

Supplementary Materials: Identification and Analysis of RNA Editing Sites in the Chloroplast Transcripts of *Aegilops tauschii* L.

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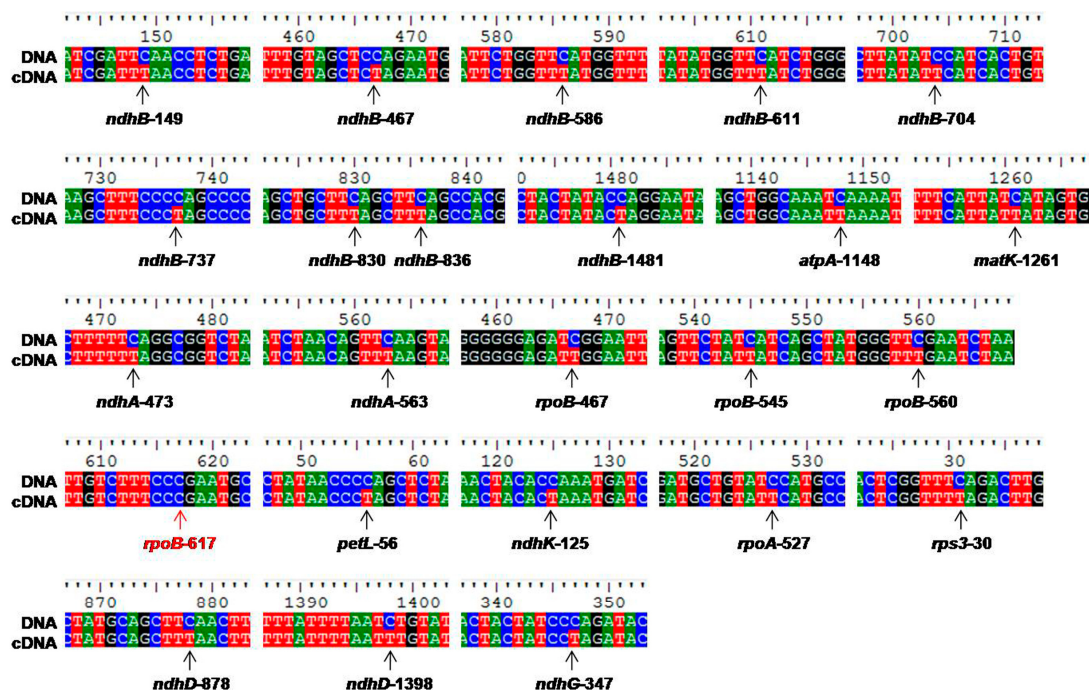


Figure S1. RNA editing sites confirmed experimentally, except *rpoB-617* which was not edited. Arrow points to the editing site.

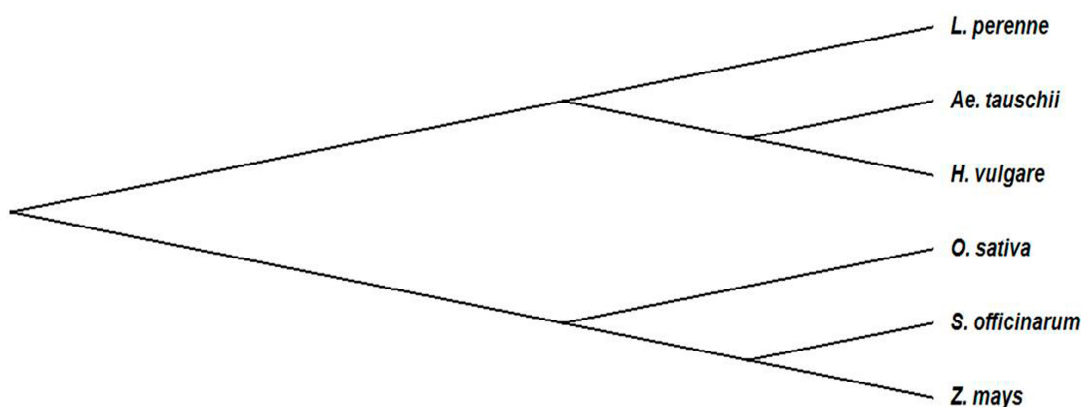


Figure S2. The phylogeny of the compared six Poaceae species, *Ae. tauschii*, *H. vulgare*, *L. perenne*, *O. sativa*, *S. officinarum* and *Z. mays*. The phylogenetic tree of the compared six Poaceae species was reconstructed using maximum likelihood (ML) based on the RNA editing sites' composition.

Table S1. Information of RNA-Seq data of *A. tauschii* (AL8/78).

Tissue	SRA Accession	Tissue	SRA Accession
Leaf1	SRX209402	Seed3	SRX209414
Leaf2	SRX209403	Sheath1	SRX209415
Leaf3	SRX209404	Sheath2	SRX209416
Leaf4	SRX209405	Sheath3	SRX209417
Leaf5	SRX209406	Sheath4	SRX209418
Pistil1	SRX209407	Spike1	SRX209419
Root1	SRX209408	Spike2	SRX209420
Root2	SRX209409	Spike3	SRX209421
Root3	SRX209410	Stamen1	SRX209422
Root4	SRX209411	Stem1	SRX209423
Seed1	SRX209412, ,	Stem2	SRX209424
Seed2	SRX209413	Stem3	SRX209425

Table S2. Primer information.

Name	Sequence (5' to 3')	Length (bp)	Tm (°C)	GC%	Product Length (bp)
ndhB-F	GAAAGCGTTTCATTTGCTTCTC	22	56.3	40.9	1477
ndhB-R	CTAAAAGAGGGTATCCTGAGCAAT	24	58.6	41.7	
atpA-F	CTGCGGATCTATTCAATGC	19	55.4	47.4	153
atpA-R	CAAAGGCTTGTAACCTGCGA	21	58.0	47.6	
matK-F	GAATCAAATGCTGGAGAAGTCA	22	56.3	40.9	338
matK-R	CCGAACCCAATCGTTGC	17	57.0	58.8	
ndhA-F	ATTGGTCTTCTTATGGCAGG	20	55.8	45.0	288
ndhA-R	TTCCTCCGCTTCTGGTAAAT	20	55.8	45.0	
rpoB-F	GGGAATTTCTATCTACACTGGCA	23	58.4	43.5	367
rpoB-R	TCGACCAATCCTTCCTAATTCAC	23	58.4	43.5	
petL-F	AACTAGTTATTTTGGTTTCTACTG	25	51.4	28.0	85
petL-R	TCAAATAAGGCGTATCTTGT	20	49.2	35.0	
ndhK-F	AAGGAAAAGATTCTATTGAGACAGT	25	53.0	32.0	312
ndhK-R	TTTGCTCGTATAATCTCACTAAAGA	25	53.0	32.0	
rpoA-F	GCCTTTATTATGGTCGTTTCA	21	51.7	38.1	510
rpoA-R	GTTTTTCATTTCCATTCCCAC	21	51.7	38.1	
rps3-F	GGACAAAAAATAAATCCACTCGGT	24	54.4	37.5	433
rps3-R	TAGGCTGTCTATAAGGTTCTTTCA	24	54.4	37.5	
ndhD-F	TCGGAGTTCTGGGTATGG	18	54.9	55.6	932
ndhD-R	TACCCTGTCAACGGATAGGGAG	22	59.5	54.5	
ndhG-F	GTAATGTTCGTAAATGGCTCAG	22	54.0	40.9	249
ndhG-R	CGAAATGAGTTCAAACGGAAGA	22	54.0	40.9	

Table S3. RNA editing sites validated by each primer pair.

Primer Pair	RNA Editing Sites to Be Validated
ndhB-F/ndhB-R	All the 9 sites in <i>ndhB</i>
atpA-F/atpA-R	<i>atpA</i> -1148
matK-F/matK-R	<i>matK</i> -1261
ndhA-F/ndhA-R	<i>ndhA</i> -473, <i>ndhA</i> -563
rpoB-F/rpoB-R	<i>rpoB</i> -467, <i>rpoB</i> -545, <i>rpoB</i> -560, <i>rpoB</i> -617
petL-F/petL-R	<i>petL</i> -56
ndhK-F/ndhK-R	<i>ndhK</i> -125
rpoA-F/rpoA-R	<i>rpoA</i> -527
rps3-F/rps3-R	<i>rps3</i> -30
ndhD-F/ndhD-R	<i>ndhD</i> -878, <i>ndhD</i> -1398
ndhG-F/ndhG-R	<i>ndhG</i> -347

Table S4. Quantity of protein secondary structures before and after editing.

Gene	Secondary Structure			Gene	Secondary Structure		
	H	E	T		H	E	T
<i>atpA</i>	B	432	209	<i>rpl2</i>	B	88	39
	A	432	209		A	91	39
<i>atpB</i>	B	349	212	<i>rpl20</i>	B	74	83
	A	349	212		A	75	84
<i>matK</i>	B	368	417	<i>rpoA</i>	B	254	147
	A	368	418		A	254	147
<i>ndhA</i>	B	306	254	<i>rpoB</i>	B	777	716
	A	309	260		A	778	729
<i>ndhB</i>	B	373	378	<i>rpoC2</i>	B	1021	945
	A	378	399		A	1027	955
<i>ndhD</i>	B	402	337	<i>rps8</i>	B	96	69
	A	402	344		A	96	69
<i>ndhF</i>	B	539	528	<i>ycf3</i>	B	105	75
	A	541	528		A	109	85
<i>petB</i>	B	153	178				
	A	153	178				

H, E and T indicates α -helix, β -sheet and turn, respectively. B, before editing; A, after editing.

Table S5. Conversion of protein secondary structure before and after editing.

Secondary Structure	Conversion	Gene	Number
α -helix	Increase	<i>ndhA, ndhB, ndhF, rpl2, rpl20, rpoB, rpoC2, ycf3</i>	8
	Reduce	None	0
	No change	<i>atpA, atpB, matK, ndhD, petB, rpoA, rps8</i>	7
β -sheet	Increase	<i>matK, ndhA, ndhB, ndhD, rpl20, rpoB, rpoC2, ycf3</i>	8
	Reduce	None	0
	No change	<i>atpA, atpB, ndhF, petB, rpl2, rpoA, rps8</i>	7
turn	Increase	<i>atpB, rpl2</i>	2
	Reduce	<i>atpA, matK, ndhA, ndhB, ndhD, rpoB, rpoC2, ycf3</i>	8
	No change	<i>ndhF, petB, rpl20, rpoA, rps8</i>	5

Table S6. RNA editing sites in chloroplast genes of *T. aestivum* cv CS (TA3008) predicted by Prep-Cp.

Gene	Nucleotide Position	Amino Acid Position	Codon Conversion	Amino Acid Conversion
<i>ndhA</i>	473	158	tCa→tTa	S→L
	563	188	tCa→tTa	S→L
	1070	357	tCt→tTt	S→F
<i>atpA</i>	1148	383	tCa→tTa	S→L
<i>atpB</i>	35	12	gCt→gTt	A→V
	1487	496	tCg→tTg	S→L
<i>matK</i>	1261	421	Cat→Tat	H→Y
	149	50	tCa→tTa	S→L
	467	156	cCa→cTa	P→L
<i>ndhB</i>	586	196	Cat→Tat	H→Y
	611	204	tCa→tTa	S→L
	704	235	tCc→tTc	S→F
	737	246	cCa→cTa	P→L
	830	277	tCa→tTa	S→L
	836	279	tCa→tTa	S→L

	1481	494	cCa→cTa	P→L
<i>ndhD</i>	878	293	tCa→tTa	S→L
<i>ndhF</i>	62	21	tCa→tTa	S→L
	1487	496	aCg→aTg	T→M
<i>petB</i>	662	221	cCa→cTa	P→L
	2009	670	cCa→cTa	P→L
	2030	677	cCa→cTa	P→L
<i>rpoC2</i>	2158	720	Ccc→Tcc	P→S
	3002	1001	cCg→cTg	P→L
	4031	1344	tCg→tTg	S→L
<i>rpl2</i>	62	21	aCt→aTt	T→I
<i>rpl20</i>	308	103	tCa→tTa	S→L
<i>rpoA</i>	1009	337	Ctc→Ttc	L→F
	467	156	tCg→tTg	S→L
<i>rpoB</i>	545	182	tCa→tTa	S→L
	560	187	tCg→tTg	S→L
	617	206	cCg→cTg	P→L
<i>rps8</i>	182	61	tCa→tTa	S→L
<i>ycf3</i>	44	15	tCc→tTc	S→F
	191	64	aCg→aTg	T→M

Capitals in column Codon Conversion indicate target nucleotides.

Table S7. RNA editing sites in chloroplast genes of *T. urartu* (PI428335) predicted by Prep-Cp.

Gene	Nucleotide Position	Amino Acid Position	Codon Conversion	Amino Acid Conversion
	473	158	tCa→tTa	S→L
<i>ndhA</i>	563	188	tCa→tTa	S→L
	1070	357	tCt→tTt	S→F
<i>atpA</i>	1148	383	tCa→tTa	S→L
<i>atpB</i>	1487	496	tCg→tTg	S→L
<i>matK</i>	1261	421	Cat→Tat	H→Y
	149	50	tCa→tTa	S→L
	467	156	cCa→cTa	P→L
	586	196	Cat→Tat	H→Y
<i>ndhB</i>	611	204	tCa→tTa	S→L
	704	235	tCc→tTc	S→F
	830	277	tCa→tTa	S→L
	836	279	tCa→tTa	S→L
	1481	494	cCa→cTa	P→L
<i>ndhD</i>	878	293	tCa→tTa	S→L
<i>ndhF</i>	62	21	tCa→tTa	S→L
	1487	496	aCg→aTg	T→M
<i>petB</i>	662	221	cCa→cTa	P→L
	2009	670	cCa→cTa	P→L
	2030	677	cCa→cTa	P→L
<i>rpoC2</i>	2158	720	Ccc→Tcc	P→S
	3002	1001	cCg→cTg	P→L
	4031	1344	tCg→tTg	S→L
<i>rpl2</i>	62	21	aCt→aTt	T→I
<i>rpl20</i>	308	103	tCa→tTa	S→L

<i>rpoA</i>	1009	337	Ctc→Ttc	L→F
	467	156	tCg→tTg	S→L
<i>rpoB</i>	545	182	tCa→tTa	S→L
	560	187	tCg→tTg	S→L
	617	206	cCg→cTg	P→L
<i>rps8</i>	182	61	tCa→tTa	S→L
<i>ycf3</i>	44	15	tCc→tTc	S→F
	191	64	aCg→aTg	T→M

Capitals in column Codon Conversion indicate target nucleotides.

Table S8. RNA editing sites in chloroplast genes of *Ae. speltoides* (AE918, TA1796 and PI487232) predicted by Prep-Cp.

Gene	Nucleotide Position	Amino Acid Position	Codon Conversion	Amino Acid Conversion
<i>ndhA</i>	473	158	tCa→tTa	S→L
	563	188	tCa→tTa	S→L
	1070	357	tCt→tTt	S→F
<i>atpA</i>	1148	383	tCa→tTa	S→L
<i>atpB</i>	35	12	gCt→gTt	A→V
<i>atpB</i>	1487	496	tCg→tTg	S→L
<i>matK</i>	1261	421	Cat→Tat	H→Y
<i>ndhB</i>	149	50	tCa→tTa	S→L
	467	156	cCa→cTa	P→L
	586	196	Cat→Tat	H→Y
<i>ndhB</i>	611	204	tCa→tTa	S→L
	704	235	tCc→tTc	S→F
	737	246	cCa→cTa	P→L
	830	277	tCa→tTa	S→L
	836	279	tCa→tTa	S→L
	1481	494	cCa→cTa	P→L
<i>ndhD</i>	878	293	tCa→tTa	S→L
<i>ndhF</i>	62	21	tCa→tTa	S→L
<i>petB</i>	1487	496	aCg→aTg	T→M
	662	221	cCa→cTa	P→L
	2009	670	cCa→cTa	P→L
<i>rpoC2</i>	2030	677	cCa→cTa	P→L
	2158	720	Ccc→Tcc	P→S
	3002	1001	cCg→cTg	P→L
<i>rpl2</i>	4031	1344	tCg→tTg	S→L
	62	21	aCt→aTt	T→I
<i>rpl20</i>	308	103	tCa→tTa	S→L
<i>rpoA</i>	1009	337	Ctc→Ttc	L→F
	467	156	tCg→tTg	S→L
	545	182	tCa→tTa	S→L
<i>rpoB</i>	560	187	tCg→tTg	S→L
	617	206	cCg→cTg	P→L
<i>rps8</i>	182	61	tCa→Ta	S→L
<i>ycf3</i>	44	15	tCc→tTc	S→F
	191	64	aCg→aTg	T→M

Capitals in column Codon Conversion indicate target nucleotides.

Table S9. RNA editing sites in chloroplast genes of *T. turgidum* ssp. *dicoccoides* (TA0073, TA0060 and TA1133) predicted by Prep-Cp.

Gene	Nucleotide Position	Amino Acid Position	Codon Conversion	Amino Acid Conversion
<i>ndhA</i>	473	158	tCa→tTa	S→L
	563	188	tCa→tTa	S→L
	1070	357	tCt→tTt	S→F
<i>atpA</i>	1148	383	tCa→tTa	S→L
<i>atpB</i>	35	12	gCt→gTt	A→V
	1487	496	tCg→tTg	S→L
<i>matK</i>	1261	421	Cat→Tat	H→Y
	149	50	tCa→tTa	S→L
	467	156	cCa→cTa	P→L
	586	196	Cat→Tat	H→Y
<i>ndhB</i>	611	204	tCa→tTa	S→L
	704	235	tCc→tTc	S→F
	737	246	cCa→cTa	P→L
	830	277	tCa→tTa	S→L
	836	279	tCa→tTa	S→L
	1481	494	cCa→cTa	P→L
<i>ndhD</i>	878	293	tCa→tTa	S→L
<i>ndhF</i>	62	21	tCa→tTa	S→L
	1487	496	aCg→aTg	T→M
<i>petB</i>	662	221	cCa→cTa	P→L
	2009	670	cCa→cTa	P→L
	2030	677	cCa→cTa	P→L
<i>rpoC2</i>	2158	720	Ccc→Tcc	P→S
	3002	1001	cCg→cTg	P→L
	4031	1344	tCg→tTg	S→L
<i>rpl2</i>	62	21	aCt→aTt	T→I
<i>rpl20</i>	308	103	tCa→tTa	S→L
<i>rpoA</i>	1009	337	Ctc→Ttc	L→F
	467	156	tCg→tTg	S→L
	545	182	tCa→tTa	S→L
<i>rpoB</i>	560	187	tCg→tTg	S→L
	617	206	cCg→cTg	P→L
	182	61	tCa→tTa	S→L
<i>rps8</i>	44	15	tCc→tTc	S→F
	191	64	aCg→aTg	T→M

Capitals in column Codon Conversion indicate target nucleotides.