



Targeted replacement of *clpP1* in the tobacco plastome with cDNA counterparts from different *Silene* species. (A) Physical map of the targeting region in the plastome of wild type tobacco cloned in pBluescript KS (+) plasmid (pBS-v3, see text for details). The chimeric selectable marker gene *aadA* conferring resistance to spectinomycin and streptomycin is driven by the rRNA operon promoter (5' *Prrn*), and the 3' untranslated region of the *psbA* gene (3' *psbA*) was added to stabilize the mRNA (Svab and Maliga 1993). (B) Plastid-targeting region with flanking homologous recombination sequences (HR regions), donor *clpP1* coding sequences, and *aadA* cassette. The *aadA* cassette was introduced in antisense direction relative to *clpP1* operon. Restriction sites used for cloning and replacement are indicated. Numbered horizontal arrows represent primers used in PCR validation of insertion and orientation (Table S1). Transcription start sites (TSSs) are indicated in the intergenic regions between the *psbB* gene and *clpP1* operon (Hajdukiewicz, et al. 1997). Certain regions of the map are expanded and not drawn to scale for the sake of clarity.

