Journal of Integrative Agriculture 2026, 25(7): 2825–2835

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

A single nucleotide mutation in *BrECB2* impaired RNA editing efficiency and early chloroplast biosynthesis in pak choi

Zifan Zhao, Feng Pan, Tianxiang Zhao, Luyao Zhang, Qingli Hou, Tian'er Tang, Nan Wang, Chong Tan, Yun Zhang[#], Zhiyong Liu[#]

Liaoning Key Laboratory of Genetics and Breeding for Cruciferous Vegetable Crops, College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China

Highlights

- A delayed chloroplast development mutant 'M136' was identified during self-crossing of the pak choi inbred line '136'.
- *BrECB2* was identified as the candidate gene by BSA-seq and InDel markers.
- *BrECB2* encodes a chloroplast-localized DYW-type pentatricopeptide repeat protein.
- *BrECB2* is involved in early chloroplast development and RNA editing.

Abstract

Chloroplasts are important organelles for photosynthesis, which is essential for increasing the yields of pak choi. In this study, we evaluated a delayed chloroplast development mutant 'M136' identified during self-crossing of the pak choi inbred line '136'. The young true leaves of 'M136' were yellow and gradually turned green with maturation. Chloroplast development, pigment contents, and photosynthetic parameters were impaired and gradually recovered with growth in 'M136', and chlorophyll fluorescence parameters were also impaired in 'M136'. Genetic analysis and bulk segregant analysis (BSA)-seq revealed that the mutant phenotype was controlled by a single recessive gene, identified as *BraA06g011520*.3.5C (*BrECB2*), which encodes a DYW-type pentatricopeptide repeat (PPR) protein. In 'M136', a T-to-C single nucleotide polymorphism (SNP) in the 4th PPR motif of BrECB2 caused a threonine-to-isoleucine amino acid substitution. *BrECB2* was mainly expressed in young leaves. The chloroplast RNA editing efficiency of 'M136' was affected and significantly recovered after the leaves turned green, and the editing efficiency was partially restored in complementation lines. The plastid-encoded RNA polymerase activity was not affected in 'M136'. Functional complementation analyses revealed that the transient overexpression of *BrECB2* partially rescued the mutant phenotype and the RNA editing efficiency in 'M136'. In summary, this study indicates that *BrECB2* is involved in early chloroplast development and RNA editing, providing a theoretical basis for understanding the regulatory network involved in chloroplast development in pak choi.

Keywords: pak choi, BSA-seq, *BrECB2*, pentatricopeptide repeat protein, RNA editing, chloroplast development

1. Introduction

Pak choi (*Brassica rapa* L. ssp. *chinensis*) is an essential leafy vegetable that originated in China and has a long history of cultivation. The leaf is the major edible organ of pak choi and determines its commercial value. Chloroplasts are the organelles of photosynthesis, and their normal function depends on both chloroplast- and nucleus-encoded proteins (Gao *et al.* 2023). Most chloroplast proteins are encoded in the nucleus and regulated by nuclear proteins, and are subsequently delivered to the chloroplast (Thomson *et al.* 2020). The identification of nucleus-encoded chloroplast proteins is essential for understanding chloroplast development. There are currently few studies on chloroplast development in pak choi, and the mechanisms regulating chloroplast development remain unclear. Therefore, the identification of chloroplast- and nucleus-encoded genes associated with chloroplast development is crucial for

Received 7 March 2025; Received in revised form 17 June 2025; Accepted 30 July 2025; Available online 3 November 2025
Zifan Zhao, E-mail: zhaozifan818@163.com; [#]Correspondence Yun Zhang, E-mail: zhangyun511@syau.edu.cn; Zhiyong Liu, E-mail: liuzhiyong99@syau.edu.cn

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.11.001

revealing chloroplast development in pak choi.

Chloroplast development mutants are important materials for uncovering genes involved in chloroplast biosynthesis, and mutations in chloroplast development and chlorophyll synthesis genes result in reduced yield. Several chloroplast development mutants have been found in different plants. *EMB1303* encodes a novel chloroplast-localized protein, and the expression of photosynthesis and chloroplast biogenesis genes is significantly reduced in the *emb1303-1* mutant (Huang *et al.* 2009). Mutations in *ALS4*, which encodes 30S ribosomal protein S1, affect transcription of plastid genes as well as early chloroplast development and chlorophyll biosynthesis in rice (Zhou *et al.* 2021). Ribonucleotide reductase large subunit SvSTL1 plays a major role in chloroplast development, and chloroplast development was disturbed in the *svstl1* mutant (Li *et al.* 2022). *SlRCM1* encodes tomato Lutescent1, and the premature termination of SlRCM1 leads to collapse of the thylakoid membranes in tomato fruits (Liu G *et al.* 2021). The *Y4063* mutant shows reduced chlorophyll content and abnormal chloroplasts during early leaf development in cabbage (Zhang *et al.* 2024). *CsUMPK* encodes a uridylate kinase and is required for chlorophyll synthesis and chloroplast integrity in cucumber (Pan *et al.* 2024). Mutation in *BoAP1* confers disrupted chloroplast development and a lethal albino phenotype in the 6-219W mutant of curly kale (Zhao *et al.* 2024).

Pentatricopeptide repeat (PPR) proteins constitute a large protein family in land plants. PPR proteins comprise tandem arrays of PPR motifs, and each PPR motif is a highly conserved unit of 35 amino acids and forms a superhelix with a groove (Small and Peeters 2000). PPR proteins are categorized into two subfamilies, P and PLS, and the latter is further divided into three subclasses, PLS, E/E+, and DYW, based on differences in their C-termini (Lurin *et al.* 2004). In *Arabidopsis*, PPR proteins belong to a superfamily that contains approximately 450 members and are primarily localized to the chloroplasts or mitochondria (Lurin *et al.* 2004). PPR proteins rely on forming complexes to mediate RNA editing in plants (Barkan and Small 2014). RNA editing is a post-transcriptional process for altering sequences through selective nucleotide insertions, deletions, and substitutions (Knoop 2011). RNA editing, which alters mRNA codons by C-to-U conversion, is common in plant chloroplasts (Hoch *et al.* 1991). To date, 37 RNA editing sites have been identified in *Arabidopsis* chloroplasts (Bentolila *et al.* 2013).

Loss of function of PPR proteins can affect chloroplast RNA editing efficiency, and several PPR protein mutations cause impaired chloroplast development in various species. In *Arabidopsis*, a mutant of *AtECB2*, *ecb2-2*, is screened and shows a delayed-green phenotype. *AtECB2* encodes a DYW-type PPR protein related to *accD* and *ndhF* editing sites (Yu *et al.* 2009; Cao *et al.* 2011). Furthermore, the delayed-green mutant *dg409* shows severe disruptions in chloroplast and mitochondrial development. A mutation in dual-localized *AtDG409* causes editing defects in *rpoA-200* and *accD-1568* (Wang H *et al.* 2023). *OsPPR16* belongs to the DYW subclass and is required for *ndhB* editing in rice (Tang *et al.* 2017). Mutations in GmPGL2, a PLS subclass PPR protein, reduce the editing of *rps16*, *rps18*, *ndhB*, *ndhD*, *ndhE*, and *ndhF* in soybean (Feng *et al.* 2021). *OsYLWS* is a member of the P-type PPR protein family, and the *ylws* mutant causes editing defects of *ndhA*, *ndhB*, and *rps14* (Lan *et al.* 2023). The P-type PPR protein BoYgl-2 participates in editing of *atpF*, *rps14*, *petL*, and *ndhD* in cabbage (Zhang *et al.* 2024).

In the present study, we characterized a delayed chloroplast development mutant, 'M136', from the pak choi inbred line '136'. Based on bulk segregant analysis (BSA)-seq and InDel markers, *BraA06g011520.3.5C* (*BrECB2*) was identified as the candidate gene, and its function was verified through complementation assays. The chlorophyll contents, chloroplast structure and photosynthetic activity were impaired in 'M136'. The point mutation affected the RNA editing efficiency and early chloroplast development in 'M136'. This study demonstrates the importance of PPR protein-mediated RNA editing in pak choi chloroplast biosynthesis, improving our understanding of the molecular regulatory mechanisms underlying chloroplast development in pak choi.

2. Materials and methods

2.1. Plant materials

The pak choi delayed chloroplast development mutant 'M136' was obtained during self-crossing of the inbred line '136'. The wild-type '136' and delayed chloroplast development mutant 'M136' were used for phenotypic evaluation. Flowering Chinese cabbage 'CX001' and pak choi 'M136' were used as mapping parents. 'CX001' (normal chloroplast development with green leaves) and 'M136' (delayed chloroplast development with yellow-to-green leaves) were crossed to generate the F_1 population, which was self-crossed to obtain the F_2 population for genetic analysis and BSA-seq. The BC_1 population was generated by crossing F_1 with 'CX001' and 'M136'. All plants were cultured in the greenhouse of Shenyang Agricultural University, China in autumn.

2.2. Determination of the chlorophyll content

Leaves from 20- and 45-d-old '136' and 'M136', respectively, were collected. Equal amount (0.1 g) of each leaf samples was placed in 80% acetone and kept in the dark for 24 h. The samples were measured for absorbance at 663, 645, and 470 nm using a spectrophotometer (DU 800, Beckman Coulter, USA). The chlorophyll contents were calculated as described by Lichtenthaler and Wellburn (1983). Three biological replicates were performed.

2.3. Transmission electron microscopy

Ture leaves of 20- and 45-d-old '136' and 'M136' were collected, sliced into 1 mm×3 mm rectangles, and fixed in 4% glutaraldehyde for 3 d at 4°C for transmission electron microscopy (TEM). The slices were then placed in OsO_4 at 4°C for 12 h, dehydrated with acetone, and embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate, and observed using a transmission electron microscope (Hitachi H-7650, Tokyo, Japan).

2.4. Photosynthetic parameters

Photosynthetic parameters, including the net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2

concentration (C_i), and transpiration rate (E), were determined using a portable photosynthesis system (PP Systems, USA) at 10:00 a.m. from the true leaves of 20- and 45-d-old '136' and 'M136', respectively. Three biological replicates were performed for each parameter.

2.5. Chlorophyll fluorescence parameters

The 20-d-old true leaves from '136' and 'M136' were subjected to dark treatment for 20 min. Parameters of the fifth true leaf of the plant were measured at a pulse intensity of 4,500 μmol m^{-2} s^{-1} and pulse time of 0.8 s. Chlorophyll fluorescence parameters were measured and recorded using FluorCam FC 1000-H/ GFP (PSI, Czech Republic). Three biological replicates were performed.

2.6. BSA-seq analysis and fine-mapping of *BrECB2*

Equal amounts of leaves from 50 green and 50 yellow 20-d-old plants of the F_2 population were used to construct the bulked green (BG) and bulked yellow-to-green (BY) pools, respectively, and the leaves from 'CX001' and 'M136' were used to construct the parent pools. DNA of each pool was extracted using a DNA Extraction Kit (Tiangen, Beijing, China). After resequencing using a NovaSeq 6000 System sequencer (Illumina, USA), high-quality reads were filtered using fastQC, and the *Brassica* reference genome was aligned using BWA software. SNPs and InDels were detected using GATK. The SNP index and Euclidean distance (ED) algorithms were performed as described previously (Abe *et al.* 2012; Hill *et al.* 2013).

InDel primers were designed based on the resequencing results of the parents using Primer 5, and polymorphisms of InDel markers were detected using the parents. Individuals with the delayed chloroplast development phenotype of the F_2 population were used to narrow the candidate region by PCR. The genetic map was constructed as previously described (Zhao *et al.* 2022).

2.7. Functional complementation of 'M136'

The *BrECB2* coding sequence (CDS) was PCR-amplified and subcloned into the pRI101 vector. The construct was transformed into GV3101 and the bacterial solution was cultured until OD_{600}=0.8. The bacteria were collected and resuspended in an equal volume of infection solution (10 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} MES, and 150 μmol L^{-1} acetosyringone, pH=5.7). The solution was used to infiltrate 100 seeds of 'M136' using the vacuum method as previously described (Ge *et al.* 2023). The leaves from 7-d-old seedlings were measured for overexpression efficiency by qRT-PCR, and the leaves from 20-d-old seedlings were examined for editing efficiency.

2.8. Cloning of *BrECB2* and phylogenetic analysis

Genomic DNA and total RNA were extracted from young leaves of '136' and 'M136' using a modified CTAB (SL2071, Coolaber, Beijing, China) method and SteadyPure Plant RNA Extraction Kit (Accurate Bio, Changsha, China), respectively. First-strand cDNA was synthesized using the reverse transcription kit (R312, Vazyme, Nanjing, China). The CDSs of *BrECB2* and *Brecb2* were cloned as previously described (Zhao *et al.* 2025). The primers used for cloning are listed in Appendix A. Sequence alignment was performed using DNAMAN. Three-dimensional structures of proteins were constructed using AlphaFold3 (https://alphafoldserver.com) and visualized using PyMOL software. The amino acid sequences of BrECB2 homologs were searched from NCBI and aligned using ClustalW. The phylogenetic tree was constructed as described previously (Song *et al.* 2023).

2.9. Subcellular localization

The CDSs of *BrECB2* and *Brecb2* without stop codons were amplified and cloned into pCAMBIA1300 fused with green fluorescent protein (GFP) at the *Kpn*I and *Sal*I sites. The 35S::*BrECB2*-GFP, 35S::*Brecb2*-GFP, and 35S::GFP constructs were transferred into tobacco leaves. Fluorescence was detected after 48 h of culturing in the dark. GFP was observed at an excitation wavelength of 490–530 nm and chloroplast autofluorescence was observed at 600–700 nm. Fluorescence was observed using a confocal laser scanning microscope TCS SP8 (Leica, Germany).

2.10. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from different tissues-roots, stems, young leaves (20-d-old), mature leaves (40-d-old), flowers, buds, and siliques of '136', and reverse transcribed to cDNA using a kit (R312, Vazyme, Nanjing, China). qRT-PCR was performed as described previously (Wang *et al.* 2018). Primer pairs were designed by Primer 5 (Appendix A). *BrActin* was used as the internal control gene (Gao *et al.* 2020). The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

2.11. Analysis of RNA editing

Total RNA was extracted from leaves of 20-d-old '136', 20-d-old 'M136', 20-d-old complementation line individuals, and turned green leaves of 45-d-old 'M136' (T136), and first-strand cDNA was reverse transcribed using the kit. Chloroplast RNA editing sites were amplified using specific primers designed using Primer 5 (Appendix A). Unpurified PCR products were used for Sanger sequencing. The editing efficiency was calculated based on the heights of the nucleotide peaks.

2.12. Statistical analysis

An unpaired *t*-test was used to evaluate the differences between the two groups. A one-way ANOVA test was used to evaluate the differences among three or more groups.

3. Results

3.1. Phenotypic characterization of 'M136'

A delayed chloroplast development mutant 'M136' was found during self-crossing of the pak choi inbred line '136'. Cotyledons of 'M136' exhibited a green phenotype after sowing. The first true leaves grew and displayed a yellow phenotype at 3 d after sowing. With growth, the apices of the yellow true leaves of 'M136' started to turn green, and

the yellow true leaves gradually turned green completely with maturation. Notably, the color of the mature 'M136' plants was slightly lighter than that of mature '136' (Fig. 1-A–C). The pigment contents of 20-d-old 'M136' were less than 30% of those of 20-d-old '136' (Fig. 1-D). The pigment contents of '136' and 'M136' increased with maturation. However, the chlorophyll and carotenoid contents in the 45-d-old 'M136' were slightly lower than those in 45-d-old '136', consistent with the phenotypes of mature '136' and mature 'M136' (Fig. 1-E). To analyze the biomass of '136' and 'M136', dry weights were determined every 5 d. The dry weight of 'M136' was lower than that of '136' until after 45 d. As 'M136' plants turned green, the difference in dry weight gradually narrowed. After 50 d, there was no significant difference in dry weight between '136' and 'M136' (Fig. 1-F).

3.2. Chloroplast biosynthesis and photosynthetic activity were impaired in 'M136'

The TEM results showed that the chloroplasts of the true leaves of 20- and 45-d-old '136' were well-structured. However, the chloroplasts of the 20-d-old 'M136' were impaired, with fewer thylakoid membranes and thinner grana thylakoids (Fig. 2-A). With development, the thylakoid membrane of the true leaves of 'M136' became comparable to that of '136' (Fig. 2-B). Compared with the true leaves of the 20-d-old '136', the P_n, G_s, and E of 'M136' were significantly reduced, and the C_i concentration was significantly increased, indicating a decrease in the photosynthetic capacity of 'M136' leaves. The photosynthetic parameters of 'M136' increased as the leaves turned green. Although the P_n and G_s of 'M136' were significantly lower than those of '136' at the 45-d-old stage, there were no significant differences in E and C_i (Fig. 2-C–F). Furthermore, F_0, F_m, F_0', and F_m' were significantly lower in 20-d-old 'M136' than in 20-d-old '136' (Fig. 2-G). However, F_v/F_m and F_v'/F_m' showed no significant difference. Compared with those of 20-d-old '136', Φ_{PSII}, q_P, and NPQ were significantly lower in 20-d-old 'M136' (Fig. 2-H), suggesting that photosynthetic electron transport in photosystem II was affected and photoprotection was reduced in 'M136', leading to impaired photosynthesis. These results showed that chloroplast development and photosynthetic activity were affected in 'M136'.

3.3. Identification of the candidate gene

To map the candidate gene for 'M136', we constructed genetic populations by crossing 'M136' and 'CX001'. The F_1 population showed the normal chloroplast development phenotype with green leaves. For the F_2 population, 2,527 plants showed the delayed chloroplast development phenotype with yellow leaves, and 820 plants showed the wild-type phenotype. The segregation ratio was 3.08:1, consistent with 3:1 (χ^2=0.45<$\chi^2_{0.05}$=3.84). All BC_1 plants (F_1×'CX001') showed the wild-type phenotype. For the BC_1 (F_1×'M136') population, 184 and 162 plants showed the wild-type phenotype and the delayed chloroplast development phenotype, respectively. The segregation ratio was 1.14:1, consistent with 1:1 (χ^2=1.40<$\chi^2_{0.05}$=3.84) (Table 1). These results indicated that the mutant phenotype in pak choi was caused by a single recessive mutation.

To preliminarily map the candidate gene, we performed BSA-seq analysis using individuals with the wild-type and delayed chloroplast development phenotypes from the F_2 population and the parental lines ('CX001' and 'M136'). In this study, 108,278,057, 117,990,179, 136,297,956, and 129,726,298 clean reads were obtained for the 'CX001', 'M136', BG, and BY pools with average sequencing depths of 31.72×, 34.39×, 40.17×, and 37.83×, respectively. The clean reads were aligned to the Brassica genome V3.5, and

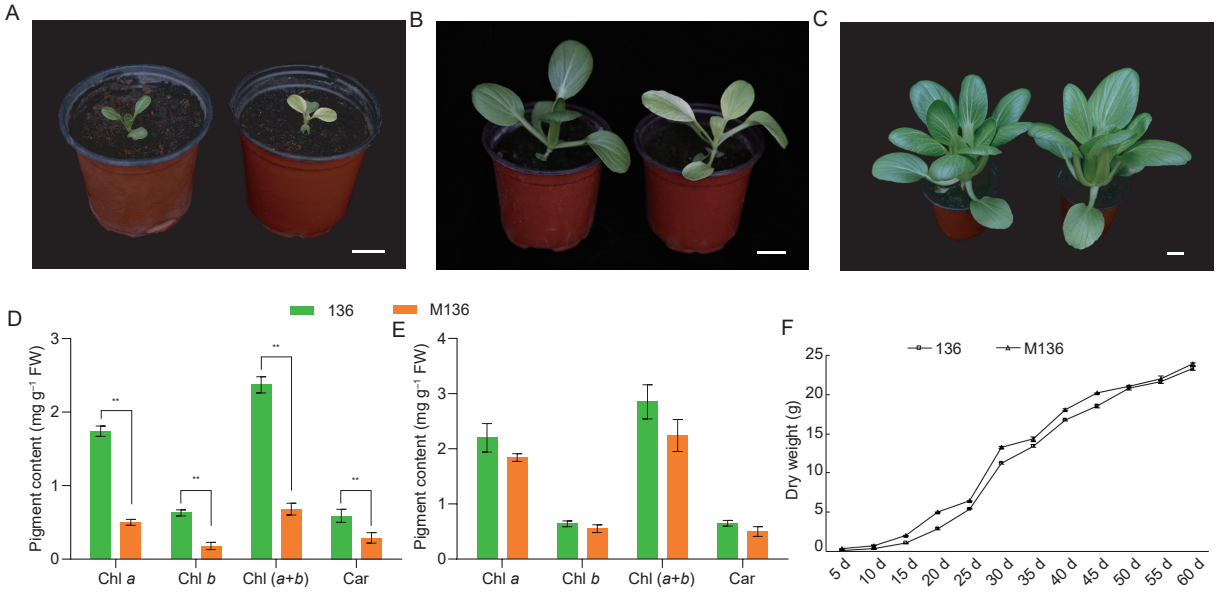


Fig. 1 Phenotypic characterization of '136' and 'M136'. A–C, phenotypes of '136' (left) and 'M136' (right) at 7 (A), 20 (B), and 45 (C) d. D and E, pigment contents of '136' and 'M136' at 20 (D) and 45 (E) d. F, dry weights of '136' and 'M136' at different stages. Bars=2 cm. Values represent means±SD of three biological replicates (*, P<0.05; **, P<0.01).

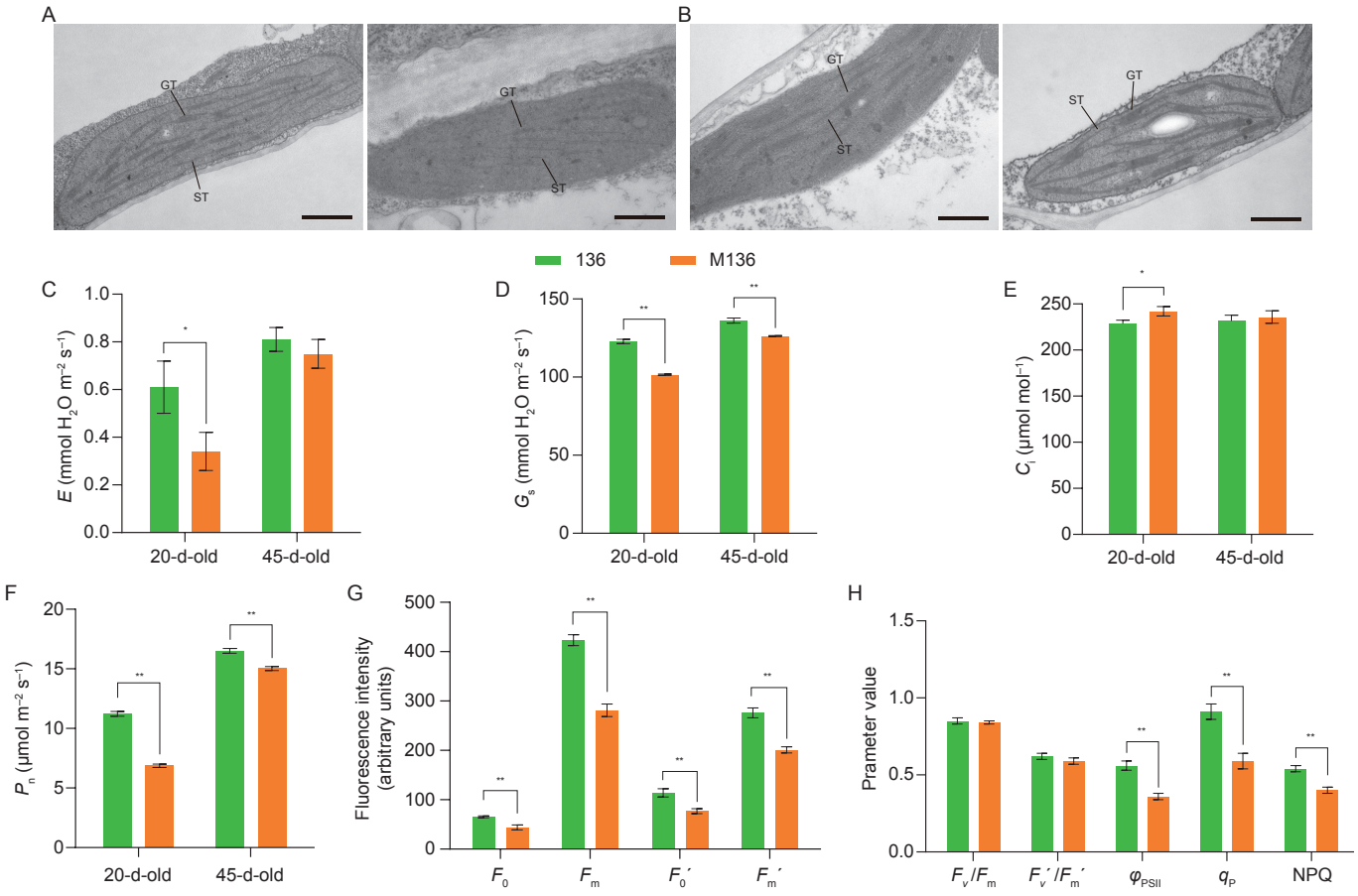


Fig. 2 Chloroplast ultrastructure, photosynthetic parameters and chlorophyll fluorescence parameters. A, chloroplast ultrastructure of leaves in '136' (left) and 'M136' (right) at 20 d. GT, granum thylakoid; ST, stroma thylakoid. Bars=500 nm. B, chloroplast ultrastructure of leaves of '136' (left) and 'M136' (right) at 45 d. C–F, transpiration rate (*E*) (C), stomatal conductance (G_s) (D), intercellular CO_2 concentration (C_i) (E), and net photosynthetic rate (P_n) (F) of '136' and 'M136' at 20 and 45 d. G and H, chlorophyll fluorescence parameters of '136' and 'M136' at 20 d. Values represent means±SD of three biological replicates (*, P<0.05; **, P<0.01).

Table 1 Genetic analysis of 'M136'

Generation	Total plants	Wild type plants	Mutant plants	Segregation ratio	X^2
P_1 (136)	50	50	0		
P_2 (M136)	50	0	50		
F_1 (P_1×P_2)	50	50	0		
F_1 (P_2×P_1)	50	50	0		
BC_1 (F_1×P_1)	50	50	0		
BC_1 (F_1×P_2)	346	184	162	1.14:1	1.40
F_2	3,347	2,527	820	3.08:1	0.45

mapping rates were 97.75%, 98.51%, 98.45%, and 98.67%, respectively. In total, 3,216,912 SNPs and 678,108 indels were identified. SNP-index and ED algorithms were used to obtain the candidate interval. The peak regions, 23.1 Mb (1.20–24.3 Mb) and 5.56 Mb (3.22–8.78 Mb), on chromosome A06 were obtained (Fig. 3-A and B). The overlap between the two regions was identified as a candidate region of 5.56 Mb (3.22–8.78 Mb) on chromosome A06.

To fine-map the candidate gene, we designed seven InDel markers in the candidate region according to the resequencing results of 'CX001' and 'M136'. Furthermore, 820 individuals with delayed chloroplast development phenotype from the F_2 population were used to narrow the target region using InDel markers. The candidate gene was finally mapped to a 144.9 kb (6,209,488–6,354,393 bp) region between InDel 11 and 14. According to the Brassica genome V3.5, 18 genes were annotated in the final region (Fig. 3-C) (Appendix B). We focused on chloroplast-targeted genes owing to the delayed chloroplast development in 'M136'. Within the interval, only *BraA06g011520.3.5C* encoded a chloroplast-localized protein. *BraA06g011520.3.5C* is homologous to *AtECB2*, which encodes a PPR protein involved in chloroplast biosynthesis. A previous study has shown that *atecb2-2* exhibits a delayed chloroplast development phenotype similar to that of 'M136' (Cao *et al.* 2011). Thus, *BraA06g011520.3.5C* was identified as the candidate gene and named *BrECB2*. The mutated *BrECB2* in 'M136' was named *Brecb2*.

3.4. Functional verification of BrECB2

To verify whether BrECB2 is associated with the delayed chloroplast development phenotype, we performed transient overexpression experiments to overexpress the *BrECB2* gene in 'M136'. The complementation lines displayed a green phenotype similar to that of '136' (Fig. 4-A). We evaluated

the overexpression efficiency using qRT-PCR, and the results indicated that *BrECB2* expression was significantly higher in the complementation lines than in '136' and 'M136' (Fig. 4-B). Additionally, the chlorophyll contents of complementation lines were significantly higher than those of 'M136' (Fig. 4-C). These results suggested that BrECB2 was involved in chloroplast development in pak choi.

3.5. Sequence analysis of *BrECB2*

The full-length sequence and 2-kb promoter region of *BrECB2* were cloned and analyzed with sequence alignment using genomic DNA from '136' and 'M136', and the coding sequences were cloned from cDNA. Sequence alignment analysis showed that *BrECB2* was 2,595 bp in length and contained no introns (Fig. 5-A). No variation was found in the promoter region of *BrECB2* between '136' and 'M136' (Appendix C). Only one SNP was identified in *BrECB2* and *Brecb2* (Fig. 5-B). The mutation occurred at 593 bp, with a change from C to T, causing a variation in the 198th amino acid from Thr to Ile and altering the three-dimensional structure of the protein (Fig. 5-C and D).

3.6. Structural and phylogenetic analysis of BrECB2

Sequence analysis revealed that *BrECB2* encodes an 865-amino-acid protein and belongs to the DYW-type subfamily according to the PPR database (https://ppr.

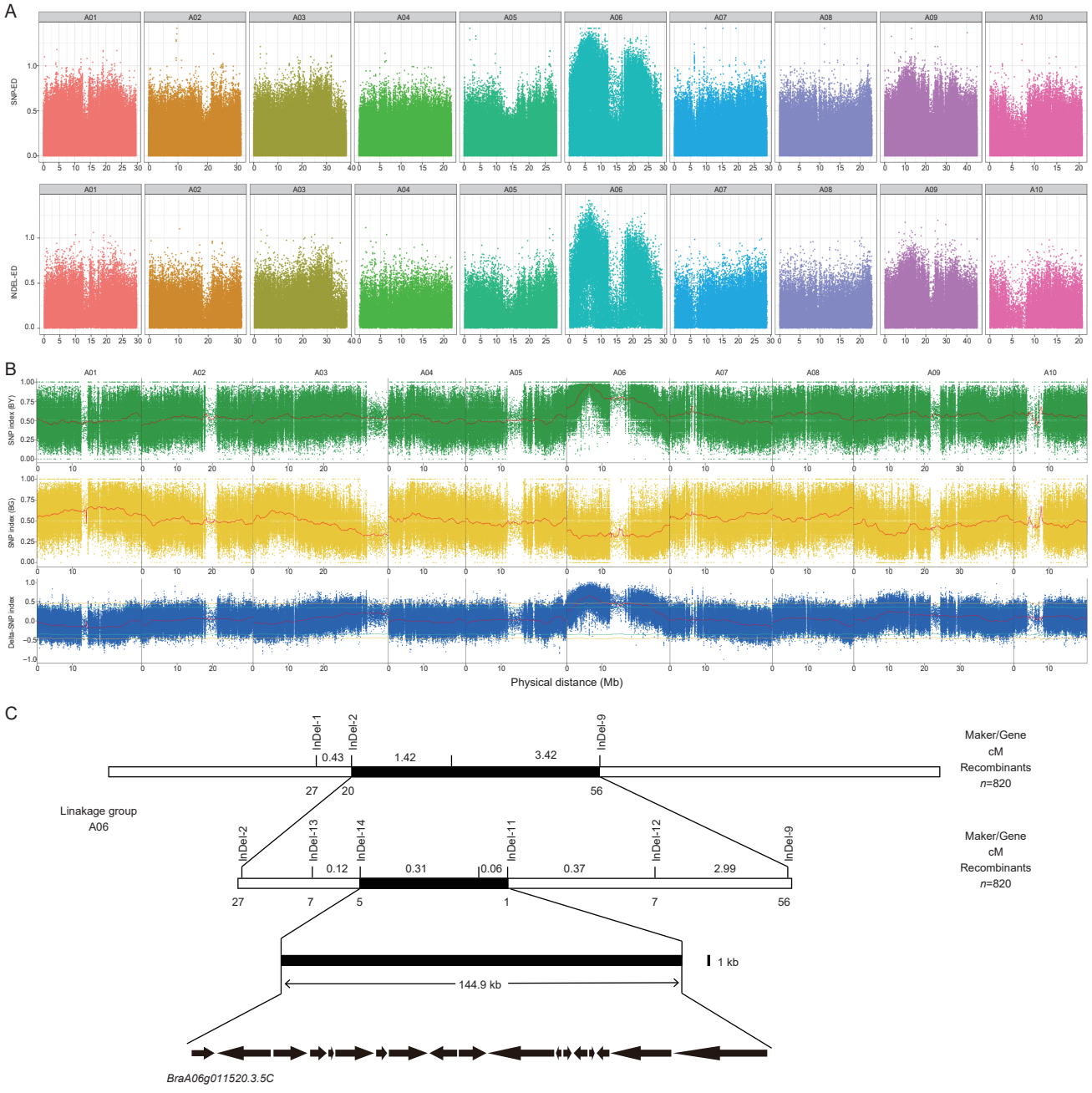


Fig. 3 Fine mapping of *BraA06g011520.3.5C* (*BrECB2*). A, Euclidean distance graph of SNPs and InDels. B, SNP index and ΔSNP index graph of two bulked pools. BY and BG, bulked yellow-to-green and green pools, respectively. C, genetic map of *BrECB2*.

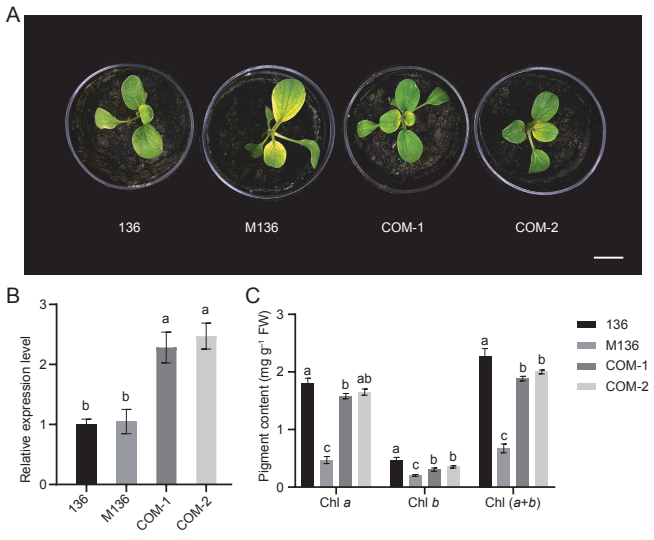


Fig. 4 Complementation verification of the *BrECB2* gene. A, phenotypes of '136', 'M136' and complementation lines (COM-1 and COM-2). Bars=2 cm. B, relative expression levels of *BrECB2* in '136', 'M136' and complementation lines (COM-1 and COM-2). C, the chlorophyll contents of '136', 'M136' and complementation lines (COM-1 and COM-2). Values represent means±SD of three biological replicates (**, $P<0.01$). Different letters indicate statistically significant differences ($P<0.05$) within the groups.

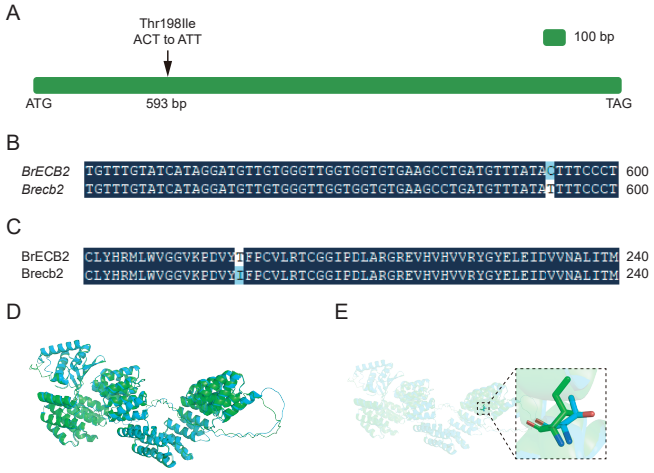


Fig. 5 Gene structure of *BrECB2* and sequence alignment. A, gene structure of *BrECB2*. B, partial alignment of the coding sequences of *BrECB2* and *Brecb2*. C, partial alignment of the amino acid sequences of BrECB2 and Brecb2. D, alignment of the BrECB2 (cyan) and Brecb2 (green) protein structures. E, comparison of the 198th amino acid structures of the BrECB2 (cyan) and Brecb2 (green) proteins.

plantenergy.uwa.edu.au). BrECB2 comprises 17 PPR motifs, an E/E+ domain, and a DYW domain (Fig. 6-A). The mutation site was in the 4th PPR motif, which is conserved in cruciferous species (Fig. 6-B). To analyze the evolutionary relationships of BrECB2 in cruciferous species, we constructed a phylogenetic tree with BrECB2 and other cruciferous homologs. These results indicate that BrECB2 is evolutionarily conserved among cruciferous species. BrECB2 was closely related to *Brassica cretica* KAF2559505.1 (97.11%) and *Brassica napus* XP_048614080.1 (96.76%), suggesting that they share a common ancestor (Fig. 6-C).

3.7. BrECB2 was localized to the chloroplasts

Most PPR proteins are chloroplast- or mitochondria-localized, and the BrECB2 protein was predicted to be chloroplast-localized by TargetP. To confirm the subcellular localization of BrECB2, we fused the BrECB2 and Brecb2 proteins with GFP. The recombinant constructs were transiently expressed in tobacco leaves, and 35S::GFP was the control. Strong green fluorescence signals were detected in the chloroplasts and coincided with the red autofluorescence of chlorophyll in 35S::BrECB2-GFP and 35S::Brecb2-GFP (Fig. 7-A). These results suggested that BrECB2 was localized to the chloroplasts and that the mutation did not change the chloroplast localization.

3.8. *BrECB2* was primarily expressed in young leaves

To explore the expression pattern of *BrECB2*, we performed a qRT-PCR analysis of different tissues of '136'. The results revealed that *BrECB2* was expressed in all tissues (Fig. 7-B). In particular, *BrECB2* was primarily expressed in the young leaves, followed by the mature leaves, stems, buds, and siliques. These results indicated that *BrECB2* is primarily expressed in young leaves, which was in accordance with its role in early chloroplast biosynthesis.

3.9. Chloroplast RNA editing was impaired in 'M136'

PPR proteins play essential roles in RNA editing, and mutations in PPR motifs cause impaired RNA editing (Kotera *et al.* 2005; Okuda *et al.* 2007). To date, 29 editing sites have been identified in *Brassica* species (Zhang *et al.* 2022, 2024). In *Arabidopsis*, *AtECB2* has been found to be involved in RNA editing of *accD* and *ndhF*. To investigate whether the mutation in *BrECB2* impaired the RNA editing efficiency, we examined the editing efficiency at sites targeted by *AtECB2* in '136' and 'M136'. The results showed that the editing efficiencies of *accD-C782*, *accD-C1391* and *ndhF-C290* were impaired to varying degrees (Fig. 8-A). The editing efficiency of *ndhF-C290* was severely impaired, decreasing to 32.49%. Moreover, the editing efficiencies of *accD-C782* and *accD-C1391* decreased to 50.70% (Fig. 8-B). To investigate variations in the editing efficiencies with leaf maturation, we examined the RNA editing levels in 'M136' after the leaves turned green and found that the editing rates were significantly restored. A previous study has shown that the RNA editing efficiency can be partially restored using complementation assays (Liu *et al.* 2023). We examined the editing efficiency of the overexpression lines and found that RNA editing efficiency in the complementation lines was partially restored (Fig. 8-A and B). These results indicated that *BrECB2* was crucial for the RNA editing efficiency in pak choi chloroplasts.

3.10. Chloroplast-encoded gene expression

The *AtECB2* mutation reduces the expression of *PsaB*, *PsbA*, *PsbB*, *PetD*, and *RbcL*, defined as plastid-encoded RNA

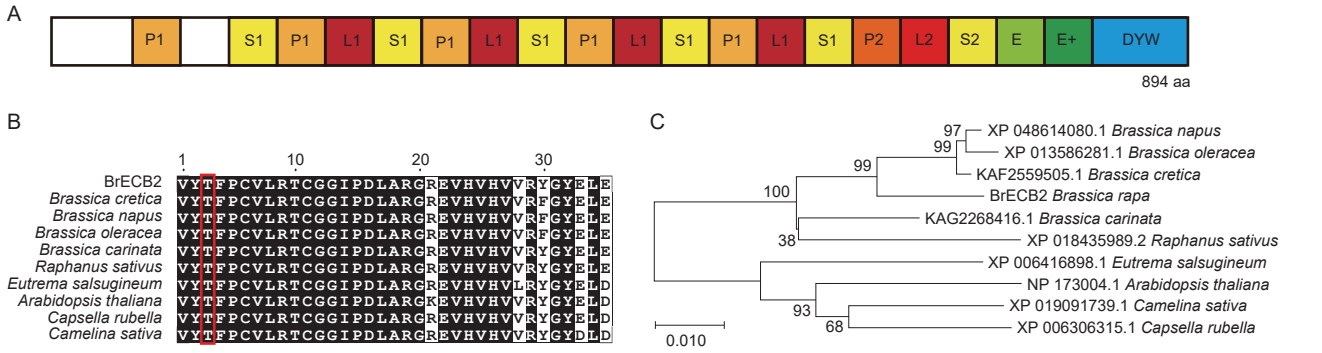


Fig. 6 Protein structure and phylogenetic analysis of BrECB2. A, diagrams of the BrECB2 protein. B, alignment of the 4th PPR motif of the BrECB2 protein and its homologs in cruciferous species. C, phylogenetic analysis of the BrECB2 protein and its homologs in cruciferous species.

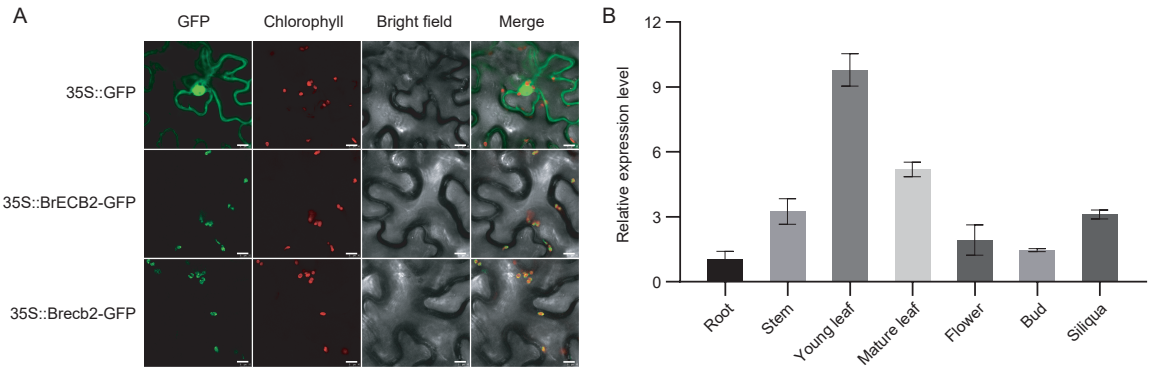


Fig. 7 Analysis of subcellular localization and expression patterns. A, subcellular localization of the BrECB2 and Brecb2 protein. Bars=10 μm. B, qRT-PCR analysis of the *BrECB2* gene in the root, stem, young leaf, mature leaf, flower, bud, and siliqua. Values represent means±SD of three biological replicates.

polymerase (PEP)-dependent genes, but increases the levels of *AccD*, *Ycf2*, and *RpoA* in *ecb2-1*, defined as the nuclear-encoded RNA polymerase (NEP)-dependent genes (Yu *et al.* 2009). However, the *AtECB2* mutation does not influence the expression levels of the chloroplast-encoded genes in *ecb2-2* (Yu *et al.* 2009; Cao *et al.* 2011). To further study whether *BrECB2* mutation affected chloroplast development genes, we compared the expression levels of chloroplast-encoded genes in 20-d-old '136' and 'M136' seedlings. The expression level of the PEP-dependent gene *PsbB* was significantly higher in 'M136' than in '136', and other PEP-dependent genes showed no significant difference in expression level between '136' and 'M136' (Fig. 9-A). In contrast, the NEP-dependent genes were significantly upregulated in 'M136' compared with '136' (Fig. 9-B). For PEP- and NEP-dependent genes, the expression of *Rrn23* and *AtpE* was significantly increased; however, the expression of *Rrn16* and *AtpB* did not change significantly in 'M136' (Fig. 9-C). Additionally, there were no significant differences in the expression levels of chloroplast-encoded genes between 45-d-old '136' and 'M136' plants (Appendix D). These results suggest that the *BrECB2* mutation did not impair the PEP transcriptional machinery in the seedling stage.

4. Discussion

4.1. *BrECB2* is the candidate gene for 'M136'

Chloroplasts are organelles that perform photosynthesis in plants and photosynthetic efficiency determines yield and quality of pak choi. The identification of chloroplast development mutants is a useful approach for revealing the regulation of leaf chloroplast biosynthesis. In our study, a delayed chloroplast development mutant 'M136' was obtained from pak choi inbred line '136'. The young true leaves of 'M136' were yellow and turned green from the apex with leaf development, whereas the leaves of '136' exhibited a green color in the seedling stage. The chloroplast structure and the thylakoid of 'M136' were smaller than those of '136'. The photosynthetic pigment contents and photosynthetic activity were reduced in the 'M136' mutant. The abnormalities in chloroplast structure and function recovered with the maturation of 'M136'. Based on BSA-seq and InDel markers, a candidate 144.9 kb region was identified on chromosome A06. The chloroplast-targeted gene *BrECB2* was identified as the candidate gene. *BrECB2* is homologous to *AtECB2* in *Arabidopsis*, which is involved in chloroplast biosynthesis. *AtECB2* is associated with chloroplast biogenesis, and *ecb2-1* and *vac1* show abnormal leaf color and fail to complete the life cycle (Yu *et al.* 2009; Tseng *et al.* 2010). However, *ecb2-2* exhibited a delayed green phenotype (Cao *et al.* 2011). In our

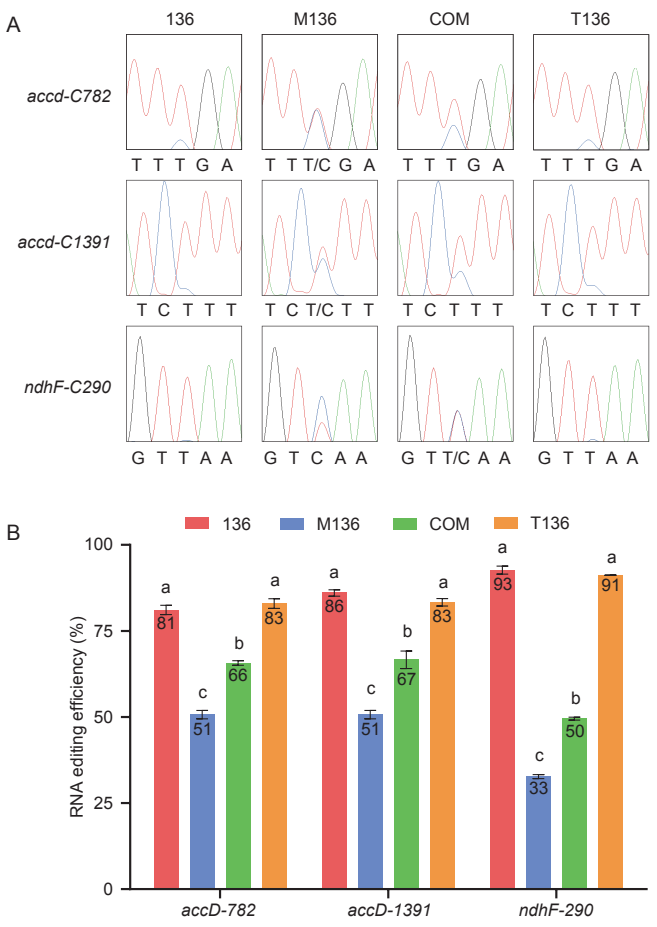


Fig. 8 RNA editing efficiency analysis. A, sequencing peak map of *accD-C782*, *accD-C1391* and *ndhF-C290* in '136', 'M136', COM, and T136. B, RNA editing efficiency of *accD-C782*, *accD-C1391* and *ndhF-C290* in '136', 'M136', COM, and T136. COM, complementation lines; T136, 'M136' after the leaves turned green. Values represent means±SD of three biological replicates. Different letters indicate statistically significant differences (*P*<0.05) within the groups.

study, similar to *ecb2-2*, 'M136' also exhibits a delayed chloroplast development phenotype. Thus, *BrECB2* is the candidate gene responsible for the delayed chloroplast development phenotype.

4.2. *BrECB2* plays a key role in early chloroplast biosynthesis

PPR proteins are a large protein family in higher plants and are involved in post-transcriptional RNA modifications, such as RNA editing (Barkan and Small 2014). Most PPR proteins are chloroplast- or mitochondria-targeted and play key roles in chloroplast and mitochondrial biogenesis (Lurin *et al.* 2004). *BrECB2* encodes a DYW-type PPR protein with 17 PPR motifs, and BrECB2 was localized to chloroplasts. This single nucleotide mutation caused a non-synonymous mutation in the 4th PPR motif. The functional complementation experiments partially restored the delayed chloroplast development phenotype of 'M136'. *BrECB2* is highly expressed in green tissues, especially in young leaves. We speculate that the high *BrECB2* expression is responsible for early chloroplast biosynthesis in young leaves. In mature leaves, *BrECB2* expression becomes low, and other chloroplast biosynthesis genes are mainly responsible for chloroplast development, resulting in the transformation of the leaf to a green color. Therefore, we conclude that *BrECB2* is involved in early chloroplast biosynthesis.

4.3. The Thr198 of the 4th PPR motif is required for the recognition of target transcripts

PPR family proteins have RNA-binding activity, and each PPR motif forms a superhelix consisting of two α-helices that bind to sequence-specific RNAs to form complexes that recruit other factors to the target RNA sites (Lurin *et al.* 2004). Our experiments demonstrated that the substitution of the Thr198 residue with Ile198 caused the mutant phenotype of 'M136', and the Thr198 residue was highly conserved in different species (Fig. 5-C). Each PPR motif consists of two α-helices (Small and Peeters 2000), and Thr198 is located in the helix of the 4th PPR motif (Appendix E). Similarly, a Thr-to-Ile substitution occurred at amino acid position 500 in *atecb2-2* (Cao *et al.* 2011). The side chains of Thr may form the central groove of the protein, which could bind to single-stranded RNA (Small and Peeters 2000; Yamazaki *et al.* 2004). In our study, the Thr-to-Ile substitution changed the protein structure of BrECB2 and may have affected the recognition capacity of BrECB2. We infer that Thr198 of the 4th PPR motif is required for the recognition

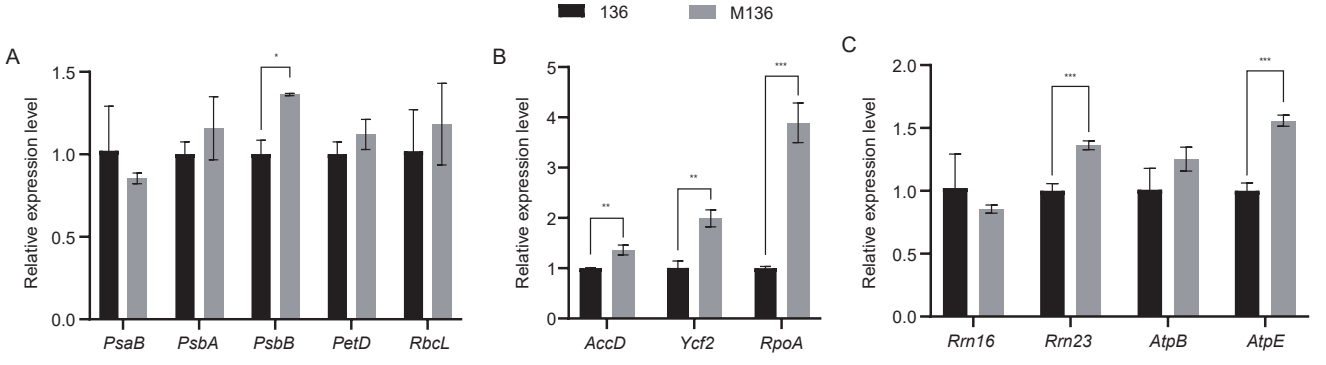


Fig. 9 Expression levels of chloroplast-encoded genes in 20-d-old '136' and 'M136' seedlings. A, relative expression levels of PEP-dependent genes. B, relative expression levels of NEP-dependent genes. C, relative expression level of PEP- and NEP-dependent genes. Values represent means±SD of three biological replicates (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001).

of target transcripts. However, this inference needs to be demonstrated in further experiments.

4.4. The mutation of *BrECB2* maintains the normal PEP expression level

Chloroplast genes are transcribed by at least two RNA polymerases, PEP and NEP, in higher plants (Hedtke *et al.* 1997). PEP plays a major transcriptional role in chloroplasts and is recruited by sigma factors to promote the initiation of gene transcription (Börner *et al.* 2015). During early development, NEP is mainly responsible for housekeeping gene transcription, and the inhibition of NEP activity and the activation of PEP promote the light-dependent maturation of chloroplasts (Börner *et al.* 2015). The impairment of PEP activity causes abnormal chloroplast development. In *Arabidopsis ecb2* and *ys1* mutants, as well as the rice *ostha8* mutant, PEP-dependent genes were downregulated, and NEP-dependent genes were upregulated (Yu *et al.* 2009; Zhou *et al.* 2009; Wang Y *et al.* 2023). However, the point mutation does not disrupt the PEP-dependent genes in *ecb2-2* (Cao *et al.* 2011). In our study, PEP- and NEP-dependent genes in '136' and 'M136' showed a similar expression pattern to those in *ecb2-2*, indicating that the *BrECB2* mutation maintains the normal PEP transcriptional machinery in 'M136'.

4.5. *BrECB2* is essential for RNA editing in pak choi

Mutations in PPR proteins cause impaired RNA editing efficiency and impaired chloroplast development. In rice, mutations in the P-type PPR protein OsTHA8 impair the editing of *ndhB* and *rps8*, resulting in pale-green leaves (Wang Y *et al.* 2023). *AtYS1* is required for editing *rpoB-25992*, and knockout alleles of *AtYS1* in *Arabidopsis* cause a virescent phenotype (Zhou *et al.* 2009). In maize, the albino seedling-lethal *zmppr26* mutant shows impaired editing of *aspA*, *ndhA*, *ndhF*, *rpoC2*, *rpl2*, *rpl20*, *rps8*, and *petB* (Liu X Y *et al.* 2021). *AtECB2* is linked to *accD* and *ndhF* editing (Huang *et al.* 2017). We detected the RNA editing sites related to *AtECB2*, and the efficiencies of *accD* and *ndhF* were impaired in 'M136'. Similar to *ecb2-2*, the same editing sites were detected in 'M136' (Cao *et al.* 2011), demonstrating that Thr may play a highly conserved role in RNA editing. The significant reduction in RNA editing efficiency in 'M136' is likely the primary cause of chloroplast developmental impairment. However, the efficiencies were significantly restored in 'M136' after the leaves turned green and were partially restored in complementation lines. Previous experimental results have shown that functional complementation cannot be used to fully restore impaired RNA editing efficiency, which explains why the delayed chloroplast development phenotype and editing efficiency were only partially restored in our experiments (Liu *et al.* 2023).

5. Conclusion

In the present study, we screened a delayed chloroplast development mutant 'M136' during self-crossing of the inbred line '136'. *BrECB2*, which encodes a DYW-type pentapeptide-repeat protein, was identified as the candidate gene for 'M136'. The mutation in the 4th PPR motif of BrECB2 caused a delayed chloroplast development phenotype. The RNA editing efficiency was impaired; however, the PEP activity was not impaired in 'M136'. A functional complementation assay was used to partially restore the delayed chloroplast development phenotype and the editing efficiency of 'M136'. Our results provide new evidence for the involvement of PPR proteins in the regulation of chloroplast biosynthesis and provide new insights into the mechanism underlying chloroplast biosynthesis in pak choi.

Acknowledgements

This work was supported by the National Key Research and Development Program, China (2022YFF1003004 and 2023YFD1200101), the Earmarked Fund for CARS-23, China, and the National Natural Science Foundation of China (32272736 and 32472762).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.11.001

References

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Yoshida K, Mitsuoka C, Tamiru M, Innan H, Cano L, Kamoun S, Terauchi R. 2012. Genome sequencing reveals agronomically important loci in rice using MutMap. *Nature Biotechnology*, **30**, 174–178.

Barkan A, Small I. 2014. Pentatricopeptide repeat proteins in plants. *Annual Review of Plant Biology*, **65**, 415–442.

Bentolila S, Oh J, Hanson M R, Bukowski R. 2013. Comprehensive high-resolution analysis of the role of an Arabidopsis gene family in RNA editing. *PLoS Genetics*, **9**, e1003584.

Börner T, Aleynikova A Y, Zubo Y O, Kusnetsov V V. 2015. Chloroplast RNA polymerases: Role in chloroplast biogenesis. *Biochimica et Biophysica Acta*, **1847**, 761–769.

Cao Z L, Yu Q B, Sun Y, Lu Y, Cui Y L, Yang Z N. 2011. A point mutation in the pentatricopeptide repeat motif of the AtECB2 protein causes delayed chloroplast development. *Journal of Integrative Plant Biology*, **53**, 258–269.

Feng X, Yang S, Zhang Y, Zhiyuan C, Tang K, Li G, Yu H, Leng J, Wang Q. 2021. GmPGL2, encoding a pentatricopeptide repeat protein, is essential for chloroplast RNA editing and biogenesis in soybean. *Frontiers in Plant Science*, **12**, 690973.

Gao L L, Hong Z H, Wang Y, Wu G Z. 2023. Chloroplast proteostasis: A story of birth, life, and death. *Plant Communication*, **4**, 100424.

Gao Y, Huang S, Qu G, Fu W, Zhang M, Liu Z, Feng H. 2020. The mutation of *ent*-kaurene synthase, a key enzyme involved in gibberellin biosynthesis, confers a non-heading phenotype to Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Horticulture Research*, **7**, 178.

Ge W, Zhang J, Feng H, Wang Y, Ji R. 2023. The root meristem growth factor *BrRGF6* positively regulates Chinese cabbage to infection of clubroot disease caused by *Plasmodiophora brassicae*. *Horticulture Research*, **10**, uhac292.

Hedtke B, Börner T, Weihe A. 1997. Mitochondrial and chloroplast phage-type RNA polymerases in Arabidopsis. *Science*, **277**, 809–811.

Hill J T, Demarest B L, Bisgrove B W, Gorsi B, Su Y C, Yost H J. 2013. MMAPPR: Mutation mapping analysis pipeline for pooled RNA-seq. *Genome Research*, **23**, 687–697.

Hoch B, Maier R M, Appel K, Igloi G L, Kössel H. 1991. Editing of a chloroplast mRNA by creation of an initiation codon. *Nature*, **353**, 178–180.

Huang C, Yu Q B, Li Z R, Ye L S, Xu L, Yang Z N. 2017. Porphobilinogen deaminase HEMC interacts with the PPR-protein AtECB2 for chloroplast RNA editing. *Plant Journal*, **92**, 546–556.

Huang X, Zhang X, Yang S. 2009. A novel chloroplast-localized protein EMB1303 is required for chloroplast development in Arabidopsis. *Cell Research*, **19**, 1205–1216.

Knoop V. 2011. When you can't trust the DNA: RNA editing changes transcript sequences. *Cellular and Molecular Life Sciences*, **68**, 567–586.

Kotera E, Tasaka M, Shikanai T. 2005. A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. *Nature*, **433**, 326–330.

Lan J, Lin Q, Zhou C, Liu X, Miao R, Ma T, Chen Y, Mou C, Jing R, Feng M, Nguyen T, Ren Y, Cheng Z, Zhang X, Liu S, Jiang L, Wan J. 2023. *Young Leaf White Stripe* encodes a P-type PPR protein required for chloroplast development. *Journal of Integrative Plant Biology*, **65**, 1687–1702.

Li H, Li L, Wu W, Wang F, Zhou F, Lin Y. 2022. SvSTL1 in the large subunit family of ribonucleotide reductases plays a major role in chloroplast development of *Setaria viridis*. *Plant Journal*, **111**, 625–641.

Lichtenthaler H K, Wellburn A R. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society Transactions*, **11**, 591–592.

Liu D, Li Z A, Li Y, Molloy D P, Huang C. 2023. The DYW domain of RARE1 plays an indispensable role in regulating *accD*-C794 RNA editing in *Arabidopsis thaliana*. *Plant Science* (Amsterdam, Netherlands), **334**, 111751.

Liu G, Yu H, Yuan L, Li C, Ye J, Chen W, Wang Y, Ge P, Zhang J, Ye Z, Zhang Y. 2021. SlRCM1, which encodes tomato Lutescent1, is required for chlorophyll synthesis and chloroplast development in fruits. *Horticulture Research*, **8**, 128.

Liu X Y, Jiang R C, Wang Y, Tang J J, Sun F, Yang Y Z, Tan B C. 2021. ZmPPR26, a DYW-type pentatricopeptide repeat protein, is required for C-to-U RNA editing at atpA-1148 in maize chloroplasts. *Journal of Experimental Botany*, **72**, 4809–4821.

Livak K J, Schmittgen T D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods*, **25**, 402–408.

Lurin C, Andrés C, Aubourg S, Bellaoui M, Bitton F, Bruyère C, Caboche M, Debast C, Gualberto J, Hoffmann B, Lecharny A, Le Ret M, Martin-Magniette M L, Mireau H, Peeters N, Renou J P, Szurek B, Taconnat L, Small I. 2004. Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. *Plant Cell*, **16**, 2089–2103.

Okuda K, Myouga F, Motohashi R, Shinozaki K, Shikanai T. 2007. Conserved domain structure of pentatricopeptide repeat proteins involved in chloroplast RNA editing. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 8178–8183.

Pan M, Xia C, Gu S, He H, Wang G, Weng Y, Pan J, Pan J. 2024. Loss-function-of a UMP kinase leads to impaired chloroplast development and photosynthesis efficiency in cucumber. *Vegetable Research*, **4**, e034.

Small I D, Peeters N. 2000. The PPR motif-a TPR-related motif prevalent in plant organellar proteins. *Trends in Biochemical Sciences*, **25**, 46–47.

Song G, Dong S, Liu C, Zou J, Ren J, Feng H. 2023. BrKCS6 mutation conferred a bright glossy phenotype to Chinese cabbage. *Theoretical and Applied Genetics*, **136**, 216.

Tang J, Zhang W, Wen K, Chen G, Sun J, Tian Y, Tang W, Yu J, An H, Wu T, Kong F, Terzaghi W, Wang C, Wan J. 2017. OsPPR6, a pentatricopeptide repeat protein involved in editing and splicing chloroplast RNA, is required for chloroplast biogenesis in rice. *Plant Molecular Biology*, **95**, 345–357.

Thomson S M, Pulido P, Jarvis R P. 2020. Protein import into chloroplasts and its regulation by the ubiquitin-proteasome system. *Biochemical Society Transactions*, **48**, 71–82.

Tseng C C, Sung T Y, Li Y C, Hsu S J, Lin C L, Hsieh M H. 2010. Editing of *accD* and *ndhF* chloroplast transcripts is partially affected in the *Arabidopsis vanilla cream1* mutant. *Plant Molecular Biology*, **73**, 309–323.

Wang H, Liu J, Zhao W, Terzaghi W, Deng L, Liu H, Zheng Q, Fan S, Hua W, Zheng M. 2023. DELAYED GREENING 409 encodes a dual-localized pentatricopeptide repeat protein required for chloroplast and mitochondrial development. *Plant Physiology*, **192**, 2768–2784.

Wang N, Liu Z, Zhang Y, Li C, Feng H. 2018. Identification and fine mapping of a stay-green gene (*Brnye1*) in pakchoi (*Brassica campestris* L. ssp. *chinensis*). *Theoretical and Applied Genetics*, **131**, 673–684.

Wang Y, Duan Y, Ai P. 2023. OsTHA8 encodes a pentatricopeptide repeat protein required for RNA editing and splicing during rice chloroplast development. *The Crop Journal*, **11**, 1353–1367.

Yamazaki H, Tasaka M, Shikanai T. 2004. PPR motifs of the nucleus-encoded factor, PGR3, function in the selective and distinct steps of chloroplast gene expression in Arabidopsis. *Plant Journal*, **38**, 152–163.

Yu Q B, Jiang Y, Chong K, Yang Z N. 2009. AtECB2, a pentatricopeptide repeat protein, is required for chloroplast transcript accD RNA editing and early chloroplast biogenesis in *Arabidopsis thaliana*. *Plant Journal*, **59**, 1011–1023.

Zhang B, Wu Y, Li S, Ren W, Yang L, Zhuang M, Lv H, Wang Y, Ji J, Hou X, Zhang Y. 2024. Chloroplast C-to-U editing, regulated by a PPR protein BoYgl-2, is important for chlorophyll biosynthesis in cabbage. *Horticulture Research*, **11**, uhae006.

Zhang H, Li X, Yang Y, Hu K, Zhou X, Wen J, Yi B, Shen J, Ma C, Fu T, Tu J. 2022. BnaA02.YTG1, encoding a tetratricopeptide repeat protein, is required for early chloroplast biogenesis in *Brassica napus*. *The Crop Journal*, **10**, 597–610.

Zhao X, Chen L, Yuan K, Liu Y, Yang L, Zhuang M, Zhang Y, Wang Y, Ji J, Fujimoto R, Lv H. 2024. Genetic mapping and gene editing reveal BoAP1 as a crucial factor regulating chloroplast development in *Brassica oleracea*. *Vegetable Research*, **4**, e031.

Zhao Y, Huang S, Wang N, Zhang Y, Ren J, Zhao Y, Feng H. 2022. Identification of a biomass unaffected pale green mutant gene in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Bioscience Reports*, **12**, 7731.

Zhao Z, Tan C, Zhang J, Zhang L, Hou Q, Tang T, Wang B, Zhang Y, Ye X, Zhang Y, Liu Z. 2025. *BrSWN* mutation reduces the H3K27me3 level at the *BrFLC2* and *BrFLC3* loci and confers a late-bolting phenotype in Chinese cabbage. *Plant Journal*, **122**, e70151.

Zhou K, Zhang C, Xia J, Yun P, Wang Y, Ma T, Li Z. 2021. Albino seedling lethality 4; chloroplast 30S ribosomal protein S1 is required for chloroplast ribosome biogenesis and early chloroplast development in rice. *Rice*, **14**, 47.

Zhou W, Cheng Y, Yap A, Chateigner-Boutin A L, Delannoy E, Hammani K, Small I, Huang J. 2009. The Arabidopsis gene *YS1* encoding a DYW protein is required for editing of *rpoB* transcripts and the rapid development of chloroplasts during early growth. *Plant Journal*, **58**, 82–96.

Executive Editor-in-Chief Sanwen Huang
Managing Editor Lingyun Weng