Supplementary data

Plant materials

Arabidopsis thaliana strain Col-0 was used as the wild-type. After 4-day incubation at 4°C, the *Arabidopsis* were cultivated in a growth chamber maintained at 22°C and long-day conditions (16-h light/8-h dark) with 80 μmol photons m^{-2} s^{-1} for 4 weeks.

Isolation of *cyo1* mutant using CRISPR/Cas9

Sequences of guide RNA (gRNA) for genome editing of *CYO1* (CYO1 gRNA F1 and CYO1 gRNA R1) were shown in Supplementary Table1. The gRNA was designed using the database CRISPR-P (Lei et al. 2014). The double stranded target sequence was cloned into the *Bbs* I sites of pEn-Chimera vector (Fauser et al. 2014) using the In-fusion HD cloning kit (TaKaRa). The gRNA cassette for *CYO1* was recombined into pDe-Cas9 vector using LR clonase (Thermo Fisher Scientific, https://www.thermofisher.com/jp/ja/home.html). Thereafter, it was transformed into EHA105 using an electroporation method. Plant transformation was performed by the floral dip method (Clough et al. 1988). For screening of *cyo1* mutants, PCR products amplified with the primers cyo1 DpnII F1 and cyo1 DpnII R1 were subjected to *Dpn* II treatment.

Construction of pCYO1 and pCYO2

The 2.3-kb genomic fragment, which contains the entire *CYO1* gene, was amplified by PCR using Prime STAR GXL polymerase and primers AtCYO1 EcoRI infF1 and AtCYO1 KpnI infR1. The amplified DNA was cloned into the *EcoR* I-*Kpn* I site of pDe-Cas9 using In-fusion HD cloning kit (pDe-EK). The 1.7-kb DNA fragment, which contains *CmR -ccdB* cassette derived from pGWB601 (Nakamura et al 2010), was amplified by PCR using primers attR1 EcoRI infF1 and attR2 EcoRI infR3. The amplified DNA was cloned into the *EcoR* I sites of pDe-EK using In-fusion HD cloning kit (pCYO1). The 3-kb DNA fragment containing CaMV35S promoter-Ω, *CmR-ccdB* cassette and Nos terminator was amplified from pGWB602Ω (Nakamura et al. 2010) by PCR using primers 35Spro EcoRI infF2 and Nos ter EcoRI infR3. The amplified DNA was cloned into the *EcoR* I sites of pDe-EK using In-fusion HD cloning kit (pCYO2).

Construction of pCYO3

The 2.3-kb genomic fragment, which contains the entire *CYO1* gene, was amplified by PCR using primers AtCYO1 SacI infF1 and AtCYO1 KpnI infR1. The amplified DNA was cloned into the *Sac* I-*Kpn* I site of pDe-Cas9 using In-fusion HD cloning kit (pDe-SK). The 2.2-kb

DNA fragment, which contains Pea3A terminator and *CmR-ccdB* cassette derived from pDe-Cas9, was amplified by PCR using the primers Pea3AT SacI infF1 and attR2 SacI infR1. The amplified DNA was cloned into the *Sac*I site of pDe-SK using In-fusion HD cloning kit (pCYO3).

pCYO constructs for *Arabidopsis* transformation

The *GFP* fragment derived from pJ4-GFP (Igarashi et al. 2001; Yamatani et al. 2013) was cloned into the *Not*I-*Asc*I site of pENTR (Thermo Fisher Scientific) using In-fusion HD cloning kit and recombined into the pCYO2 destination vector for the transformation experiment in Figure 2. Sequences of gRNA for genome editing of *CTR1* (CTR1 gRNA F1 and CTR1 gRNA R1) can be found in Supplementary Table 1. The gRNA was designed using the database CRISPR direct (http://crispr.dbcls.jp/) (Naito et al. 2015). The double stranded target sequence was cloned into the *Bbs*I sites of pEn-Chimera. The gRNA cassette for *CTR1* was recombined into the pCYO3 destination vector.

Transformation and screening

All the pCYO transformants were obtained with floral dip method using *cyo1-11* plants and *Agrobacterium tumefaciens* strain EHA105. GFP images were captured with the fluorescence microscope MZ10F (Leica, http://www.zeiss.com). For screening of *cyo1* mutants, PCR products amplified with the primers ctr1 BamHI F1 and ctr1 BamHI R1 were subjected to *Bam*HI treatment.

References

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