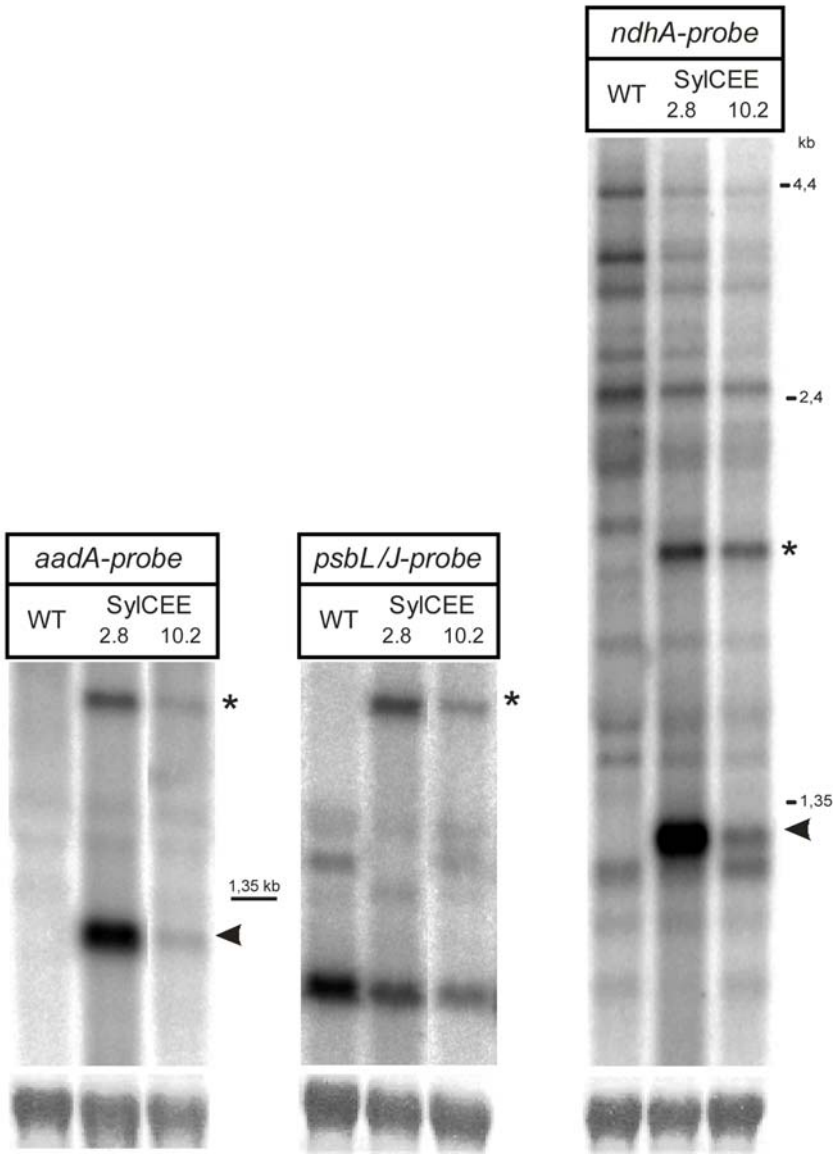


Supplemental Figure 1: Accumulation of transgenic *ndhA* transcripts in lines SylCEE-2.8 and 10.2

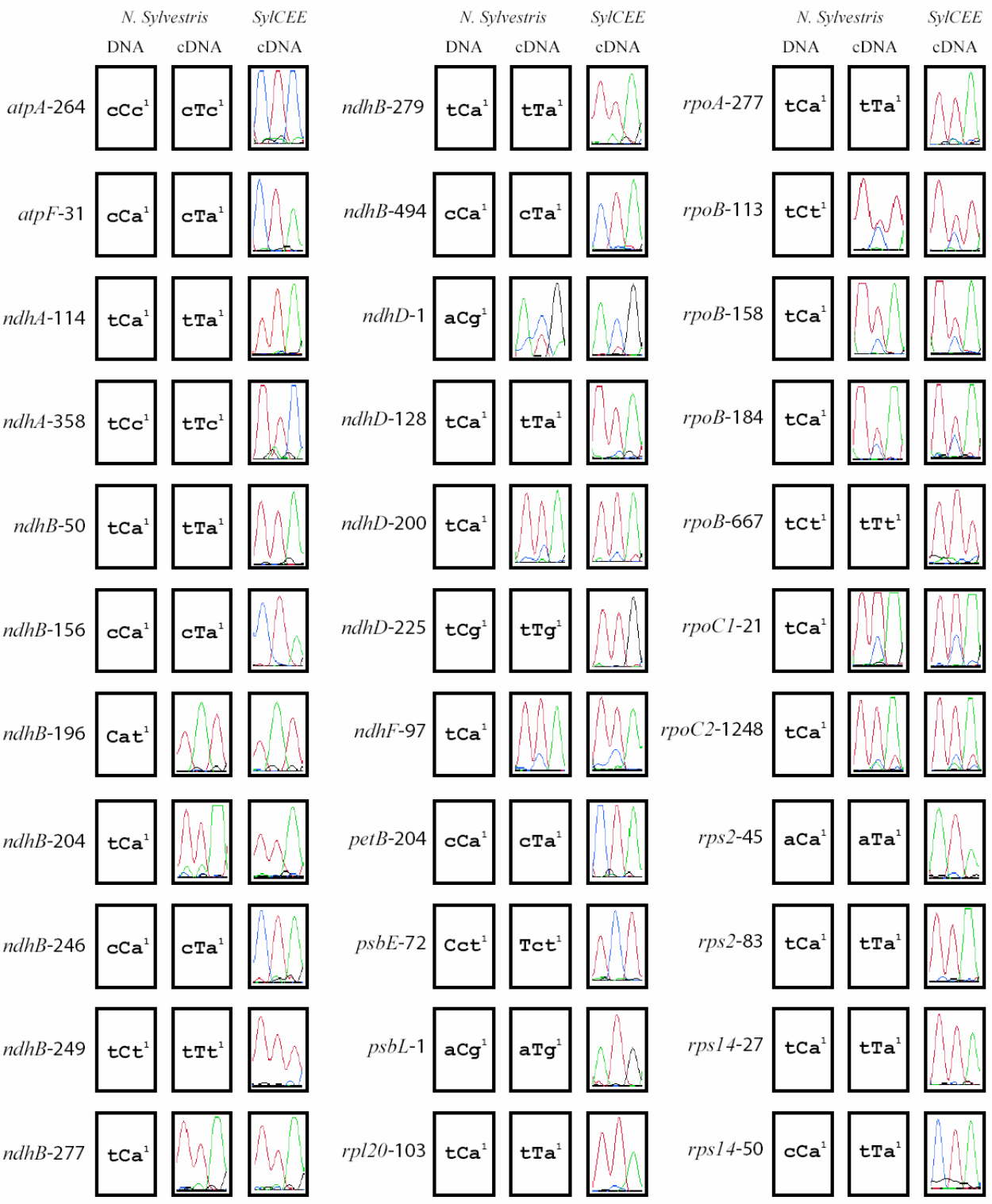
Five micrograms of total plant RNA from the indicated plant lines were analysed by RNA blot hybridization using strand-specific *aadA*, *psbL*/*J* and *ndhA* probes. Arrow heads = monocistronic *aadA*/CEE transcripts. Note the stronger signal intensity of the band corresponding to this transcript in line SylCEE-2.8 in comparison to line 10.2. Quantification of the signals for the monocistronic *aadA* transcripts indicated a 4-fold higher amount of these transcripts in SylCEE-10.2. Importantly, the difference in transcript levels is accompanied by the differences in editing efficiency in the two lines (Fig. 1D). Transcripts of ~2.1 kb length detected in all three

hybridization experiments (marked by asterisks) result from read-through transcription initiating at the *psbE* promoter (Bock et al., 1994). Methylene blue stainings of the 16S rRNA shown below each panel demonstrate comparable sample loading in all lanes.

CEE *ndhA*-189	ATCTCT-ACTATCTAATA-GTTCAA
tobacco *ndhF*-97:	CCCACTTACT-TCTATTATG-TCAA

Supplemental Figure 2: Homology of the introduced spinach *ndhA* editing site to the endogenous tobacco *ndhF* site.

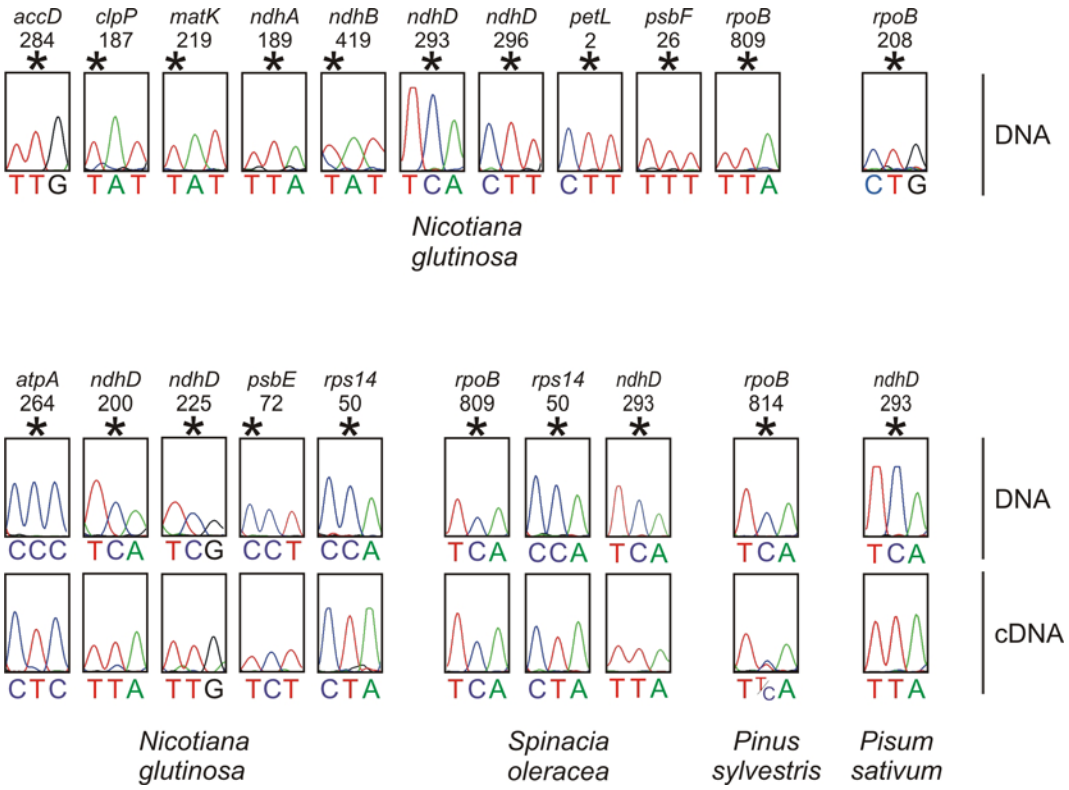
The nucleotide regions surrounding the described editing sites of *N. tabacum* and *N. sylvestris* were extracted and subsequently compared with the 20 nt. upstream of CEE *ndhA*-189. Allowing gaps ("-"), the highest similarity was found for site *ndhF*-97 (editing site: bold "C", identical nt labelled in grey). However, the fact that editing of *ndhF*-97 is not reduced in either *N. tabacum* (Schmitz-Linneweber et al., 2001) or *N. sylvestris* plants (Supplementary Figure 4) overexpressing CEE *ndhA*-189 argues against the two sites being co-edited by the same factors.



Supplemental Figure 3: Analysis of all known editing sites in SylCEE plants.

Analysis of all known editing sites from the SylCEE transcriptome according to Sasaki *et al.* (2003). Editing status was determined by sequencing cDNA-derived PCR products covering the respective areas of interest. Where partial editing was found, complementary data from wild-type

N. sylvestris plants were obtained as well. Altogether, no significant reduction in editing relative to wild-type was observed in SylCEE plants.



Supplemental Figure 4: Test for the presence and processing status of several plastid editing sites in different plant species.

Sequence analysis of *Nicotiana glutinosa* DNA. The chromatogram excerpts show the indicated codon of the given gene. Asterisks mark the position of editing sites known from different angiosperm species and tested for their presence and – if necessary - processing status in species indicated below the chromatograms. The sites analysed in *N. glutinosa* are absent from *Nicotiana tabacum*. For cDNA analysis of *ndhD*-293 in *N. glutinosa* see Fig. 2A.

Supplemental Table 1: Oligonucleotides

Plant(s)	Target	Name	Sequence	Purpose
SylCEE	ORF103	P1a	CACACAATTTAAGTAGATGCG	check of integration at left border
	aadA	P1b	ACTGCGGAGCCGTACAAATG	
	aadA	P2a	CTGTTCTTATTTTACCGGAGG	check of Integration at right border
	petA	P2b	CTTTCCAAAGCTTCAATCTCTCGCATTCTG	
	psbL	ApsbL	TACGACACAATCAAACCCGA	probe *psbL/J*
	psbJ	AT7psbJ	GATAATACGACTCACTATAGGGTACTAGAGGGATGAACCCAAT	
	aadA	aadAli	GAAGCGGTTATCGCCGAAG	probe *aadA*
	aadA	aadAT7	GTAATCGACTCACTATAGGGAACCGGATCAAAGAGT	
	ndhA	ndhAfor	ACAGGAGATACTCGTTTATT	probe *ndhA*
	ndhA	AT7ndhA	GATAATACGACTCACTATAGGGACGAGGTTGTCAATAATAGAT	
	aadA	P2a	see above	amplification and sequencing of introduced *ndhA* editing site
	CEE	hindndhAex2rev	CTTTCCAAAGCTTCAATCTCTCGCATTCTG	
Syl CEE and Nicotiana sylvestris	*atpA*	EcoatpAfor	TTCCAGAATTCACATTACAATACCTTGCTCC	amplification and sequencing of *N. sylvestris* and *N. sylvestris* cDNA
		Ecoatparev	TTCCAGAATTCTTTCCAAAAGGCGTGAATGC	
	atpF	atpFforP	ATGAAAAACGTAACCGATTC	
		atpFrevP	TTGTCGGACTTGATTAATCG	
	ndhA	ndhAfor	ACAGGAGATACTCGTTTATT	
		ndhAntex2173 rev	TGCTACTAATTCTTCTTCTGCTTCTG	
		tommunedfor	GATTATCTAACAGTTTAAGTACAGTTGATATAG	
		ndhA3'rev	CTTGTTTGAGACAAGTCGTG	
	ndhB	ndhBfor2	TCATGATCTGGCATGTACAG	
		ndhBedIII	ATTTCTTGAAGCTCAATCTCTCCCCCGGAT	
		nb11	TTCATGCTTGTTTGAGTAATAGC	
		ndhBP12	GATATACCAAGAAAGATGTACG	
		AndhB	GTCGTTGCTTTTCTTTCTG	
		ndhB3for	TCTCTTATCCCTAGGAGGTC	
		ndhBrev2	CTAAAAAAGGCTATCCTGAGC	
	ndhD	ndhDStart5'	GGTCCAAGTGTATCTTGTC	
		Zagreb7	GCTCCCATTTTTAATAAAATTCC	
		ndhDMitterev	CTAAAGTAGTGATAAATCCTG	
	ndhF	ndhFforP	GGAACAGACATATGAATATG	
		ndhFrevP	ATCCCAACATGGAAGTACTG	
	petB	petBPfor	TTCCTGTAATAGGATCACCC	
		petB3'revP	AATGATTGATTACAAATATTTAG	
	psbE/L	ApsbE	AAGCACAGGAGAACGTTCGT	
		AT7psbL	GATAATACGACTCACTATAGGGTCTTAATTGAAGAAATAATTGGA	
	rpl20	rpl20edfor	ATTCGCGAAAGGGGAGTATC	
		rpl20rev3	ATTTAAATTATTCTGGTGG	
	rpoA	ArpoA	GGATTACAAATAGAGAGAAAT	
		AT7rpoA	GTAATACGACTCACTATAGGGATTCAAAAGGTCCAACAATGT	
	rpoB	rpoBforP	GGAAAAACAGTAGGGATATGC	
		rpoBrevP	GAAAATACCGGATCGCCACC	
		rpoBfor2	TCTTTTAGCAGGTAATGGAG	
		rpoCb4	GGTTCAAATACCCATGGATT	
	rpoC1	rpoC15'for	AATAATTTTTCTTCTATGATCG	
		rpoC15'rev	AAATAATCCATCTTTTTCCG	
	rpoC2	ArpoC2	AGCTAAGCCTTATTTGGCCAC	

		AT7rpoC2	GTAATACGACTCACTATAGGGCTCGCTTCAGATATGAAACTTTG	
	rps2	rps2forP	AATGGAATCCTAAAATGGCG	
		rps2revP	CTGGAATTGAAATATCTGCG	
	rps14	Arps14	ATGGCAAGGAAAAGTTTGAT	
		AT7rps14	GTAATACGACTCACTATAGGGCCTTACCAACTTGATCTTGTT	
Solanaceans and cybrids	*ndhD*	ndhDrev	CTCCATTAAGTCCCGTGTCG	editing of *ndhD*-293
		ndhDmittefor	CTAAAGTAGTGATAAATCCTG	
	ndhA	ndhA.nt.ex2.173.rev	TGCTACTAATTCTTCTTCTGCTTCTG	editing of *ndhA*-189
		ndhAfor	ACAGGAGATACTCGTTTATT	
Nicotiana glutinosa	*accD*	psbL14	TTCTCCGAATGAAATTCAAT	amplification and sequencing of *N. glutinosa* plastid DNA
		accDrev	AATGGGATCTAGAGAGACC	
		accDfor2	TAATGATCTCGAGGTAACTC	
		AT7accD	GTAATACGACTCACTATAGGGTGATTTTCTCTCCGACTAC	
	atpA	atpAMittefor	GAGATCAGAAGCAAATTGTGC	
		EcoatpAfor	TTCCAGAATTCACATTACAATACCTTGCTCC	
		EcoatpArev	TTCCAGAATTCTTTCCAAAAGGCGTGAATGC	
	clpP	clpPtKo	GGGTTGGTTTAGATTGATCC	
		clpPex2for	GTGCGACCAGATGTCCATAC	
		clpP.AT.for	GTAATGATCCATCAACCCGC	
		clpP.AT.2rev	TGAACCGCTACAAGATCAAC	
	matK	AbmatKfor	CTTTATCTTCTTTCGAAGGC	
		K7	AAAAATATCCAAATACCAAA	
	ndhA	ndhA.nt.ex2.173.rev	TGCTACTAATTCTTCTTCTGCTTCTG	
		ndhA.nt.in.174.rev	CCTTTTTACTGCAAATTTAGAAGCCG	
		Tommunedfor	GATTATCTAACAGTTTAAGTACAGTTGATATAG	
		ndhA3'rev	CTTGTTTGAGACAAGTCGTG	
		ndhAfor	ACAGGAGATACTCGTTTATT	
	ndhB	AndhB	GTCGTTGCTTTTCTTTCTG	
		ndhBrev2	CTAAAAAAGGCTATCCTGAGC	
		ndhB3for	TCTCTTATCCCTAGGAGGTC	
	ndhD	ndhDstart5´	GGTCCAAGTGTATCTTGTC	
		ndhDrev	CTCCATTAAGTCCCGTGTCG	
		Zagreb7	GCTCCCATTTTTAATAAAATTCC	
		ndhDMittefor	AGTACTTGTATGCTTCTAGC	
		ndhDrev	CTCCATTAAGTCCCGTGTCG	
		Zagreb6	GAATTTTATTAAAAATGGGAGCG	
	petL	petL5'forP	ATTTGAAACTTAGGTAAGTGC	
		AT7petL	GATAATACGACTCACTATAGGGAATCTCAATGACAATGAACA	
		AT7petG	GATAATACGACTCACTATAGGGTTAATTAATCAAAGGTCCAA	
	psbF	ApsbE	AAGCACAGGAGAACGTTCGT	
		AT7psbL	GATAATACGACTCACTATAGGGTCTTAATTGAAGAAATAATTGGA	
	rpoB	rpoBforP	GGAAAAACAGTAGGGATATGC	
		rpoBrevP	GAAAATACCGGATCGCCACC	
		rpoBfor2	TCTTTTAGCAGGTAATGGAG	
		rpoCb4	GGTTCAAATACCCATGGATT	
	rps14	Arps14	ATGGCAAGGAAAAGTTTGAT	
		AT7rps14	GTAATACGACTCACTATAGGGCCTTACCAACTTGATCTTGTT	