold (log2) | P-value |
|-------|-------------|------------------|------------------|-------------|---------|
| rps10 | Root vs stem| 69.2             | 100              | 0.53        | 0.004009|
| atp9 (ATP synthase F0 subunit 9) | Leaf vs flower | 78.9             | 100              | 0.34        | 0.006948|
|       | Root vs stem| 97.5             | 69.2             | −0.49       | 0.001773|
|       | Stem vs flower| 69.2         | 100              | 0.53        | 0.000187|
|       | 191          | Leaf vs root     | 66.7             | 100         | 0.58     | 0.007966|
|       | 20           | Root vs stem     | 100              | 91.1        | −0.13    | 0.006911|
| rpl16 (ribosomal protein L16) | 488          | Leaf vs stem     | 54.5             | 100         | 0.87     | 0.000301|
|       | Root vs stem| 77.3             | 100              | 0.37        | 0.005747|
|       | Stem vs flower| 100          | 82               | −0.29       | 0.008943|
| nad5 (NADH dehydrogenase subunit 5) | 539          | Stem vs flower   | 95.2             | 57.7        | −0.72    | 0.001711|
|       | 608          | Leaf vs flower   | 100              | 55.3        | −0.85    | 0.002249|
|       | 629          | Leaf vs flower   | 94.7             | 43.1        | −1.13    | 7.98E-05|
| orf125e | 153         | Leaf vs flower   | 57               | 28          | −1.03    | 3.26E-06|
|       | Leaf vs root| 57               | 31.9             | −0.84       | 0.00667 |
|       | Leaf vs stem| 57               | 32.1             | −0.83       | 0.000116|
|       | 192          | Leaf vs flower   | 70.4             | 86.1        | 0.29     | 0.000353|
|       | Leaf vs root| 70.4             | 90.3             | 0.36        | 0.002421|
|       | Leaf vs stem| 70.4             | 84.8             | 0.27        | 0.002654|
| rps4 (ribosomal protein S4) | 1004         | Stem vs flower   | 100              | 61.1        | −0.71    | 0.001416|
orf105 gene, which was highly expressed in stem, but only edited in root. Pairwise differential expression analysis was conducted for these RNA edited genes, a total of 16 genes were differentially expressed (Table S7), several of them were tissue-specific edited genes or contained differentially editing sites, such as orf306, rps14, rps4, nad5, orf125e, however, no associations between them was detected. One striking example is rps14 gene, which showed vastly different expression levels between tissues, highest in leaf, lowest in root, but only edited in flower. The above observation suggested that although both expression and editing of transcripts are tissue specifically regulated in different tissues, however, there was no causality between these two events, different types of tissues may generate different kinds of signals to affect RNA editing and expression of organelles genes separately. Our observations also agree with our previous study about the dynamic response of RNA editing to heat stress in grape, no expression difference of RNA edited genes were detected under different temperatures.

Expression analysis of RNA editing factors in different tissues

To investigate the correlation between RNA editing pattern and editing factors or their interacting proteins, we also evaluated the expression of RNA editing factors, including PPR and MORF proteins, which function as catalytic machines and play key roles in RNA editing process (Yan et al. 2018). After blast searching, a total of 17 MORF and 170 PPR proteins were identified in tobacco. We found that approximately 20 RNA editing factors were differentially expressed between leaf and other three tissues, whereas only 10 RNA editing factors were differentially expressed between tissues of flower, stem and root, see Table S8. Based on the normalized expression matrix of all RNA editing factors and differentially expressed factors, heatmap plottings were performed, as is shown in Figure 6, Figure S3, which demonstrated an uneven distribution among different tissues. A batch of RNA editing factors was highly expressed in leaf significantly, we found a large proportion (8 out of 17) of MORF proteins were differentially expressed, five of them were highly expressed in leaf, the other two morf genes were highly expressed in leaf and flower, including multiple organellar RNA editing factor 2 (morf2, gene symbol: LOC107777961) and multiple organellar RNA editing factor 3 (morf3, gene symbol: LOC107760068). However, there were still five genes that were expressed in leaf at a low level, but highly expressed in root, like gene LOC107787019, LOC107782030. MORF protein family were once considered to be essential components of plant editosomes, a recent study has proved that RNA editing events in rosette leaves and flowers were reduced by morf9 mutation (Tian et al. 2019). Hence, the above results suggested that these RNA editing factors also exhibited tissue-specific expression, especially morf
genes, which may partly explain the reason for the varied RNA editing events among different tissues.

Discussion

As an important epigenetic mechanism that modified genome-encoded transcripts, RNA editing diversifies genomically encoded information to expand the complexity of the transcriptome (Takenaka et al. 2013). Previous studies revealed that RNA editing has various biological functions, including promoting RNA splicing by affecting the intron structures, and playing a central role in plant development and evolutionary adaptation (Ichinose and Sugita 2017; Tang and Luo 2018). If the proteins produced by edited and unedited transcripts had different functions, it would be beneficial for plants to have regulated RNA editing. Hence, to obtain an overview of the distribution of RNA editing in the model plant tobacco, we used the transcriptome data and performed bioinformatics analysis to examine the editing profiles of RNA editing and gene expression in root, stem, leaf, and flower tissue. A total of 473 RNA editing sites in 60 mitochondrial genes were identified. The tissue specificity of RNA editing was examined, we found 106 tissue-specific RNA editing sites and 11 tissue-specific edited genes in the four tissues. Despite tissue-specific editing, we also observed that a batch of sites demonstrated varied editing efficiency among different tissues. Our results demonstrated the fact of heterogeneity of RNA editing patterns among different tissues. However, the tissue-specific edited genes were mostly expressed at a low level, no associations were detected between their expression and editing. Oppositely, considering that the differentially expressed RNA editing factors between tissues, we proposed that the tissue-specific RNA editing may be mediated by them via expression regulation.

Compared to the other three tissues, we also found that root had not only the least number of editing sites (204), but also the lowest average editing efficiency, although we observed that nearly all the RNA edited genes were expressed in four tissues and the overall expression level was the lowest in root (Figure 5A). Hence, transcript abundance might be an important limiting factor in identifying editing sites and the increase of sequencing depth of RNA-seq will effectively avoid loss of editing sites. However, given the observation that a few sites occurred in low-level expressed genes, we still consider that they were tissue-specific edited and irrelevant to expression. Although the overall average editing efficiency was presented at a high level (~ 80%), certain sites still had varied editing efficiency among different tissues. For example, the editing efficiency of rps10_2 in root was 0.69, whereas it was nearly fully edited in the other tissues (≥0.95), as shown in Table S4. Compared with root, the other three tissues had more editing events and higher editing efficiency on the main respiratory chain (consisting of complexes I, III and IV), indicating that root tissues may differ from other tissues in terms of energy requirements. Root is an underground organ, functions in fixation of plant to the soil and absorption of water, it does not perform photosynthesis, and simultaneously lose the necessity of correction of normal functions of certain genes. Moreover, compared with root, transcripts of ribosomal genes and maturase-related...
cytochrome oxidase subunit genes. Conservative editing sites that are highly edited, up to 0.9, in all tissues, such as cytochrome oxidase subunit genes (cox1, cox2, cox3), play key roles in the respiratory electron transport chain, their stable and high editing ensures their edited proteins are homogeneous in assembling into functional complexes that are equally important for any tissues.

The current hypothesis is that several types of RNA editing factors constitute an editosome to catalyze the C-to-U conversion (Schmitz-Linneweber and Small 2008). MORF proteins were demonstrated to affect RNA editing event, and they were necessary for seedling survival in rice and seed development in maize (Liu et al. 2020; Zhang et al. 2021). Therefore, it is probable that RNA editing is regulated by the expression of editing factors and their interacting proteins, such as PPR, MORF proteins. In our study, we also observed that RNA editing factors demonstrated tissue-specific expression pattern, especially morf genes, which may partly explain the reason for tissue specificity of RNA editing. However, more ppr genes were highly expressed in leaf, but not flower, indicating that the regulation relationship between RNA editing factors and editing sites were not one-to-one, a striking feature of the plant RNA editosome is its diversity and complexity in the composition without consistency between individual RNA targets, hence, further studies are still needed to support this points.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

References

Alon S, Garrett SC, Levanon EY, Olson S, Graveley BR, Rosenthal JJ, Eisenberg E (2015) The majority of transcripts in the squid nervous system are extensively recoded by A-to-I RNA editing. Elife. https://doi.org/10.7554/eLife.05198
Bahn JH, Lee JH, Li G, Greer C, Peng G, Xiao X (2012) Accurate identification of A-to-I RNA editing in human by transcriptome sequencing. Genome Res 22(1):142–150
Benne R, Van den Burg J, Brakenhoff JP, Sloof P, Van Boom JH, Tromp MC (1986) Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. Cell 46(6):819–826
Blanc V, Xie Y, Kennedy S, Riordan JD, Rubin DC, Madison BB, Mills JC, Nadeau JH, Davidson NO (2019) Apohe1 complementation factor (A1CF) and RBM47 interact in tissue-specific regulation of C to U RNA editing in mouse intestine and liver. RNA 25(1):70–81
Bock R, Hagemann R, Kossel H, Kudla J (1993) Tissue-and stage-specific modulation of RNA editing of the psbF and psbL transcript from spinach plastids—a new regulatory mechanism? Mol Gen Genet 240(2):238–244
Brenner WG, Mader M, Müller NA, Hoenicka H, Schroeder H, Zorn I, Fladung M, Kersten B (2019) High level of conservation of mitochondrial RNA editing sites among four populus species. G3 Genes Genomes Genetics 9(3):709–717
Brown J, Pirrung M, McCue LA (2017) FQC dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinformatics 33(19):3137–3139
Chen TC, Liu YC, Wang XY, Wu CH, Huang CH, Chang CC (2017) Whole plastid transcriptomes reveal abundant RNA editing sites and differential editing status in Phalaenopsis aphrodite subsp. formosana. Bot Stud 58:14
Chepelev I (2012) Detection of RNA editing events in human cells using high-throughput sequencing. Methods Mol Biol 815:91–102
Covello PS, Gray MW (1989) RNA editing in plant mitochondria. Nature 341(6243):662–666
Daneczek P, McCarthy SA (2017) BCFTools/csq: haplotype-aware variant consequences. Bioinformatics 33(13):2037–2039
Daneczek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021) Twelve years of SAMtools and BCFTools. GigaScience. https://doi.org/10.1093/gigascience/gia0008
Edra AA, Gandini CL, Sanchez-Puerta MV (2018a) Towards a comprehensive picture of C-to-U RNA editing sites in angiosperm mitochondria. Plant Mol Biol 97(3):215–231
Grimes BT, Sisay AK, Carroll HD, Cahoon AB (2014) Deep sequencing of the tobacco mitochondrial transcriptome reveals expressed ORFs and numerous editing sites outside coding regions. BMC Genomics 15:31

Guo WH, Grewe F, Mower JP (2015) Variable frequency of plastid RNA editing among ferns and repeated loss of uridine-to-cytidine editing from vascular plants. PLoS ONE 10(1):e0117075

Haag S, Schindler M, Berndt L, Brennicke A, Takenaka M, Weber G (2017) Crystal structures of the Arabidopsis thaliana organellar RNA editing factors MORF1 and MORF9. Nucleic Acids Res 45(8):4915–4928

Hiesel R, Wissinger B, Schuster W, Brennicke A (1989) RNA editing in plant mitochondria. Science 246(4937):1632–1634

Hoch B, Maier RM, Appel K, Iglói GL, Kissel H (1991) Editing of a chloroplast mRNA by creation of an initiation codon. Nature 353(6340):178–180

Ichinose M, Sugita M (2017) RNA editing and its molecular mechanism in plant organelles. Genes 8(1):5

Kim D, Langebead M, Salzberg SL (2015) HISAT: a fast spliced aligner level expression analysis of RNA-seq experiments with HISAT, scriptome from RNA-seq reads. Nat Biotechnol 33(3):290–295

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, S. Genome Project Data Processing (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25(16):2078–2079

Liscovitch-Brauer N, Alon S, Porath HT, Elstein B, Unger R, Ziv (2017) Trade-off between transcriptome plasticity and genome evolution in cephalopods. Cell 169(2):191–202

Liu R, Cao SK, Sayed A, Yang HH, Zhao J, Wang XM, Jia RX, Sun F, Tan BC (2020) The DYW-subgroup pentatricopeptide repeat protein PPR27 interacts with ZmMORF1 to facilitate mitochondrial RNA editing and seed development in maize. J Exp Bot 71(18):5495–5505

Lukes J, Kaur B, Speijer D (2021) Plant organellar RNA editing and its specificity factors: enhancements of analyses and new database features in PREPACT 3.0. BMC Bioinformatics 19(1):255

Manna S (2015) An overview of pentatricopeptide repeat proteins and their applications. Biochimie 113:93–99

Miyata Y, Sugita M (2004) Tissue- and stage-specific RNA editing of rps 14 transcripts in moss (Physcomitrella patens) chloroplasts. J Plant Physiol 161(1):113–115

Nawae W, Yundaeng P, Sangkat C, Nongta C, Nangkachana W, Yoocha T, Sonnihero C, Naronong N, Sompa T, Laosatit K, Tangphatsornruang S, Pootakham W (2020) The genome and transcriptome analysis of the vigna mungo chloroplast. Plants (basel) 9(9):1247

Palladino MJ, Keegan LP, O’Connell MA, Reenan RA (2000) A-to-I pre-mRNA editing in Drosophila is primarily involved in adult nervous system function and integrity. Cell 102(4):437–449

Peng X, Xu X, Wang Y, Hawke DH, Yu S, Han L, Zhou Z, Mohumdar K, Jeong KJ, Labrie M, Tsang YH, Zhang M, Lu Y, Hwu P, Scott KL, Liang H, Mills GB (2018) A-to-I RNA editing contributes to proteomic diversity in cancer. Cancer Cell 33(5):817–828

Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol 33(3):290–295

Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat Protoc 11(9):1650–1667

Riemondy KA, Gillen AE, White EA, Bogren LK, Hesseltberg JR, Martin SL (2018) Dynamic temperature-sensitive A-to-I RNA editing in the brain of a heterothermic mammal during hibernation. RNA 24(11):1481–1495

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26(1):139–140

Ruchika C, Okudaira MS, Tsukahara T (2021) Genome-wide identification of U-To-C RNA editing events for nuclear genes in Arabidopsis thaliana. Cells. https://doi.org/10.3390/cells10030635

Schmitz-Linneweber C, Small I (2008) Pentatricopeptide repeat proteins: a scaffold set for organelle gene expression. Trends Plant Sci 13(2):663–670

Shikanai T (2015) RNA editing in plants: machinery and flexibility of site recognition. BBA-Bioenergetics 1847(9):779–785

Sierro N, Battey JN, Ouadi S, Bakaher N, Bovet L, Willig A, Goepter S, Peitsch MC, Ivanov NV (2014) The tobacco genome sequence and its comparison with those of tomato and potato. Nat Commun 5:3833

Small ID, Schallenberg-Rüdinger M, Takenaka M, Mireau H, Oster- setzer-Biran O (2020) Plant organellar RNA editing: what 30 years of research has revealed. Plant J 101(5):1040–1056

Sugiyama Y, Watase Y, Nagase M, Makita N, Yagura S, Hira i A, Sugimura M (2005) The complete nucleotide sequence and multiparticle organization of the tobacco mitochondrial genome: comparative analysis of mitochondrial genomes in higher plants. Mol Genet Genomics 272(6):603–615

Sun Y, Li X, Wu D, Pan Q, Ji Y, Ren H, Ding K (2016) RED: a Java- MySQL software for identifying and visualizing RNA editing sites using rule-based and statistical filters. PLoS ONE 11(3):e0150465

Takenaka M, Zehrmann A, Verbitskiy D, Kugelmann M, Hartel B, Brennicke A (2012) Multiple organellar RNA editing factor (MORF) family proteins are required for RNA editing in mitochondria and plastids of plants. Proc Natl Acad Sci U S A 109(13):5104–5109

Takenaka M, Zehrmann A, Verbitskiy D, Hartel B, Brennicke A (2013) RNA editing in plants and its evolution. Annu Rev Genet 47(47):335–352

Tan MH, Li Q, Shannumag R, Piskol R, Kohler J, Young AN, Liu K, Zhang R, Ramaswami G, Ariyoshi K, Gup te A, Keegan LP, George CX, Ramamurthy MN, Pollina EA, Leeman DS, Rusti ghi A, Goh YPS, Chawla A, Del Sal G, Peliz G, Brunet A, Conrad DF, Samuel CE, O’Connell MA, Walkley CR, Nishikura K, Li JB (2017) Dynamic landscape and regulation of RNA editing in mammalians. Nature 550(7675):249–254

Tang W, Luo C (2018) Molecular and functional diversity of RNA editing in plant mitochondria. Mol Biotechnol 60(12):935–945

Tian F, Yu J, Zhang Y, Xie Y, Wu B, Miao Y (2019) MORP9 functions in plastid RNA editing with tissue specificity. Int J Mol Sci. https://doi.org/10.3390/ijms20184635

Tillich M, Lehwark P, Morton BR, Maier UG (2006) The evolution of chloroplast RNA editing. Mol Biol Evol 23(10):1921–1921

Tseng CC, Lee CJ, Chung YT, Sung TY, Hsieh MH (2013) Differential regulation of Arabidopsis plastid gene expression and RNA editing in non-photosynthetic tissues. Plant Mol Biol 82(4–5):375–392

Walkley CR, Li JB (2017) Rewriting the transcriptome: adenosine-to-inosine RNA editing by ADARs. Genome Biol 18(1):205

Wu S, Liu W, Aljohi HA, Alromaih SA, Almazin H, Lin Q, Yu J, Hu S (2018) REDO: RNA editing detection in plant organelles based on variant calling results. J Comput Biol 25(5):509–516

Yagi Y, Tachikawa M, Noguchi H, Satoh S, Obokata J, Nakamura T (2013) Pentatricopeptide repeat proteins involved in plant organellar RNA editing. RNA Biol 10(9):1419–1425

Yagi Y, Nakamura T, Small I (2014) The potential for manipulating RNA with pentatricopeptide repeat proteins. Plant J 78(5):772–782
Yan J, Zhang Q, Yin P (2018) RNA editing machinery in plant organelles. Sci China Life Sci 61(2):162–169
Zahn LM (2017) The evolution of edited RNA transcripts. Science 355(6331):1278–1279
Zaidan H, Ramaswami G, Golubic YN, Sher N, Malik A, Barak M, Galiani D, Dekel N, Li JB, Gaïsler-Salomon I (2018) A-to-I RNA editing in the rat brain is age-dependent, region-specific and sensitive to environmental stress across generations. BMC Genomics 19(1):28
Zeltz P, Hess WR, Neckermann K, Borner T, Kossel H (1993) Editing of the chloroplast rpoB transcript is independent of chloroplast translation and shows different patterns in barley and maize. EMBO J 12(11):4291–4296
Zhang A, Jiang X, Zhang F, Wang T, Zhang X (2020) Dynamic response of RNA editing to temperature in grape by RNA deep sequencing. Funct Integr Genomics 20(3):421–432
Zhang Q, Wang YL, Xie W, Chen CZ, Ren DY, Hu J, Zhu L, Zhang GH, Gao ZY, Guo LB, Zeng DL, Shen L, Qian Q (2021) OsMORF9 is necessary for chloroplast development and seedling survival in rice. Plant Sci 307:110907
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