he accession numbers communicated in the paper are: GQ221700 (mzYFP), GQ221701 (mzGFP) and GQ221702 (mzBFP) – From clemente



label leaf plastids outside of bundle sheath or mesophyll (for example, in leaf epidermis), most of the plastids in other leaf cell types are either smaller or do not contain chlorophyll and therefore most could potentially be sorted away by size or chlorophyll content from either mesophyll or bundle sheath chloroplasts.

## Experimental procedures

### Maize transformation

Maize transformations were carried out via an *Agrobacterium*-mediated transformation protocol. Immature ears, genotype Hi II (Armstrong *et al.*, 1991), were harvested approximately 12 days post-pollination. Whole ears were surfaced sterilized by applying 70% ethanol spray and allowing to air dry within a laminar flow hood. Immature embryos were isolated and placed immediately in liquid isolation medium composed of 1/2 MS salts with full strength MS vitamins 115 mg/L proline, 6.9% sucrose, 3.6% glucose, 200 μM acetosyringone buffered with 10 mM MES (pH 5.4). Following the isolation of 100 immature embryos, the isolation medium was replaced with inoculation medium, *A. tumefaciens* transconjugant suspended in isolation medium to an $OD_{660}$ of 0.3–0.5. The embryos were inoculated for 5 min, after which they were transferred, scutellum side up, to co-cultivation medium solidified with 0.6% low EEO agarose. Co-cultivation medium consisted of 1/2 MS salts, full strength MS vitamins, 0.5 mg/L thiamine, 1 mg/L 2,4-D, 115 mg/L proline, 1% glucose, 2% sucrose, 20 μM AgNO3, and 200 μM acetosyringone. The medium was buffered with 20 mM MES (pH 5.4). The embryos were co-cultivated for 2 days at 24 °C.

Following the co-cultivation step the embryos were transferred to delay medium composed of N6 salts (Chu *et al.*, 1975), Eriksson's vitamins (Eriksson, 1965), with 1 mg/L 2,4-D, 25 mM proline, 100 mg/L casamino acids, 2% sucrose, 1.7 mg/L $AgNO_3$, and 250 mg/L carbenicillin. The medium was solidified with 0.7% phytagar and buffered with 3 mM MES (pH 5.8). The delay step was carried out for 5 days in the dark at 28 °C, after which developing coleoptiles were removed from the embryos and the explants transferred to selection medium.

The selection phase was carried in a stepwise fashion using the delay medium supplemented with 25 mg/L paramomycin for 3 weeks, followed by a transfer to 50 mg/L paramomycin for 3 weeks, and finally 100 mg/L paramomycin. Embryogenic tissue was subcultured three times on to fresh 100 mg/L selection regime until the proliferating embryogenic culture mass reached approximately 2 cm in diameter.

Paromomycin-tolerant embryogenic tissue was regenerated in a three-step process. The first step was carried out in the dark at 28 °C for a period up to 14 days, where the tissue was cultured on medium composed of MS salts, Fromm vitamins (Fromm *et al.*, 1990), 0.1 mg/L 2,4-D, 100 μM abscisic acid (ABA), 2% sucrose, 50 mg/L paromomycin, and 250 mg/L carbenicillin. The medium was solidified 0.7% phytagar and buffered with 3 mM MES (pH 5.8). The second stage of regeneration involved culturing embryos for a period up to 14 days in the dark at 28 °C on N6 salts, Eriksson's vitamins, 6% sucrose, 50 mg/L paromomycin and 250 mg/L carbenicillin. The embryo conversion step was carried out in culture vessels under an 18 h light regime on medium with MS salts, Fromm vitamins, 100 mg/L inositol, 150 mg/L asparagine, 2% maltose, 1% glucose, and 50 mg/L paromomycin. The conversion medium was solidified with 0.7% phytagar and buffered with 3 mM MES (pH 5.8).

### Fluorescent marker gene cassettes

Three cassettes that harboured a non-maize codon-optimized version (Chiu *et al.*, 1996) were assembled under the control of either the 35S CaMV (Benfey and Chua, 1990), rice actin promoter, coupled with a 5′-intron (Zhong *et al.*, 1996) or the 1.7 kb maize C4 *PepC* promoter. (Yanagisawa and Izui, 1989) (*ZmPpc1*) The respective promoters were fused with translational enhancer element from the maize PPDK-A gene (Sheen, 1993), and GFP was targeted to plastids via the maize chloroplast RNA polymerase RpoTp transit peptide (Chang *et al.*, 1999) for the 35S CaMV and rice actin cassettes, or the pea *RBCS1* transit peptide (Van den Broeck *et al.*, 1985; von Heijne *et al.*, 1991) for the *PEPC* cassette. The non-codon-optimized GFP cassettes were subcloned into either the binary plasmid pPZP211 or pPZP212 (Hajdukiewicz *et al.*, 1994), and the resultant vectors were referred to as pPTN343, pPTN372 and pPTN442, for the 35S, rice actin and *PEPC* promoters, respectively.

A set of maize codon-optimized fluorescent marker genes encoding blue (mzBFP), green (mzGP) or yellow (mzYFP) were commercially synthesized (Genscript Corporation, Piscataway, NJ, USA). The respective markers were assembled in plastid-targeted expression cassettes. The binary vector pPTN448 carries mzBFP ORF under the control of the 1.9 kb *PstI* fragment directly upstream the ATG of the maize *ubiquitin1* promoter coupled with its first intron (Christensen *et al.*, 1992). The mzBFP peptide is targeted to plastid via the pea Rubisco small subunit transit peptide (von Heijne *et al.*, 1991). The binary vectors pPTN458 and pPTN469 are identical to pPTN448, except they harbour the mzGFP and mzYFP ORFs, respectively. Sequences are available through Genbank under accession numbers 1218408 (mzYFP), 1218425 (mzGFP) and 1218426 (mzBFP).

The binary vector designated pPTN512 has the mzYFP ORF under the control of the 1.7 kb maize C4 *PepC* promoter (Yanagisawa and Izui, 1989) coupled with the pea SSU transit peptide. The binary plasmid pPTN533 has the mzYFP ORF under the control of a 0.9 kb region upstream of the initiation codon comprising the maize *RBCS1* promoter (Lebrun *et al.*, 1987; Viret *et al.*, 1994), and is plastid localized via the pea SSU transit peptide, and has the terminator region from the *RBCS1* gene believed to contribute to cell-type specificity (Viret *et al.*, 1994). Finally, the binary vector pPTN629 is identical to pPTN533, except it utilizes the maize *RBCS1* transit peptide (Lebrun *et al.*, 1987). All but the pPTN533 and PTN629 constructs use the standard *Nos* terminator.

### Preparation of bundle sheath strands and mesophyll protoplasts

Mesophyll protoplasts were prepared from third and fourth leaf blades after digestion of their cell walls, and bundle sheath

 