

Supplementary Figures

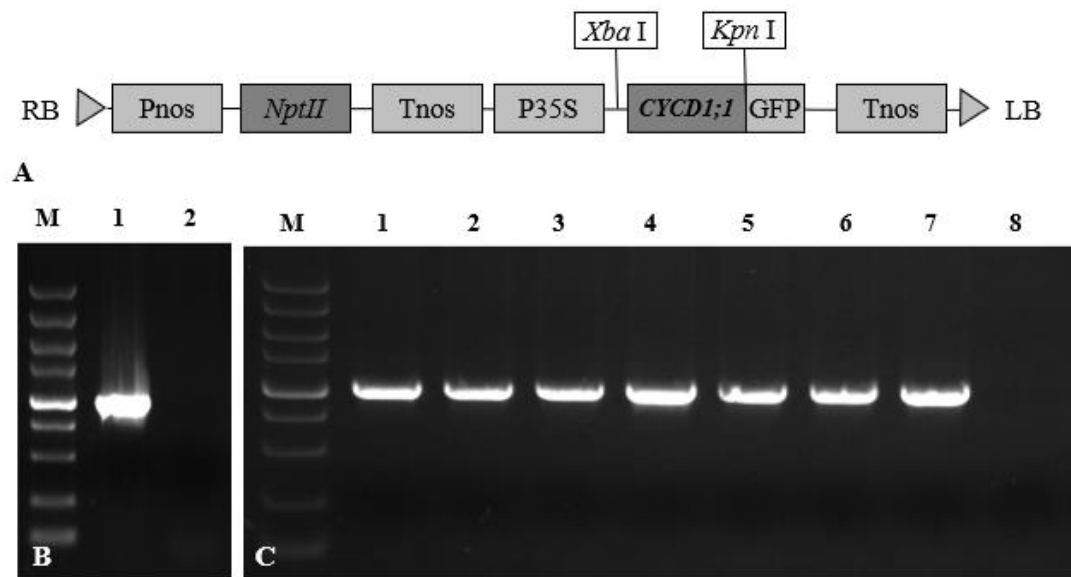


Fig. S1 The construction of pROKII-*PsnCYCD1;1*-GFP vector

A. Model of plant overexpression vector pROKII-*PsnCYCD1;1*-GFP. B. Cloning of *PsnCYCD1;1* gene. M, DNA marker DL 5000; 1, PCR product of *PsnCYCD1;1*; 2, negative control. C. PCR validation of pROKII-35S::*PsnCYCD1;1*-GFP transformants. M, DNA marker DL 5000; 1-7, PCR products of pROKII-35S::*PsnCYCD1;1*-GFP transformants; 8. negative control.

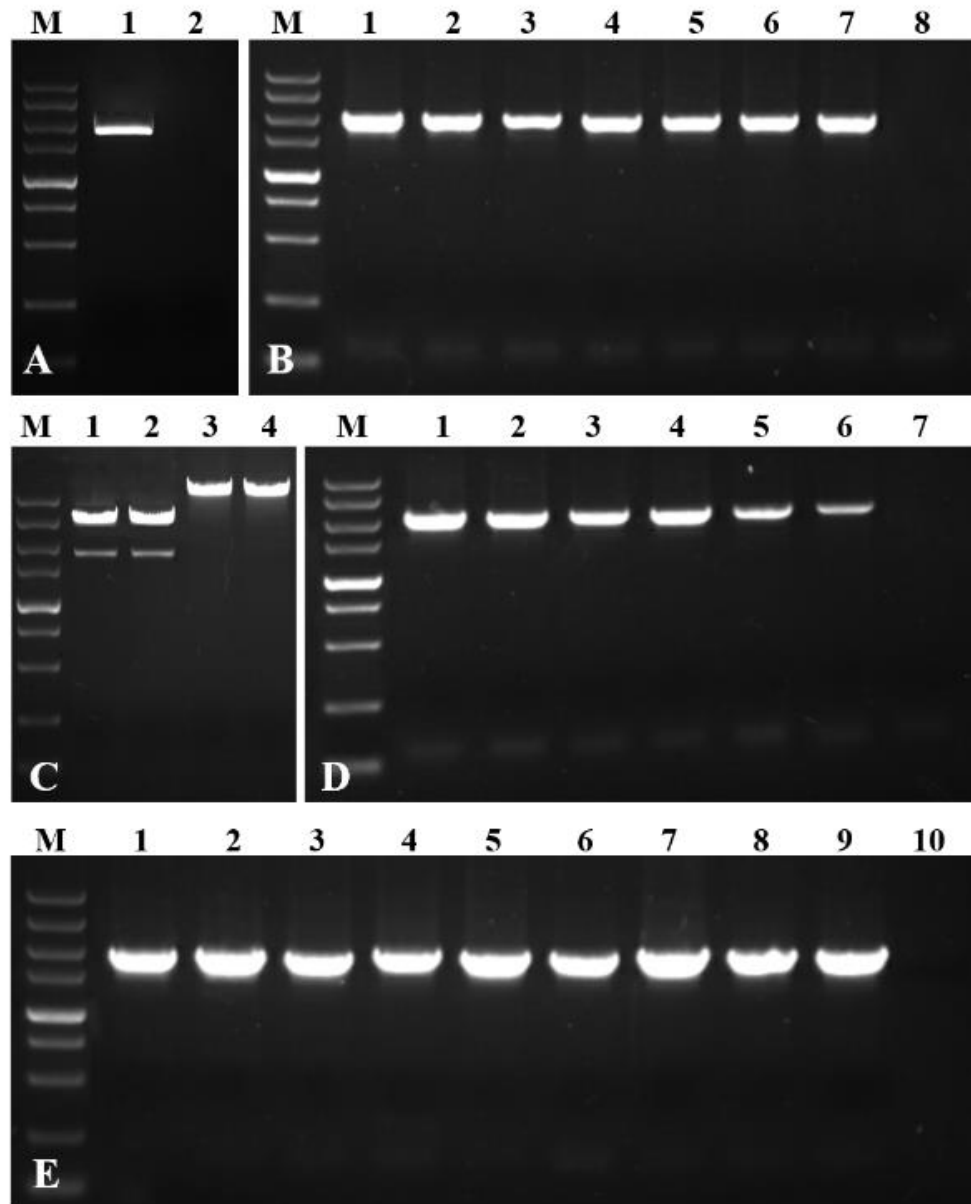


Fig. S2 The construction of pBI121-*PsnCYCD1;1*-promoter-*GUS* vector

A. Cloning of *PsnCYCD1;1* promoter. M, DNA marker DL 5000; 1, PCR product of *PsnCYCD1;1* promoter; 2, negative control. B. PCR detection of pEASY-*PsnCYCD1;1*-promoter transformants. M, DNA marker DL 5000; 1-7, PCR products of pEASY-*PsnCYCD1;1*-promoter transformants; 8, negative control. C. Double enzyme analysis of pEASY-*PsnCYCD1;1*-promoter and pBI121-*GUS*. M, DNA marker DL 5000; 1-2, digested results of pEASY-*PsnCYCD1;1*-promoter; 3-4, digested results of pBI121-*GUS* vectors. D. PCR detection of pBI121-*PsnCYCD1;1*-promoter transformants. M, DNA marker DL 5000; 1-6, PCR products of pBI121-*PsnCYCD1;1*-promoter transformants; 7, negative control. E. PCR validation of pBI121-*PsnCYCD1;1*-promoter transformants in GV3101. M, DNA marker DL 5000; 1-9, PCR products of pBI121-*PsnCYCD1;1*-promoter transformants in GV3101; 10, negative control.

+ TGATCCCGTG GTGGTGTATCA GCACTAATTG ATTTCTGGAT TTTGCGAGAT AGCGAATAGG AGCGGAAACA -1871
- ACTAGGGCAC CACCACTAGT CGTGATTAAC TAAAGACCTA AAACGCTCTA TCGCTTATCC TCGCCTTTGT -1871

AE-box

+ CGTTCGTGGA GACTTAGCAG ACTGATTTTA CAGTTTTCCT ATGTACTTTT GAATCCTTAC AAGTAGAAA -1801
- GCAAGCACCT CTGAATCGTC TGACTAAAA GTCAAAAGGA TACATGAAA CTTAGGAATG TTCATCTTTG -1801

MBS **ABRE**

+ AACTGTAACG CCTATGATGA TGGCTACGTG TACTGGTGTC TTAATTCGGA AAGGAACAGT AATTCCTGTC -1731
- TTGACATTGC GGATACTACT ACCGATGCAC ATGACCACAG AATTAAGCCT TTCTTTGTCA TTAAGGACAG -1731

CAT-box **Box 4**

+ TCTTCATGTC AGGAATTTAT TGTGGGTTT CGTTATTTAG CCACTGGATT GATTAATGAG ATTCATTATT -1661
- AGAAGTACAG TCCTTAAATA ACAACCCAAA GCAATAAATC GGTGACCTAA CTAATTACTC TAAGTAATAA -1661

5UTR Py-rich stretch

+ GAATTTTACA GTAAAAATCAT GGACAGATTG GTAATCTTCT TGAAGATTTT TCTACTCTTT TTCCCTTTTA -1591
- CTTAAAAATG CATTTTAGTA CCTGTCTAAG CATTAGAAGA ACTTCTAAAA AGATGAGAAA AAGGGGAAAT -1591

+ TATTATCATT TATCATTCTT CACCTATTAA ATCATGGACA GTAGTCAGTC CTCTCTGCA TGCAACACAG -1521
- ATAATAGTAA ATAGTAAAGA GTGGATAATT TAGTACCTGT CATCAGTCAG GAGGAGACGT ACGTTTGTGC -1521

+ TCATTAATAA AATAATTTTG ATTGTAATGC ACATTAAATA TTTATTTTTC ATGATTGAT ATGTATTATG -1451
- AGTAATTTTT TTATTAAAC TAACATTACG TGAATTTAT AAATAAAAG TACTAAACTA TACATAATAC -1451

CAAT-box

+ TTTTTCCTCA TGGTATTTT TATTTCAATA AAATAAAATA ATACATTAA GTTAGATTTT TACAGATATT -1381
- AAAAAAAGT ACCATAAAAA ATAAAGTTAT TTTATTTTAT TATGTAAAT CAATCTAAAA ATGTCCTATA -1381

+ GTACTGTATC GTAGTTTATG TATATTTTT AAAAGTATTT TTTTATTTAA AAATATATTA AAATAATTTT -1311
- CATGACATAG CATCAATAC ATATAAAAA TTTTCATAAA AAAATAAATT TTTATATAT TTTATATAAA -1311

ACE **Box 1**

+ AATTTTAAAT ATTAACGTAT TAATAACTTT TCAAAATAAA TATATTTTAA AGATCACACA ACTTTATGAG -1241
- TTAATAACCTA TAATTGCATA ATTATTGAAA AGTTTATTTT ATATAAAAT TCTAGTGTGT TGAAATACG -1241

+ GTGAAATAAC CTTACTGTC CCTCTGTTAA AATCAAAGCT GTCTTGCTCT TTGTGTTTTC GAACCTGGTC -1171
- CACTTTTATG GAATGACAAG GGAGACAATT TTAGTTTCGA CAGAACAGGA AACACAAAAG CTTGAACCAG -1171

TCT-motif **GT1-motif**

+ TCTTACACCA AAATGGTAA GATGTTGGTC AATTAACCTAC TTTTAAATAG TCGTTTAAAC AGGTTAACAT -1101
- AGAATGTGGT TTAAACCATT CTACAACGAG TTAATTGATG AAAATTATTC ACGCAAATG TCCAATTGTA -1101

Box-W1 **LTR**

+ GTTAGAATAG GACTAAATG CACATTTTAA AATACGAGTA CTTGATTGAA TTTTGTCTCT TTTTCCGAA -1031
- CAATCTTATC CTGATTAAAC GTGTAAATTT TTAGTCTCAT GAACCTAACT AAAACGAGAA AAAAAAGCCT -1031

GC-motif **CCGTCC-box** **HD-Zip 3** **circadian**

+ AAACCTCCCC GTCCACTCAG TGTGCTGGTT ATTCGTTGGC TCGTAATCAT TCCCGCAACG AAATCTCCCA -961
- TTTGAGGGGG CAGGTGAGTC ACAGCACCAA TAAGCAACCG AGCATTAGTA AGGGCGTTGC TTTAGAGGGT -961

+ GGTGCGCGC CGGACATGAA AGATCCGAGC GTCGAGGTTG TTGGAAGTAT TATAAAAGT TCGATTCACT -891
- CCAACGCGCG GCCTGTACTT TCTAGGGTCG CAGCTCCAAC AACCTTCATA ATATTTTTC AAGTAAGTCA -891

CAAT-box

+ CACCGCTTTT CTGAGACTG CAATTTGGCC TATTTCTAAG CATCTAACT ATGCAATTTA TTGATGGGAC -821
- GTGGCAGAAA GAATCTGTAC GTTAAACCGG ATAAAGATTC TAGATTGAA TACCGTAAAT AACTACCTG -821

TGA-element **G-Box**

+ AAATTAACCC AGTTTTGTAA ATTTTGTAT AACGACAC GTTTGAAACA CAAGGATTGA GACTGTAAGG -751
- TTTAATTGGG TCAAAACATT TAAACACATA TTTGCTGGTG CAACTTTGT GTTCCCTAACT CTGACATTCC -751

CAAT-box

+ AAATCAATTT TACTGAAAA ATAGAAAGGC TCAACATAAA AATGAAAACT TCGAAAAATC ATATCTATGA -681
- TTTAGTTAAA ATGACTTTTT TATCTTCCG AGTTTGTATT TTACTTTTGA AGCTTTTGTAG TATAGATACT -681

MBS

+ AAAATTATAC ATTCAACAAA TAAAAATTAT TCAACTGTAA ATAATATATT TAAATTAGC GGGAAAAGGA -611
- TTTTAAATAG TAAGTTGTTT ATTTTAAATA AGTTGACATT TATTATATAA ATTTTAAATCG CCTTTTCCCT -611

E2Fa

AT1-motif

+ TGTAGAGCGG GTTTAAGACT CTGAACAAAA TATTATTTTT TTAATAATTA ATTTTTTAAAT TATTTTAAAT -541
- ACATCTCGCC CAAATCTGTA GACTTGTTTT ATAATAAAAA AATTTTAAAT TAAAAAATTA ATAAAAATTA -541

+ TGTTTTAAAT TTATAATTTT TAAAAATAAA AATATATTAT TTTAATATAA AAAAAACACT TTAATAAGT -471
- AAAAAATTAC AATATTAAAA ATTTTATTTT TTATATAATA AAATTATATT TTTTGTGTA AATTTTATCA -471

+ ATTGGAATAA ATCTCTACAG CGCTTTGATT AGATGTTAAA AATAAATGAA AAAAAACAG GGAGTAGAGT -401
- TAAACCTTTT TAGAGATGTC GCGAAACTAA TCTACAATT TTATTACTT TTTTTTTGTG CCTCATCTCA -401

CAAT-box **Sp1**

+ AGGGGCTAGG AAGGAGTAGG CAATGATAAA TCAGATGCCA CCCTAOCAGG TCGCTTCACC TCCGTTCTTG -331
- TCCCGCATCC TTCTCATCCG GTTACTATTT AGTCTACGGT GGGATGGTCC AGCGAAGTGG AGCGAAGAAG -331

CAAT-box **TCT-motif**

+ AAATGAAAA TCAATTTTCC CGGTAAACAA AAGAACCCTA CACTGTCTTA CCTTCTCTCC CCTTTCCTTC -261
- TTTACCTTTT AGTTAAAAAG GCCATTTTGT TTCTTGGGGT GTGACAGAAT GGAAGAGAGG GGAAGGAAG -261

Box 4 **TATA-box** **HSE**

+ ATTAATCTAC TTCCACTCTA AAATAATATAA AACAAAAAG AAAAGCAAAA AAAATTGCA TCCCTTGATA -191
- TAATTAGATG AAGGTGAGAT TTTATATATT TTTGTTTTTC TTTTCGTTTT TTTTAAACGT AGGGAACAT -191

+ TTTATATGGC TACAACACCA AACACTGATG GACATCCCTC CTCCATCTCT CTCTAGCTC AACTTCGAAA -121
- AAATATACCG ATGTTGTGGT TTGTGACTAC CTGTAGGGAG GAGGTAGAGA GAGGATCGAG TTGAAGCTTT -121

circadian **GAG-motif**

+ GCCTCTCTAG CTTAGOCCTT CACTACACAA GAGCATCCCG ATCACTACCC GGTGGAGTCT CCGGCCATCA -51
- CGGAGAGATC GAATCGGGAA GTGATGTGTT CTGTAGGGC TAGTGATGGG CCACCTCAGA GGCCGGTAGT -51

-3-2-1 +1+2+3

+ TCGCGGCAAG ATGAGAGGAG AATGAGAAAC TATTATCATC AAAGGCTGCG ATG -
- AGCGCGTTTC TACTCTCTCT TTACTCTTTG ATAATAGTAG TTTCCGACGC TAC

Fig. S3 Sequence and cis-elements in *PsnCYCD1;1* promoter

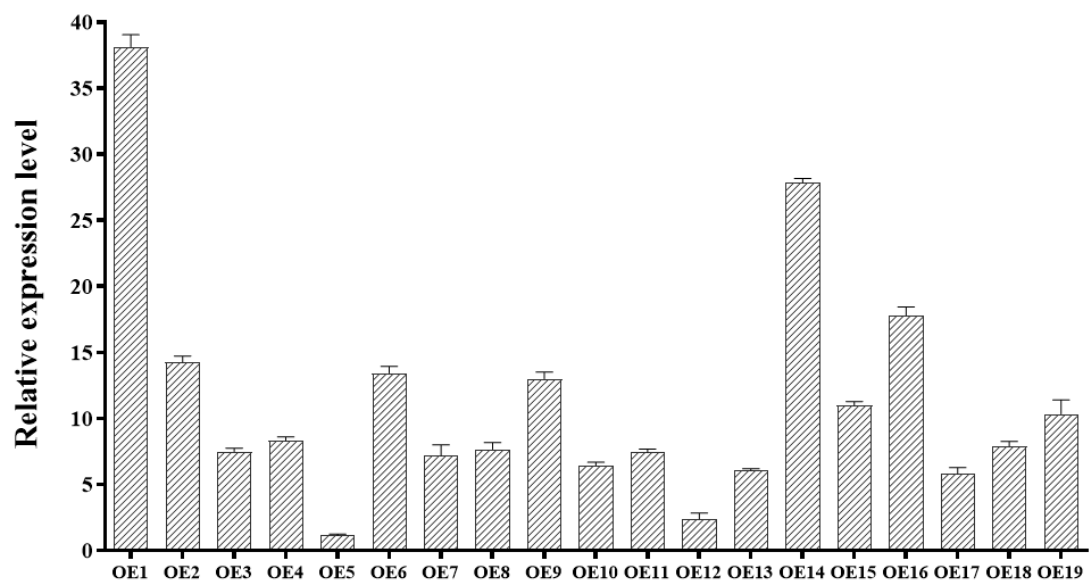


Fig. S4 The expression level of *PsnCYCD1;1* gene in transgenic tobacco

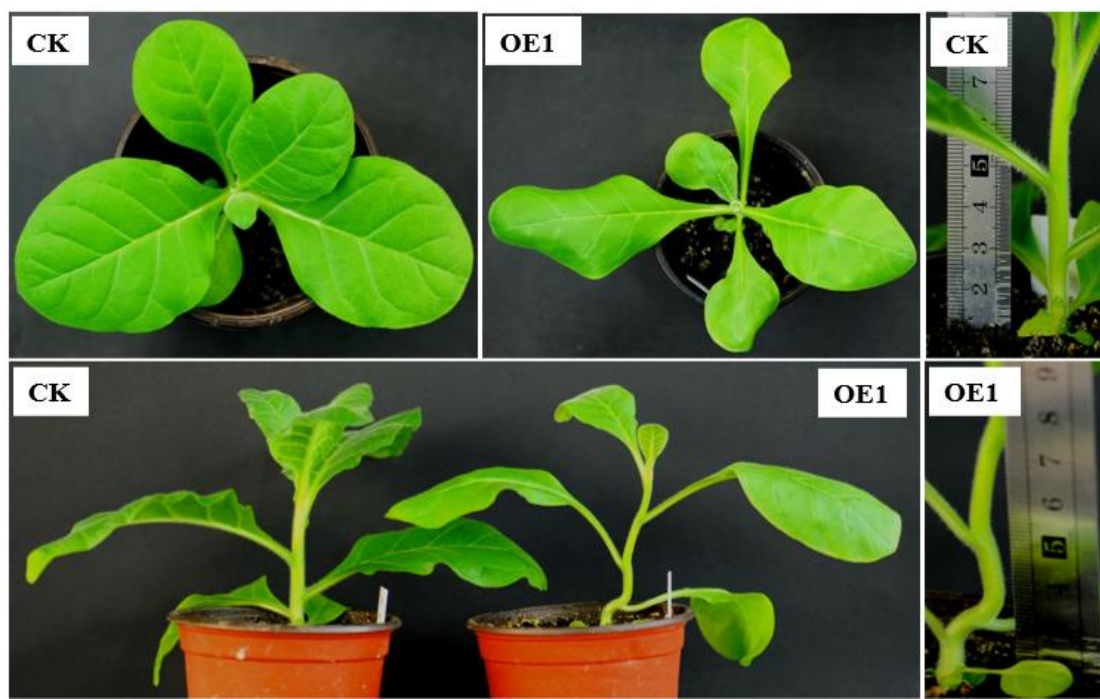


Fig. S5 Phenotype of 30-d seedlings of T₂ generation of transgenic tobacco

CK. Empty vector control; OE1: The transgenic tobacco (*35S::PsnCYCD1;1-GFP*) line 1

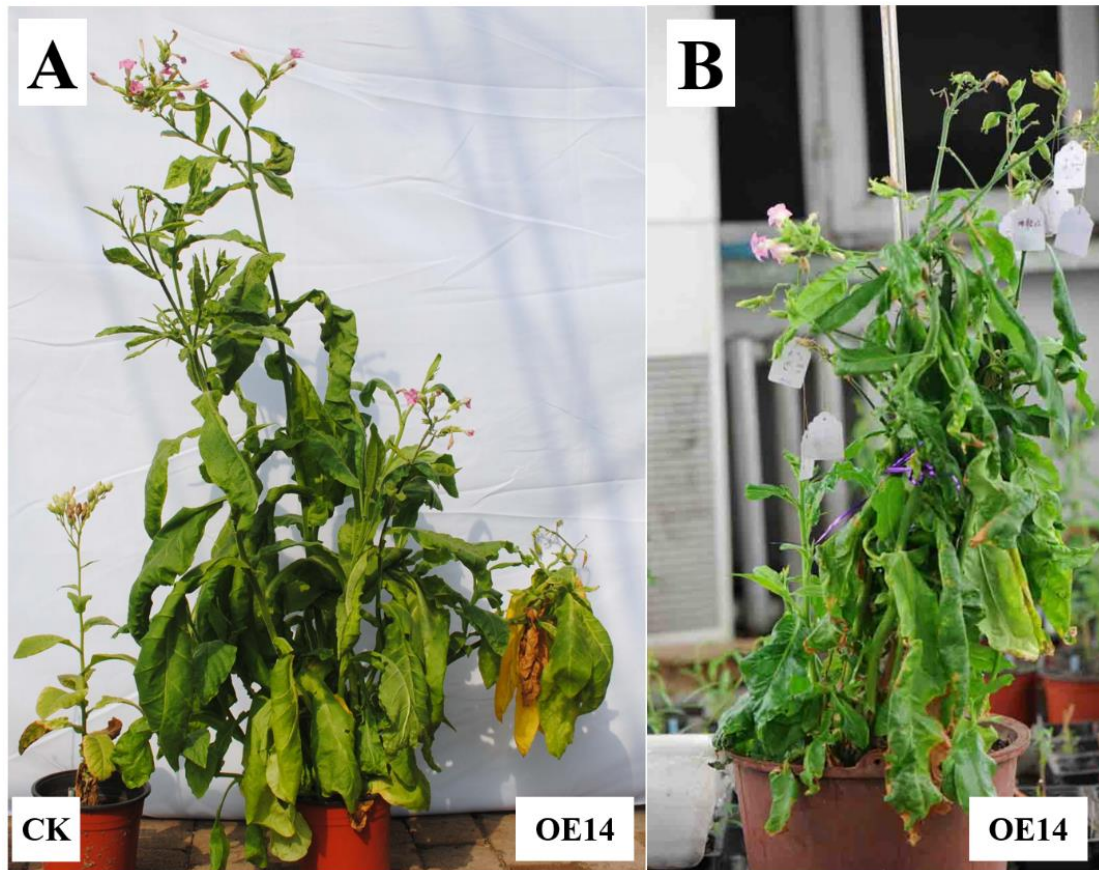


Fig. S6 Special transgenic lines (OE14) that maintain sustained growth
CK. Empty vector control; OE14: The transgenic tobacco (*35S::PsnCYCD1;1-GFP*) line 14

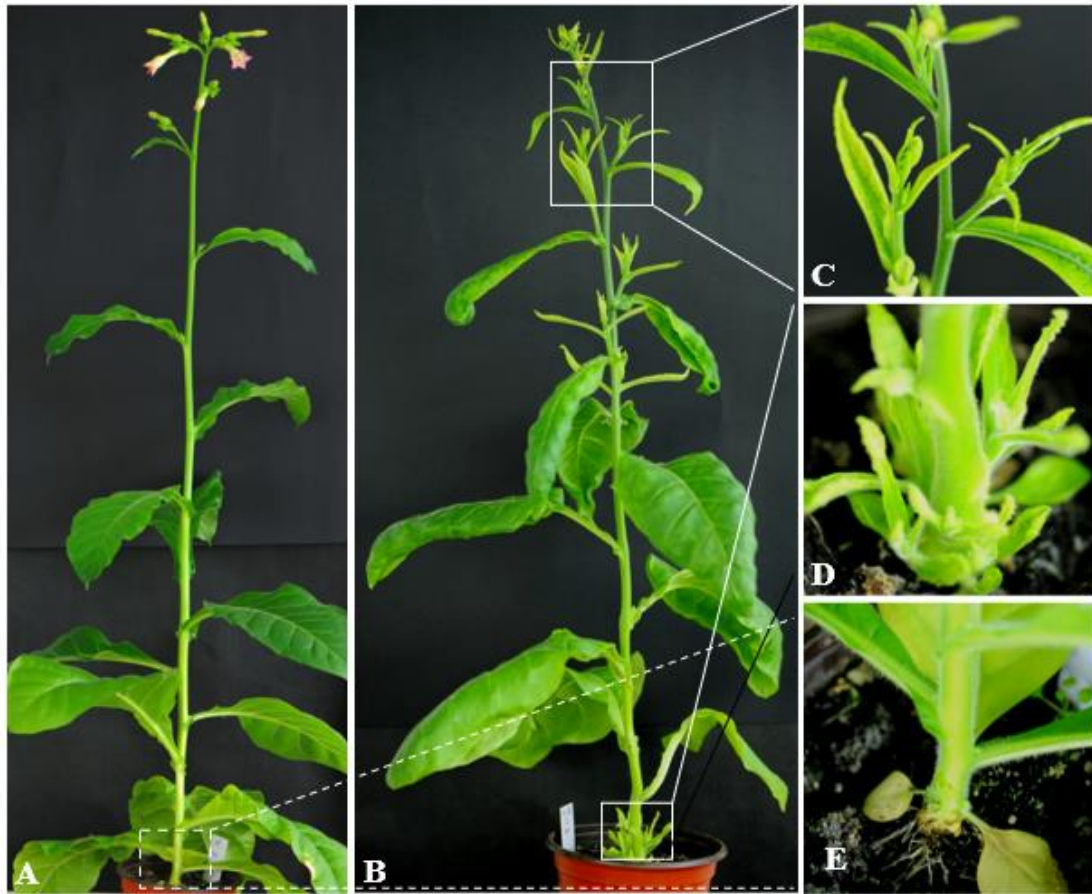


Fig. S7 Phenotype of 60-d seedlings of T₂ generation of transgenic tobacco

A 60 d old control tobacco; B 60-d transgenic line 2; C, D, E Close-up of parts of A or B

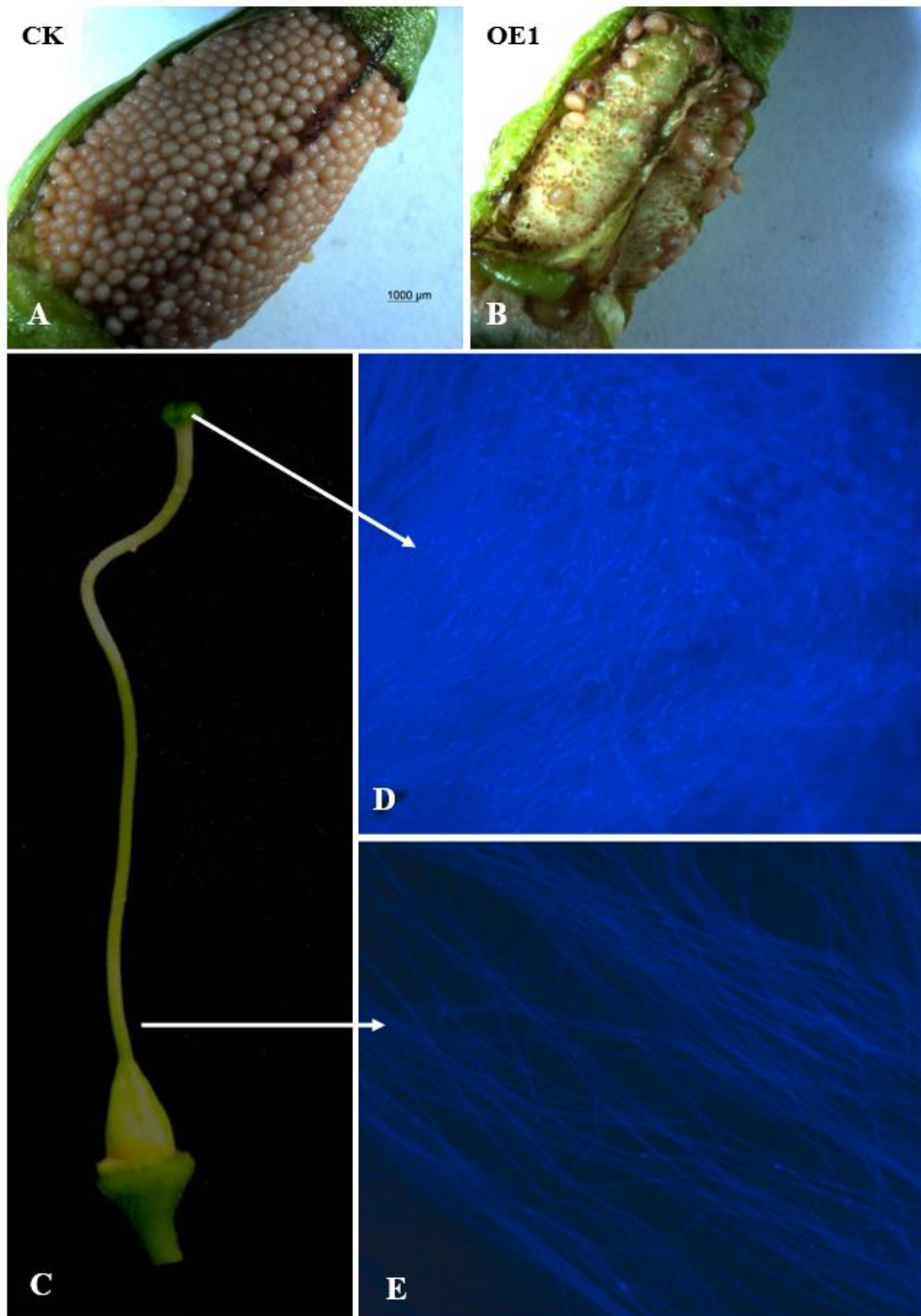


Fig. S8 Fertility analysis of transgenic tobacco

A and B. Microscopic observation of seeds in the ovary 10 days after pollination with own pollens.
 C. Schematic diagram of pistil of transgenic tobacco. D. Staining of pollen tubes on stigma using water-soluble aniline blue staining. E. Staining of pollen tubes on base of style using water-soluble aniline blue staining.

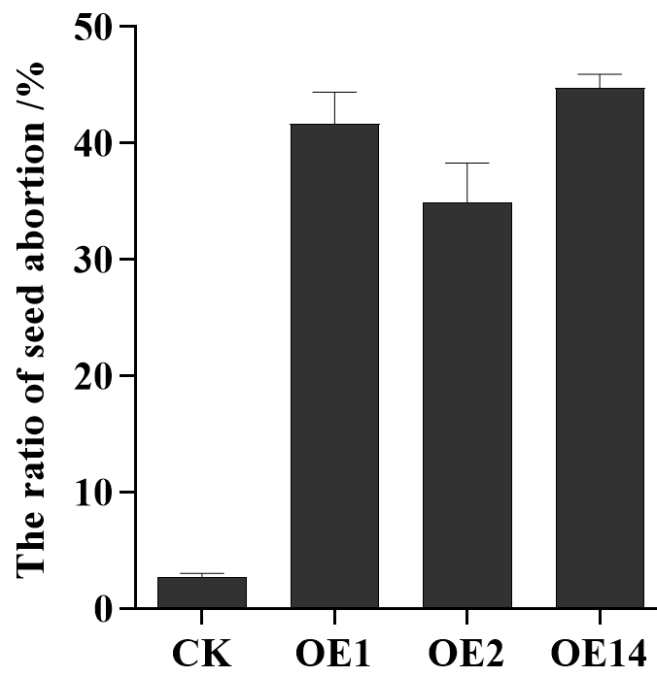


Fig. S9 Comparative analysis abortion rates between control and transgenic tobacco
OE1/2/14: Three individual transgenic lines.

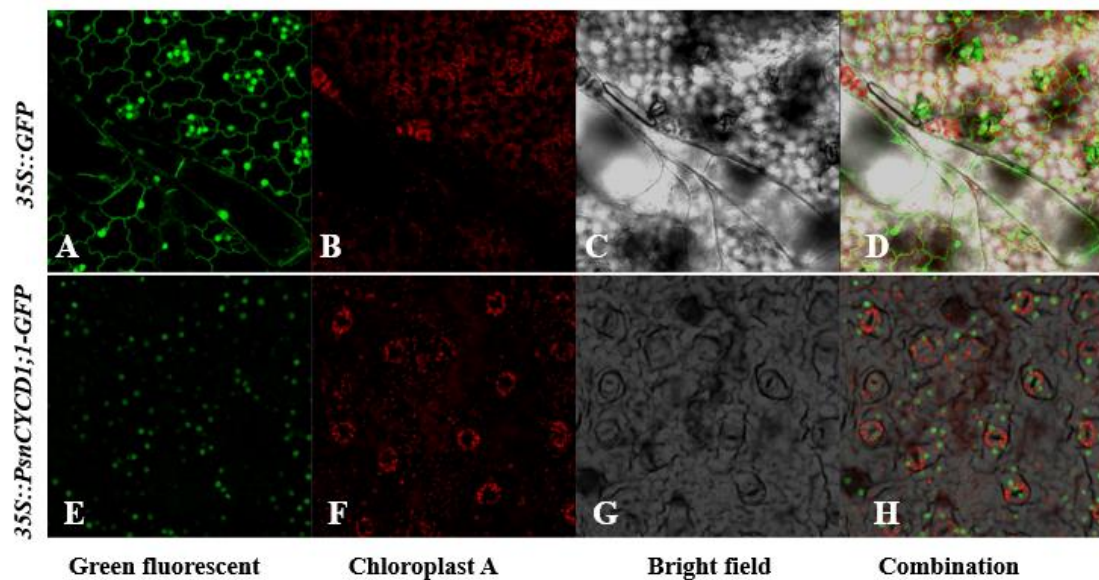


Fig. S10 GFP observation of tobacco leaf lower epidermis
35S::GFP: pROKII-35::GFP empty vector control; *35S::PsnCYCD1;1-GFP*: fusion vector of *PsnCYCD1;1* and *GFP*; Green fluorescent: green fluorescence signal; Chloroplast A: Chloroplast autofluorescence; Bright field: white light; Combination: Combined signals of different fluorescence.

Supplementary Tables

Table S1 The primer sequences used in this study

Name	Sequence (5'--3')	Usage
PsnCYCD1;1-GFP-F	ATCGTCGACGAGATGGCCTACTCTGATTGCTTATCAG	Plant expression vector
PsnCYCD1;1-GFP-R	ATCCCATGGCCTCGGAATTTTCCTTTGTCA	
PsnCYCD1;1-Pro-F	ATCAAGCTTCTGTAAACGCCTATGATGATGGCTAC	
PsnCYCD1;1-Pro-R	ATCGGTACCCGCAGCCTTTGATGATAATAGTTTCTC	Promoter cloning
GUS-F	GGGCGAACAGTTCCTGATTA	
GUS-R	CGAAATATTCCCGTGCACCT	
PsnCYCD1;1-RT-F	CCTCTGGTTCCTTCTCTATTGG	qRT-PCR
PsnCYCD1;1-RT-R	CAGAAACCCAGTATAGGCTCC	
NtActin-RT-F	TGTGTTGGACTCTGGTGATG	
NtActin-RT-R	CGCTCGGTAAGGATCTTCATC	
NtCYCD1-F	GTGCTTCTCCGACTTACTCTG	
NtCYCD1-R	TCTCGAAATAGTCAAATCCAGGG	
NtCYCD2-F	TGGAATACAAGCCTTCTGAGATAG	
NtCYCD2-R	GATGGAATTGGTGCACCTTGG	
NtCYCD3-F	TTTGTTTTGGGAAGATGAAGAGC	
NtCYCD3-R	GCAGTCAAAGCAGAGAAACC	
NtCYCD5-F	ATCGCCAACCCTATACTCAATG	
NtCYCD5-R	TGTCCGTGTAGCAAGAAACC	
NtCYCD6-F	AGCTAATGCGGGATGTTACAG	
NtCYCD6-R	AACCACTCAATCTTCGCCTC	
NtCYCD7-F	GCTTGAATTACAGATGGAAGGTG	
NtCYCD7-R	AGATGGGTAATTTGAGGGACTTG	
NtCDKA1-F	CTGGTGAATCCGAGATTGATG	
NtCDKA1-R	CCAAGCGGAGCATTTTATCAAG	
NtCDKB1-F	GATTGCTGATTTGGGTCTTGG	
NtCDKB1-R	AGAGTCACCAGGAAATAAGGC	
NtE2F-F	GGGAGATGTGGAATAATTGACG	
NtE2F-R	CCATGTGGAGCTTTGATTGC	
NtEXP10-F	AGGATCAAGAAGTGGTTGGC	
NtEXP10-R	TCCAAAAGACCAATGAGCAGG	
NtSTM-F	CCTCGTCTCTTGTCTGCTTATG	
NtSTM-R	CCACTGTTACGGCCTATTGTAG	

NtKNAT1-F	GCTCATCCTCAGTGTTCTAACC
NtKNAT1-R	GATCAAGTTCTGGGTCCTTAGC
NtAS1-F	AGATTGTGAAAGAGCGGAGTG
NtAS1-R	TGACGGTTGAAGTAGCAGTG
NtAS2-F	GGCCGAACTGCTAAAAGATTG
NtAS2-R	CGTATTTTCCCTCGTCTACTGG
