Supporting Information

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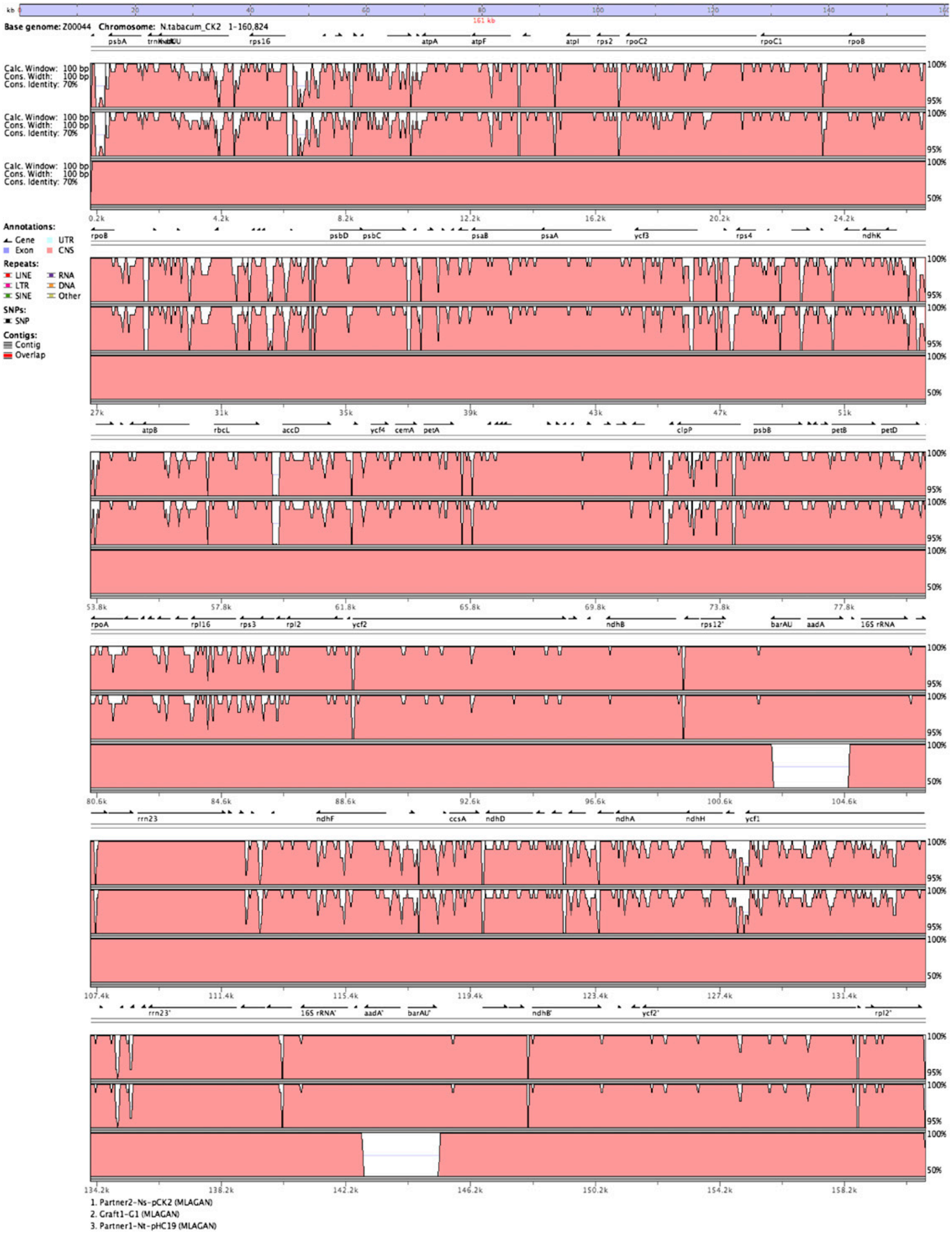

Fig. S1. Identification of the *N. undulata* plastids in the PGT plants. mVISTA-based identity plots (100-bp sliding window) showing sequence identity between sequenced chloroplast genomes of the transplastomic P2 partner carrying *N. undulata* ptDNA (u) and the *aadA* and *bar*^{au} transgenes (GenBank accession no. JN563930) (*Top*); the G1, G3, and G4 (G) PGT plants (*Middle*); and the P1 partner *N. tabacum* ptDNA (t) (GenBank accession no. Z00044) (*Bottom*) as the reference. Shown above the map are the positions of the genes.

Table S1. Plastid primers for testing ptDNA polymorphic sites between *N. tabacum* and *N. undulata*

Pair	Primer	Position	Strand	Gene	Sequence
*1	12upF	12907	F	*atpF*	TCTTACTTAGAATAGGTCGTCGATTCAGCA
*1	14upR	14098	R	*atpF*	CCACTGATTTCTGCCGCTTCCGTT
*2	27upF	27875	F	*rpoB-trnC*	ACACATTCCAACCTGCTTGAATACCA
*2	29upR	29210	R	*rpoB-trnC*	TCTTCCGCCCCCTTCCACAACTAT
*3	48upF	48971	F	*trnL*	GAGACATTCCTCCGCTTTCAGGCG
*3	49upR	49945	R	*trnL*	TGGAACCGCTAAGGAAAGGGGGTC
*4	60upF	60806	F	*accD*	AACGGCATTCCCGTAGCAATTGGG
*4	62upR	62222	R	*accD*	GGATGAGATTGGGTCCCAGCGGAT
*5	83upF	83888	F	*ndhF*	TTTCCACCACGACGTGCATTTCGT
*5	85upR	85414	R	*ndhF*	TACAAATTGCGGGGCGTATCGACG
*6	111upF	111916	F	*ndhE-ndhG*	TCGGAAGAAAGGTGGGATCCGGAC
*6	113upR	113293	R	*ndhE-ndhG*	TGGTATGGGGTCTTATCGAAGCGC

F, forward; R, reverse.

Table S2. Mitochondrial primers for testing mtDNA polymorphic sites between *N. tabacum* and *N. undulata*

Pair	Primer	Position	Strand	Gene	Sequence
1	mt-0-F	690	F	*orf125a*	CCCCGCCCAGTAGTGCCTCT
1	mt-4-R	4334	R	*orf125a*	CCGCGGGCATCGCGATAAGT
2	mt-100-F	100070	F	*orf129b*	CGGCCATCCTGGTCCTCAGGA
2	mt-104-R	104811	R	*orf129b*	TGGGGACTCGCACGAGGAGG
3	mt-180-F	180316	F	*nad4*	GGCAGGAGCGCAACGACCTT
3	mt-183-R	183813	R	*nad4*	AGTCGGGTTGCTCACGCAGC
4	mt-201-F	201586	F	*nad2*	TGGTGTGCTTCCTGCTCGCG
4	mt-204-R	204759	R	*nad2*	TTTCTCCGTGCCCGTTCCGC
5	mt-222-F	222140	F	*nad5*	AGGTGCCCGTAGTAGGCCGG
5	mt-226-R	226463	R	*nad5*	TTGGGCTTGGCTCTGCTCGC
6	mt-306-F	306203	F	*orf115-ccmFc*	CACGACTCCCCCTCTCCCCG
6	mt-309-R	309623	R	*orf115-ccmFc*	TGCCCGATTCCCCGACCCAT

F, forward; R, reverse.

Table S3. Plastid primers for PCR amplification of the *N. tabacum* and *N. undulata* plastid genomes

Pair	Primer	Position	Strand	Gene	Sequence
1	0F	14	F	*trnH*	ACGGGAATTGAACCCGCGCA
1	4R	4410	R	*trnK*	CGGGTTGCTAACTCAACGG
2	3F	3704	F	*trnK*	TCAAATGATACATAGTGCGATACA
2	8R	8653	R	*trnS*	CGAATCCCTCTCTTTCCG
3	7F	7989	F	*psbK*	GCCTTTGTTTGGCAAGCTGCTGTAAG
3	12R	12042	R	*atpA*	GGCATTGCTCGTATTCACGGTCTTG
4	11F	11052	F	*atpA*	CCACTCTGGAAACGGAGATACCC
4	16R	16791	R	*rps2*	CTCGTTTTTTATCAGAAGCTTGTG
5	15F	15267	F	*atpI*	GATGGCCCTCCATGGATTCACC
5	20R	20888	R	*rpoC2*	GAGGATTAATGTCAGATCCTCAAGG
6	19F	19971	F	*rpoC2*	GATAGACATCGGTACTCCAGTGC
6	24R	24612	R	*rpoB*	GTTACACAACAACCCCTTAGAGG
7	24F	24069	F	*rpoC1*	GCACAAATTCCGCTTTTTATAGG
7	29R	29568	R	*ycf6*	GCCCAAGCAAGACTTACTATATCCAT
8	28F	28849	F	*trnC*	CCAGTTCAAATCCGGGTGTC
8	34R	34493	R	*psbD*	TACCAAGGGCTATAGTCAT
9	33F	33186	F	*trnT*	GCCCTTTTAACTCAGTGGTA
9	38R	38115	R	*trnG*	AACCCGCATCTTCTCCTTGG
10	37F	37147	F	*trnS*	GAGAGAGAGGGATTCGAACC
10	43R	43484	R	*psaA*	TTCGTTCGCCGGAACCAGAA
11	41F	41267	F	*psaA*	AAGAATGCCCATGTTGTGGC
11	46R	46162	R	*ycf3*	CCTATTACAGAGATGGTGCGATTT
12	45F	45083	F	*ycf3*	CGATGCATATGTAGAAAGCC
12	51R	51022	R	*ndhJ*	TTTTTATGAAATACAAGATGCTC
13	49F	49312	F	*trnL*	CGAAATCGGTAGACGCTACG
13	54R	54971	R	*atpE*	GAAGGAAGGAGACAAAAAATTGAGGC
14	53F	53776	F	*trnV*	CGAACCGTAGACCTTCTCGG
14	58R	58198	R	*rbcL*	GTAAAATCAAGTCCACCGCG
15	57F	57272	F	*atpB*	TCTAGGATTTACATATACAACAT
15	62R	62754	R	*ycf4*	CTAATAAGAAGCCTAATGAACC
16	61F	61145	F	*accD*	GCAGGTAAAAGAGTAATTGAAC
16	66R	66664	R	*psbL*	TACTCATTTTTGTACTTGCTGT
17	65F	65219	F	*petA*	GCATCTGTTATTTTGGCACA
17	71R	71704	R	*clpP*	ACCATAGAAACGAAGGAACCCACT
18	70F	70727	F	*rps18*	GCTCGTATTTTATCTTTGTTACC
18	76R	76301	R	*psbB*	CCCCTTGGACTGCTACGAAAAACACC
19	74F	74963	F	*psbB*	TGCCTTGGTATCGTGTTCATAC
19	78R	78846	R	*petB*	CCCAGAAATACCTTGTTTACG
20	77F	77212	F	*psbH*	TGGGGAACTACTCCTTTGAT
20	82R	82676	R	*rps8*	CGAGGTATAATGACAGACCGAG
21	81F	81880	F	*rpl36*	ATTCTACGTGCACCCTTACG
21	86R	86576	R	*rps19*	GGGCATCTACCATTATACCC
22	85F	85864	F	*rps3*	AGTCTGAAACCAAGTGGATTTATT
22	89R	89311	R	*YCF2*	GAAGATACAGGAGCGAAACAATCAAC
23	88F	88062	F	*rpl2*	GCTTATGACCTCCCCCTCTATGC
23	93R	93140	R	*YCF2*	TCTTCTAGAGAATCTCCTAATTGTTC
24	91F	91131	F	*YCF2*	CTTCGAATATGGAATTCAAAGGGATC
24	97R	97636	R	*ndhB*	CTCAAACAAGCATGAAACGTATGC
25	96F	96469	f	*trnL*	GAGATTTTGAGTCTCGCGTGTC
25	100R	100782	R	*rps12*	TCACTGCTTATATACCCGGTATTGGC
26	99F	99552	F	*rps7*	GTGCAAAAGCTCTATTTGCCTCTGCC
26	104R	104797	R	*oriA*	ATCGAAAGTTGGATCTACATTGGATC
27	103F	103454	F	*rrn16*	CGACACTGACACTGAGAGACGAAAGC
27	108R	108280	R	*rrn23*	CGCTACCTTAGGACCGTTATAGTTAC
28	107F	107056	F	*rrn23*	GAAACTAAGTGGAGGTCCGAACCGAC
28	111R	111882	R	*ORF350*	AGTGGATCCCTCTTGTTCCTGTTTAG
29	110F	110672	F	*trnN*	ACAGCCGACCGCTCTACCACTGAGC
29	114R	114269	R	*ndhF*	GGATCATACCTTTCATTCCACTTCC
30	113F	113036	F	*ndhF*	ATTTCATCTTTGGACCAAAAACAAGC
30	119R	119286	R	*psaC*	GCTAAACAAATTGCTTCTGCTCC
31	117F	117227	F	*ycf5*	GGTCAATCTTTTAGGAATAGGGTTAC
31	123R	123506	R	*ndhA*	GGACTTCTTATGTCGGGATATGGATC

Table S3. Cont.

Pair	Primer	Position	Strand	Gene	Sequence
32	122F	122194	F	*ndhA*	CTGCGCTTCCACTATATCAACTGTAC
32	128R	128835	R	*ycf1*	TGAAACCTTGGCATATATCT
33	127F	127391	F	*ycf1*	AATTTCGAGGTTCTTATTTACT
33	132R	132957	R	*trnR*	GACGATACTGTAGGGGAGGTC
34	154F	154629	F	*rpl2*	CCATAGAATACGACCCTAAT
34	1R	1533	R	*psbA*	CTAGCACTGAAAACCGTCTT

F, forward; R, reverse.

Table S4. Nuclear SSR primers

Chromosome	Primer	Strand	Sequence
1	PT30307	F	AAAGAAGCACGGTCAAATAGG
1	PT30307	R	GCAACAACAAGGTGTCATGG
2	PT30242	F	TGTGTACTACCGGCCTACTGC
2	PT30242	R	TTCTGCTAAACCGATCGTGG
3b	PT30205	F	GGTCGATCCACAATTTAAACG
3b	PT30205	R	GCACTTGCTCCTTTGTACCC
4	PT30272	F	GAACCTAACCTCGCTCCACA
4	PT30272	R	AAATGGTAGCTGCGAGGAGA
5	PT30471	F	GTCTGTACCTTCGCCAAAGC
5	PT30471	R	TCCTCAGAGAACTCCAGCGT
6	PT30087	F	CTTCTTCCTAAGCCGAGGGT
6	PT30087	R	TTGATGATAGAACGCAACTCG
7	PT30138	F	AGTTGCAGGATTGTTCGCTT
7	PT30138	R	CGACTGCAAGAGTTGGCAAT
8a	PT30167	F	TGATACAGAATATGGCGAACTTT
8a	PT30167	R	CCGCTTCATCATTGAGGTTT
9	PT30140	F	AAGATGGCATATGGGATTGG
9	PT30140	R	TGAATCGGAGGAAGTGAATG
10	PT30482	F	CTTCTCTCTCCACCGCAGAC
10	PT30482	R	ACAGTTGGATATGGTGGCGT
11	PT30008	F	CGTTGCTTAGTCTCGCACTG
11	PT30008	R	GGTTGATCCGACACTATTACGA
12	PT30098	F	TTGTTGCTCTCTCGAGTTCTTT
12	PT30098	R	GCAGTCGACTCATTGGCA
13	PT30342	F	GACAACAATCAGTAAAGGAAACGA
13	PT30342	R	AATGCAAGACCCTGTCAACC
13	PT30420	F	AACAAACCGCTTTCCATTCT
13	PT30420	R	GAATTAGGCGCTTTGGGAAT
14a	PT30175	F	TTAGGCGGCGGTATTCTTAT
14a	PT30175	R	TATGCCTCAATCCCTTACGC
15	PT30463	F	AAGCTGCCCTAGCTCAATCA
15	PT30463	R	AACATCACCATTTCCACAAGTTT
16	PT30412	F	CATTTAGCCGGGAACATTCA
16	PT30412	R	CATGGGATACACACGCAAAG
17	PT30274	F	TGACAGCTAAGCTAATAACAGTAAATG
17	PT30274	R	GGACTTTGGAGTGTCAAATGC
18	PT30111	F	AGCCAGCCACCAAATTTATC
18	PT30111	R	GGAACATTGCTCAAGCCCTA
19	PT30230	F	TTTCTTTCTGTCTGATGCTTCAAT
19	PT30230	R	TTGTCCATCTCACTTGCTGC
20	PT20286	F	ACGCTAGAGCATCCAACA
20	PT20286	R	TAGTGAAAGGCAAGCAGG
21	PT30378	F	TCAAATGAGGGTTGTAGCCA
21	PT30378	R	TGCAATGGCTACACAAGAAGA
22	PT30168	F	TTGAACACCAATTGCGGTAA
22	PT30168	R	AAATTCTTGGGTCATGGTGG
23	PT30231	F	AGGAGGCGAAGAAAGAGGAG
23	PT30231	R	CCCATGAATTCGTAACAGCA
24	PT40024	F	AATGTCTGCCCAATCGAAAG
24	PT40024	R	CGAATAACGACACTCGAACG

F, forward; R, reverse.