

Supporting Information

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1 MSPQETKASVGFKAGVKYKLTYTPEYTKDTDLAAFRVTPOPGVPPPEAGAAVAEESSTGTWTTW tobacco LSu
1 MSPQETKASVGFKAGVKYKLTYTPEYTKDTDLAAFRVTPOPGVPPPEAGAAVAEESSTGTWTTW F bidentis LSu
1 MSPQETKASVGFKAGVKYKLTYTPEYTKDTDLAAFRVTPOPGVPPPEAGAAVAEESSTGTWTTW F pringlei LSu
1 MSPQETKASVGFKAGVKYKLTYTPEYTKDTDLAAFRVTPOPGVPPPEAGAAVAEESSTGTWTTW F floridana LSu

71 TDGLTSLDRYKGRCYRIERVWGEKQDQIAYAVYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDRI tobacco LSu
71 TDGLTSLDRYKGRCYRIERVWGEKQDQIAYAVYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDRI F bidentis LSu
71 TDGLTSLDRYKGRCYRIERVWGEKQDQIAYAVYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDRI F pringlei LSu
71 TDGLTSLDRYKGRCYRIERVWGEKQDQIAYAVYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRLEDRI F floridana LSu

149
141 PPAYVKTBGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECRLRGGLDFTKDDENVNSQP tobacco LSu
141 PPAYVKTBGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECRLRGGLDFTKDDENVNSQP F bidentis LSu
141 PPAYVKTBGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECRLRGGLDFTKDDENVNSQP F pringlei LSu
141 PPAYVKTBGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECRLRGGLDFTKDDENVNSQP F floridana LSu

265
211 FMRWRDRFLCAEAELYKAQAEETGEIKGHYLNATAGTCCEEMIKRAVFARELGVPIIHDYLTGGFTANTSL tobacco LSu
211 FMRWRDRFLCAEAELYKAQAEETGEIKGHYLNATAGTCCEEMIKRAVFARELGVPIIHDYLTGGFTANTSL F bidentis LSu
211 FMRWRDRFLCAEAELYKAQAEETGEIKGHYLNATAGTCCEEMIKRAVFARELGVPIIHDYLTGGFTANTSL F pringlei LSu
211 FMRWRDRFLCAEAELYKAQAEETGEIKGHYLNATAGTCCEEMIKRAVFARELGVPIIHDYLTGGFTANTSL F floridana LSu

309
281 AHYCRDNGLLLHHIRHAMHAVIDRQKNHQHFRVLAKALRMSGGDHISHTVVGKLEGERDITLGFV DLLR tobacco LSu
281 SHYCRDNGLLLHHIRHAMHAVIDRQKNHQHFRVLAKALRMSGGDHISHTVVGKLEGERDITLGFV DLLR F bidentis LSu
281 SHYCRDNGLLLHHIRHAMHAVIDRQKNHQHFRVLAKALRMSGGDHISHTVVGKLEGERDITLGFV DLLR F pringlei LSu
281 SHYCRDNGLLLHHIRHAMHAVIDRQKNHQHFRVLAKALRMSGGDHISHTVVGKLEGERDITLGFV DLLR F floridana LSu

351 DDFVEQDRSRGIYFTQDWVSLPGVLPVASGGIHVWHMPALTEIFGDDSVLQFGGGTLGHPWGNAPGAVAN tobacco LSu
351 DDFVEQDRSRGIYFTQDWVSLPGVLPVASGGIHVWHMPALTEIFGDDSVLQFGGGTLGHPWGNAPGAVAN F bidentis LSu
351 DDFVEQDRSRGIYFTQDWVSLPGVLPVASGGIHVWHMPALTEIFGDDSVLQFGGGTLGHPWGNAPGAVAN F pringlei LSu
351 DDFVEQDRSRGIYFTQDWVSLPGVLPVASGGIHVWHMPALTEIFGDDSVLQFGGGTLGHPWGNAPGAVAN F floridana LSu

421 RVALEACVKARNEGRDLAEGNEIIIREACKWSPELAACEVWKEIVFNAAVDVLLK. tobacco LSu
421 RVALEACVKARNEGRDLAEGNEIIIREACKWSPELAACEVWKEIKFEEFQAMDLTDKDCKDKKR. F bidentis LSu
421 RVALEACVKARNEGRDLAEGNEIIIREACKWSPELAACEVWKEIKFEEFQAMDLTDKDCKDKKR. F pringlei LSu
421 RVALEACVKARNEGRDLAEGNEIIIREACKWSPELAACEVWKEIKFEEFQAMDLTDKDCKDKKR. F floridana LSu

Fig. S1. Sequence comparison of tobacco and *Flaveria ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco)* large subunits (L-subunits). Sequences were aligned using ClustalW (1). Amino acids residues differing from the tobacco L-subunit (GenBank accession number Z00444) are shaded blue. The positions of the divergent residues in the *Flaveria* L-subunits (at residues 149, 265, and 309) are boxed in red.

- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.

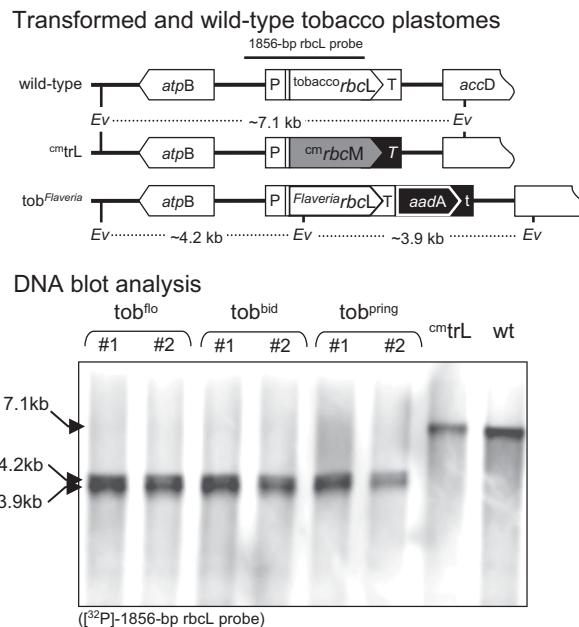


Fig. S2. DNA blot analysis of homoplasmicity. Unlike the *rbcL* in wild-type tobacco and the tobacco master line ^{cm}trl (1), the *Flaveria rbcL* genes introduced into the tobacco plastome contain an *EcoRV* site (Ev, Lower). Total genomic DNA from mature leaves of transformed T₁ progeny (germinated on Murashige and Skoog medium containing spectinomycin before transfer to growth in soil) and control plants (wild-type and ^{cm}trl) was isolated using the DNeasy Plant Mini Kit (Qiagen), digested with *EcoRV*, and examined by Southern (DNA) blot analysis as described (2) using a [³²P]-labeled 1,856-bp *rbcL* probe as shown (Upper). In wild-type and ^{cm}trl plants the *rbcL* probe recognizes a single fragment of ~7.1 kb. Correct insertion of a *Flaveria rbcL* and *aadA* gene is indicated by hybridization to two fragments of 3.9 and 4.2 kb, respectively. No 7.1-kb band was present in the transformed lines, indicating they were homoplasmic.

- Whitney SM, Sharwood RE (2008) Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *J Exp Bot* 59:1909–1921.
- Whitney SM, Andrews TJ (2001) Plastome-encoded bacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) supports photosynthesis and growth in tobacco. *Proc Natl Acad Sci USA* 98:14738–14743.

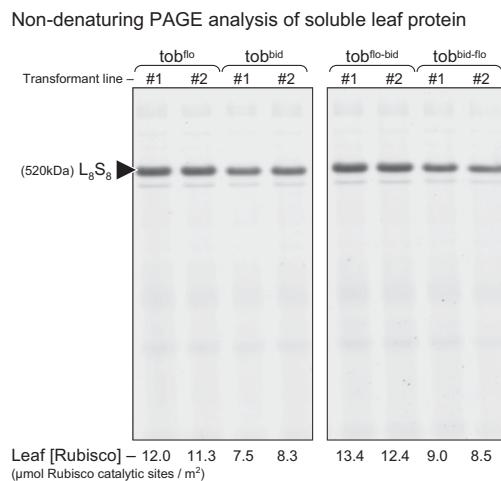


Fig. S3. Nondenaturing PAGE analysis of soluble protein from 2 mm² of leaf. Soluble protein from 2 mm² of young leaves (12 cm wide) from the same position in the upper canopy of plants ~15 cm high was separated by nondenaturing PAGE as described (1). Samples were taken from the T₂ *tob*^{flo} and *tob*^{bid} plants and the T₁ progeny of the *tob*^{flo-bid} and *tob*^{bid-flo} lines. In each sample, the L₈S₈ rubisco was the prominent band evident by Coomassie blue staining. The rubisco content in each sample was quantified by [¹⁴C]2-carboxyarabinitol-1,5-bisphosphate binding as described (2).

- Whitney SM, Sharwood RE (2008) Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *J Exp Bot* 59:1909–1921.
- Ruuska S, et al. (1998) The interplay between limiting processes in C₃ photosynthesis studied by rapid-response gas exchange using transgenic tobacco impaired in photosynthesis. *Aust J Plant Physiol* 25:859–870.