

## ***New Phytologist* Supporting Information**

### **Functional understanding of secondary cell wall cellulose synthases in *Populus trichocarpa* via the Cas9/gRNA-induced gene knockouts**

Wenjing Xu, Hao Cheng, Siran Zhu, Jiyao Cheng, Huanhuan Ji, Baocai Zhang, Shenquan Cao, Chong Wang, Guimin Tong, Cheng Zhen, Liqiang Mu, Yihua Zhou, Yuxiang Cheng

Article acceptance date: 02 March 2021

#### **Supplemental Figures**

**Fig. S1** Cas9/gRNA system and stepwise protocol in Nisqually-1.

**Fig. S2** Cas9/gRNA-induced mutations in *PtrCHL11* gene and *PtrCHL11/2* family genes of *Populus trichocarpa*.

**Fig. S3** Cas9/gRNA-induced mutations in *PtrCesA7A*, *PtrCesA7B*, *PtrCesA8A*, and *PtrCesA8B* genes of *Populus trichocarpa*.

**Fig. S4** Production of anti-PtrCesA4, -7A/B, and -8A/B polyclonal antibodies in rabbits.

**Fig. S5** Transcriptional levels of five SCW *PtrCesA* genes in *ptrcesa* mutants using RT-PCR analysis.

**Fig. S6** Characterization of the *ptrcesa4*, *7a/b*, *8a/b*, *7a*, *7b*, *8a*, and *8b* mutants.

**Fig. S7** Observation of stem internodes, mature leaves, and roots of 3-month-old *ptrcesa4*, *7a/b*, and *8a/b* mutants.

**Fig. S8** Anatomic analysis of different stem internodes from WT, *ptrcesa4*, *7a/b*, and *8a/b* mutants.

**Fig. S9** Anatomic analysis of different stem internodes from WT, *ptrcesa7a*, *7b*, *8a*, and *8b* mutants.

**Fig. S10** Observation of pith parenchyma in WT, *ptrcesa4*, *7a/b*, and *8a/b* mutants.

**Fig. S11** Wall thickness of xylem and phloem fibres in the basal stems of 6-month-old WT and *ptrcesa* mutants.

**Fig. S12** Microscopic analysis of the disaggregated xylem fibres and vessels in WT, *ptrcesa4*, *7a/b*, and *8a/b* mutants.

**Fig. S13** Induction of tension wood (TW) in WT and *ptrcesa* mutants under gravi-stimulation.

**Fig. S14** Immunolocalization of crystalline cellulose in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S15** Immunolocalization of the xylan in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S16** Immunolocalization of  $\beta$ -(1 $\rightarrow$ 4)-galactan in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S17** Immunolocalization of the mannan in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S18** Immunolocalization of crystalline cellulose in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S19** Immunolocalization of  $\beta$ -(1 $\rightarrow$ 4)-galactan in phloem fibres of WT, *ptrcesa4*, *7ab* and *8ab* mutants.

**Fig. S20** Immunolocalization of the mannan in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S21** Immunolocalization of the xylan in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.

**Fig. S22** Lignin phloroglucinol staining in WT, *ptrcesa4*, *7ab* and *8ab* mutants.

**Fig. S23** Crystalline cellulose and lignin content in woods of WT, *ptrcesa7a*, *7b*, *8a*, and *8b* mutants.

## Supplemental methods

**Methods S1** Analysis of putative Cas9/gRNA off-target sites.

**Methods S2** RNA extraction and RT-PCR analysis.

**Methods S3** Scanning electron microscopy (SEM) of leaf epidermal cells.

**Methods S4** Wood fibre and vessel cell length analysis.

## Supplemental tables

**Table S1.** Primers used in this study.

**Table S2.** The Cas9/gRNA-targeted mutations in single *PtrCHL11* gene of *Populus trichocarpa*.

**Table S3.** The Cas9/gRNA-targeted mutations in both *PtrCHL11* and 2 genes of *Populus trichocarpa*.

**Table S4.** Analysis of potential off-target sites among the Cas9/gRNA-induced *ptrchli1* mutants.

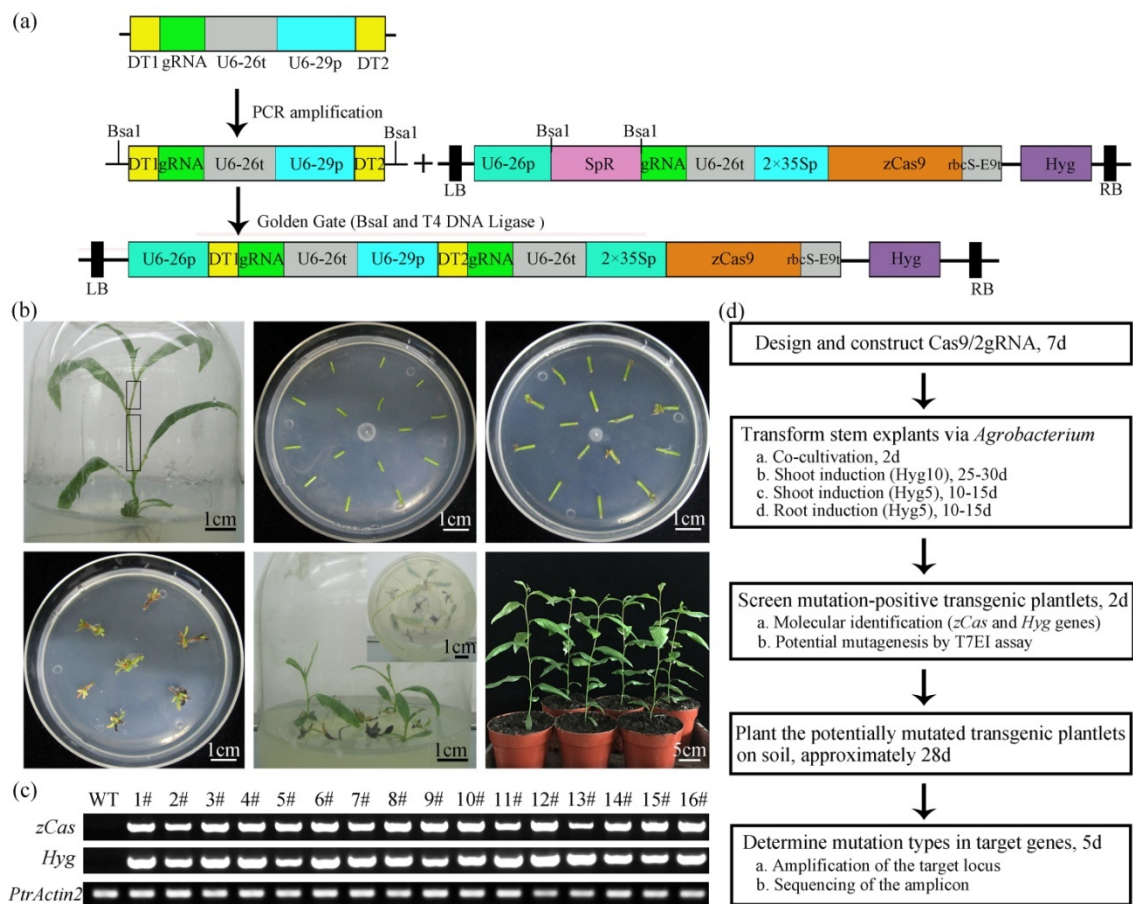
**Table S5.** The Cas9/gRNA-targeted mutations in *PtrCesA4*, *7A*, *7B*, *8A*, *8B*, *7A/B* and *8A/B* genes of *Populus trichocarpa*.

**Table S6.** Analysis of potential off-target sites among the Cas9/gRNA-induced *ptrcesa* mutants

**Table S7.** Inheritance of the Cas9/gRNA-induced mutations in progeny of *ptrcesa4*, *7a/b* and *8a/b* mutants through asexual propagation methods.

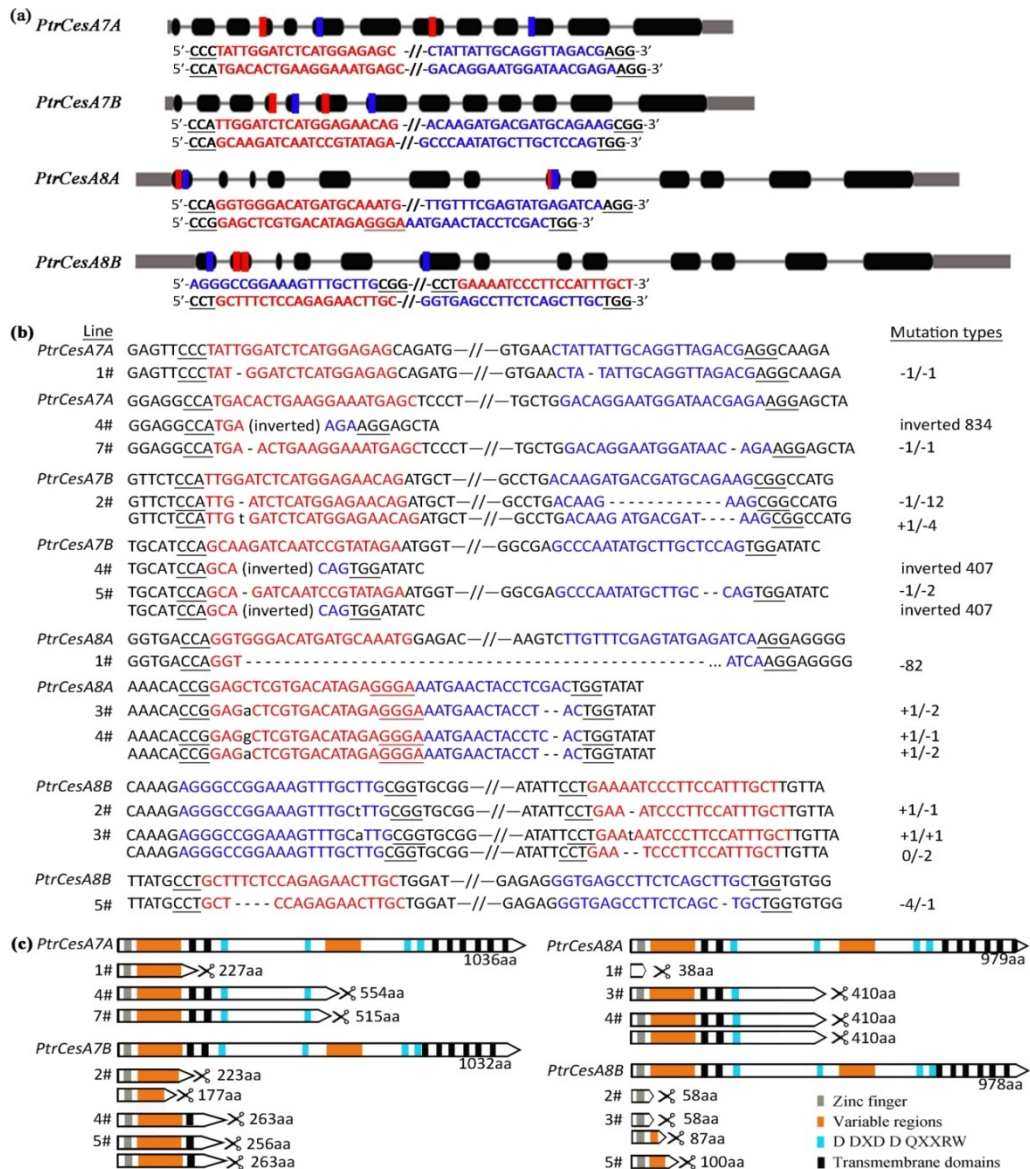
**Table S8.** Inheritance of the Cas9/gRNA-induced mutations in the progeny of the *ptrcesa* lines.

**Fig. S1 Cas9/gRNA system and stepwise protocol in Nisqually-1.** (a) Illustration of the cloning of two gRNA expression cassettes into the Cas9/gRNA binary vector using Golden Gate ligation. *zCas9*, Zea mays codon-optimized Cas9; U6-26p and U6-29p, two *Arabidopsis* U6 gene promoters; U6-26t, U6-26 terminator with the downstream sequence; SpR, spectinomycin resistance gene; Hyg, hygromycin resistance gene. (b) Production of hygromycin-resistant transgenic plantlets using *Agrobacterium*-mediated transformation of Nisqually-1. Scale bars show 1 or 5 cm. (c) Identification of transgenic plantlets by using a genomic DNA PCR analysis of the *zCas* and *Hyg* genes in all 16 transgenic lines (#) and wild-type (WT). *Actin2* was used as an internal control. (d) A flow chart of the Cas9/gRNA-based mutagenesis in Nisqually-1.



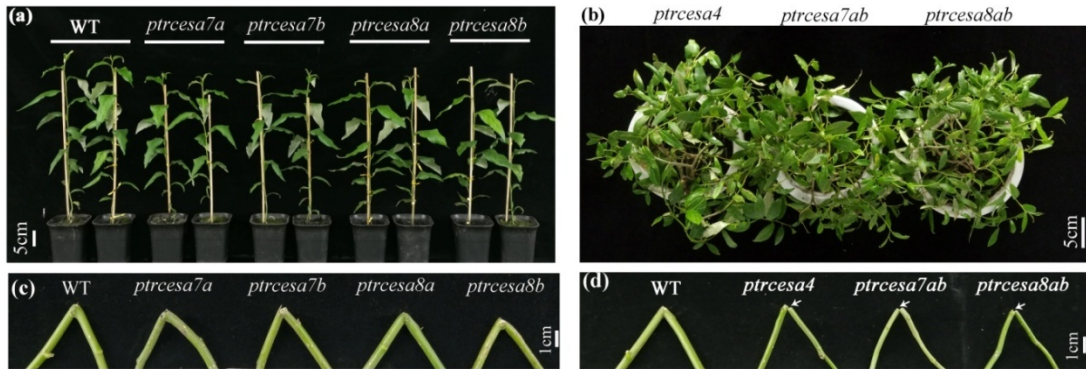


**Fig. S3 Cas9/gRNA-induced mutations in *PtrCesA7A*, *PtrCesA7B*, *PtrCesA8A*, and *PtrCesA8B* genes of *Populus trichocarpa*.** (a) Sixteen gRNAs were designed in *PtrCesA7A*, *PtrCesA7B*, *PtrCesA8A* and *PtrCesA8B* genes. Nucleotides in blue and red represent the target sites. (b) Characterization of the target site mutations in 12 *ptrcesa* mutants (*ptrcesa7a-1#*, -4# and -7#; *ptrcesa7b-2#*, -4# and -5#; *ptrcesa8a-1#*, -3# and -4#; *ptrcesa8b-2#*, -3# and -5#). Dashes and lowercase letters represent the deletion and insertion of nucleotides. Mutation types are indicated on the right. (c) The deduced amino acids of protein-coding regions from the Cas9/gRNA-edited genes in 12 *ptrcesa* mutants (*ptrcesa7a-1#*, -4# and -7#; *ptrcesa7b-2#*, -4# and -5#; *ptrcesa8a-1#*, -3# and -4#; *ptrcesa8b-2#*, -3# and -5#). The scissors indicate protein-coding termination.



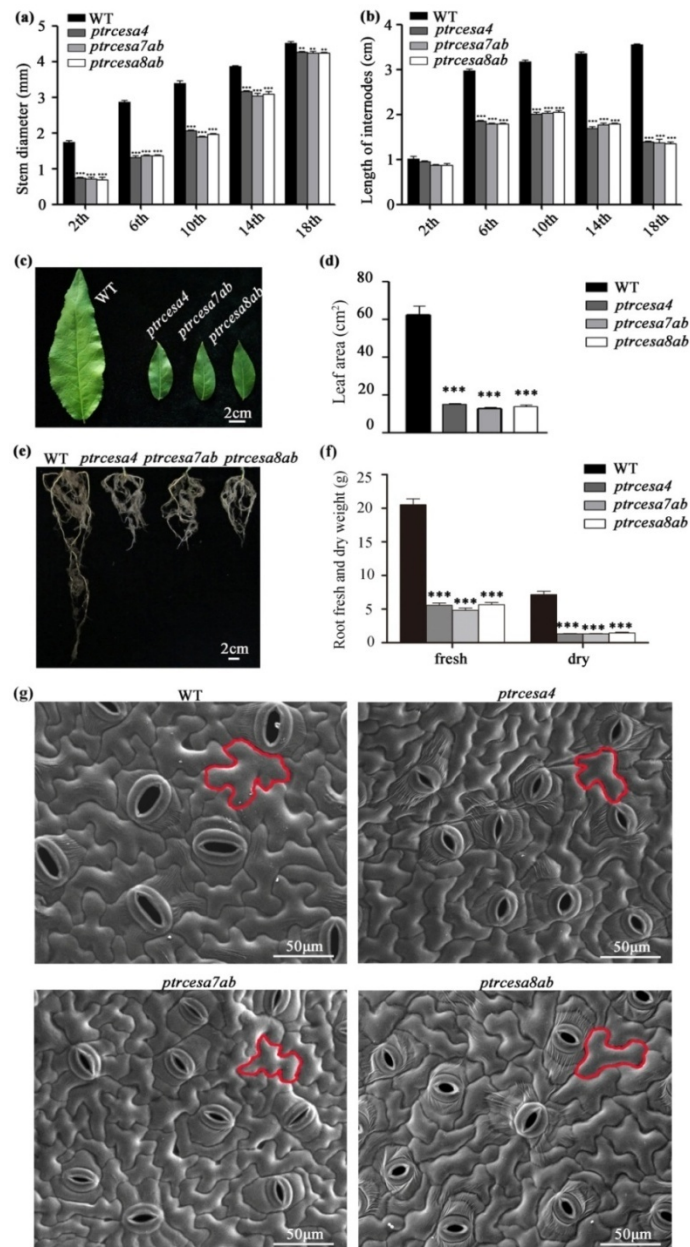


**Fig. S6 Characterization of *ptrcesa4*, *7a/b*, *8a/b*, *7a*, *7b*, *8a*, and *8b* mutants.** (a) Morphology of *ptrcesa7a*, *7b*, *8a*, *8b*, and wild-type (WT) young trees grown for 3 months in a greenhouse. (b) 6-month-old *ptrcesa4*, *7a/b* and *8a/b* mutant trees grown in a greenhouse. (c-d) Brittleness properties in stems of WT and *ptrcesa* mutants. Scale bars: (a-b) 5 cm; (c-d) 1 cm.

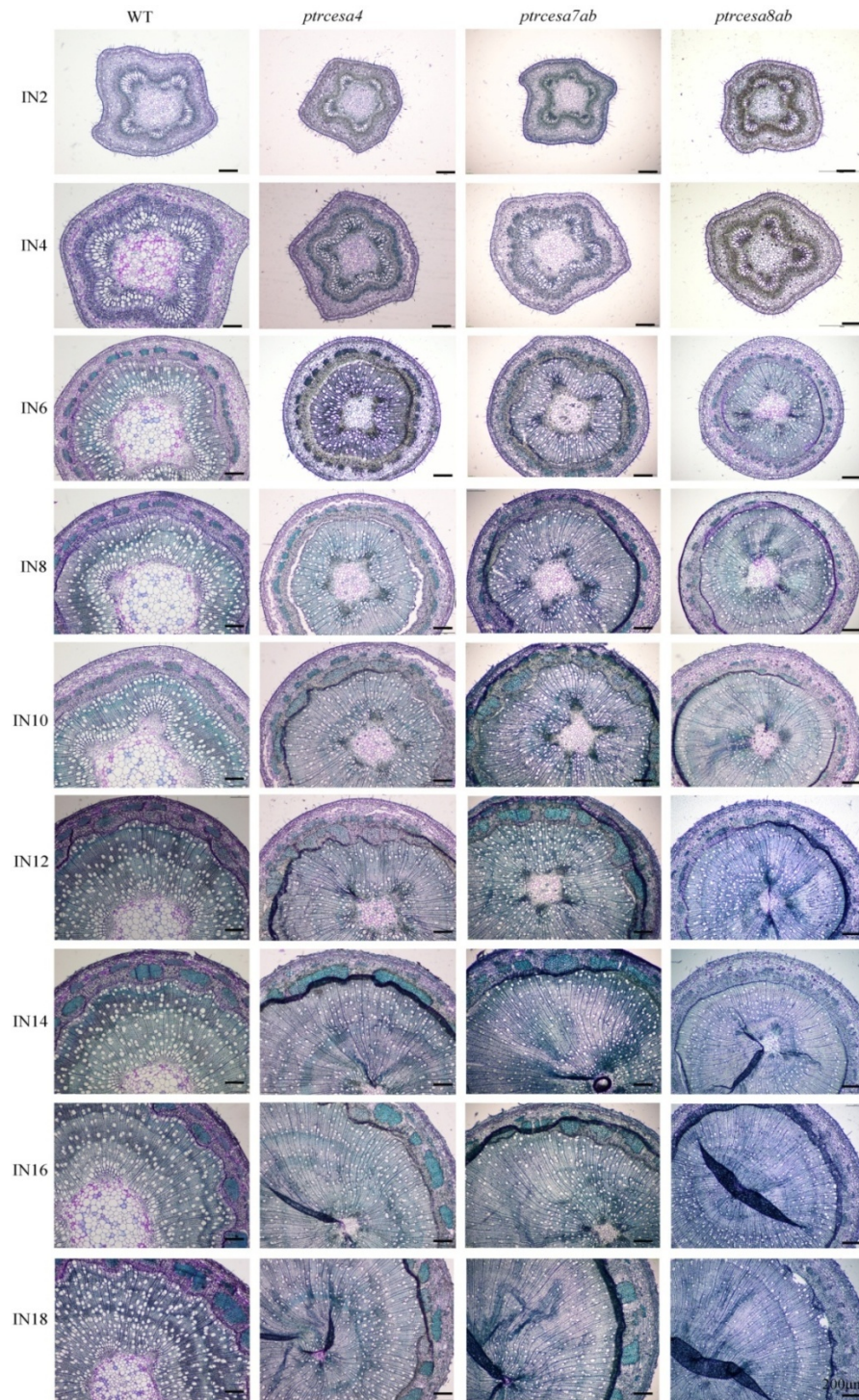




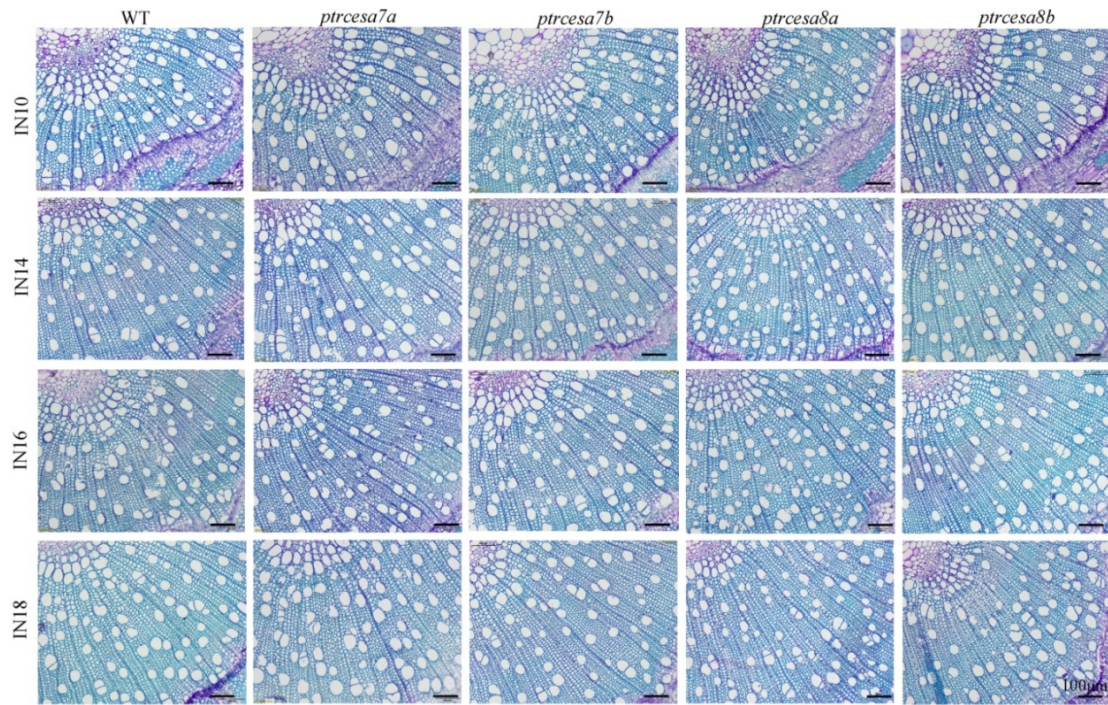
**Fig. S7 Observation of stem internodes, mature leaves, and roots of 3-month-old *ptrcesa4*, *7a/b*, and *8a/b* mutants.** (a-b) Stem diameter and internode length of wild-type (WT) and *ptrcesa* mutants. (c-d) Mature leaves and leaf areas of WT and *ptrcesa* mutants. (e-f) Roots and root biomass of WT and *ptrcesa* mutants. (g) Scanning electron microscopy of leaf epidermal cells in WT and *ptrcesa* mutants. The pavement cells were indicated in red. Scale bars: (c, e) 2 cm; (g) 50  $\mu$ m. Values are means  $\pm$  SD (n = 3). Asterisks denote significant difference from WT by Student's *t*-test: \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



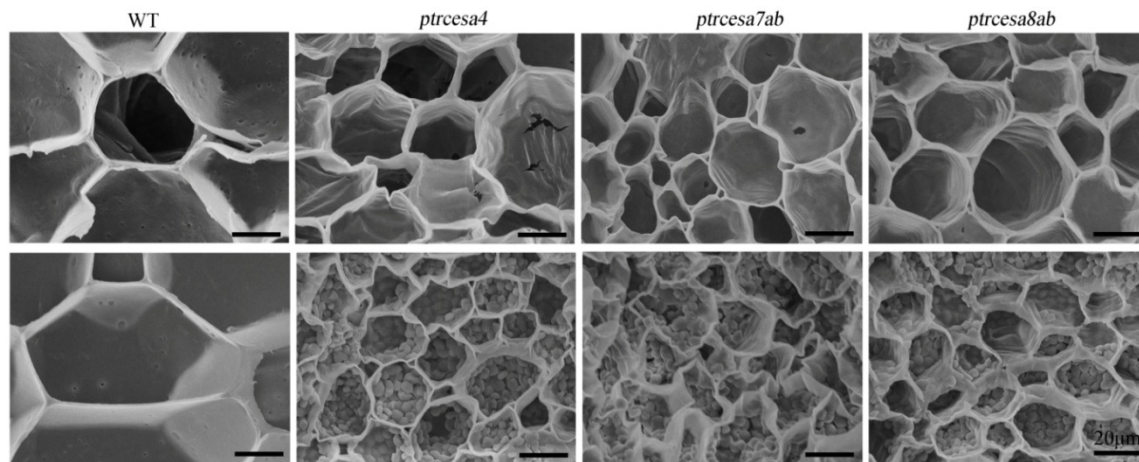
**Fig. S8 Anatomic analysis of different stem internodes from WT, *ptrcesa4*, *7a/b*, and *8a/b* mutants.** Cross-sections from the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup>, 16<sup>th</sup>, and 18<sup>th</sup> stem internodes (IN) were stained with toluidine blue. Scale bars: 200  $\mu$ m.



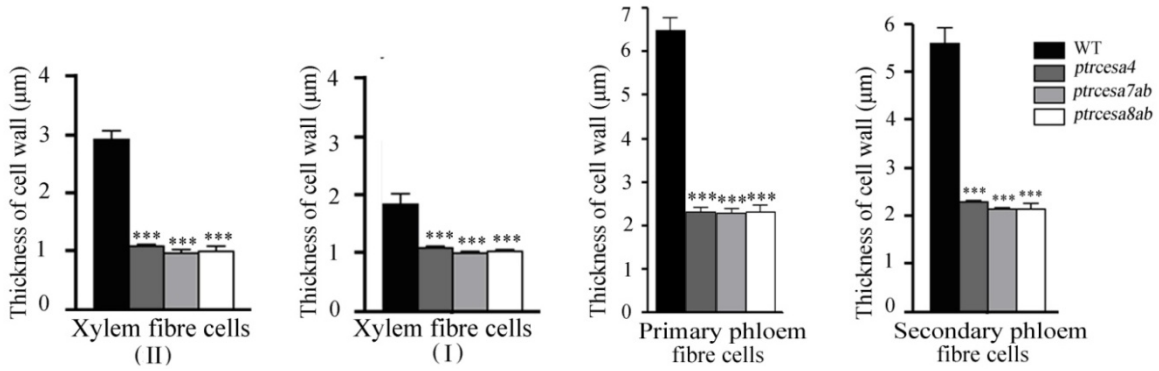
**Fig. S9 Anatomic analysis of different stem internodes from WT, *ptrcesa7a*, *7b*, *8a*, and *8b* mutants.** Cross-sections from the 10<sup>th</sup>, 14<sup>th</sup>, 16<sup>th</sup>, and 18<sup>th</sup> stem internodes (IN) were stained with toluidine blue. Scale bars: 100  $\mu$ m.



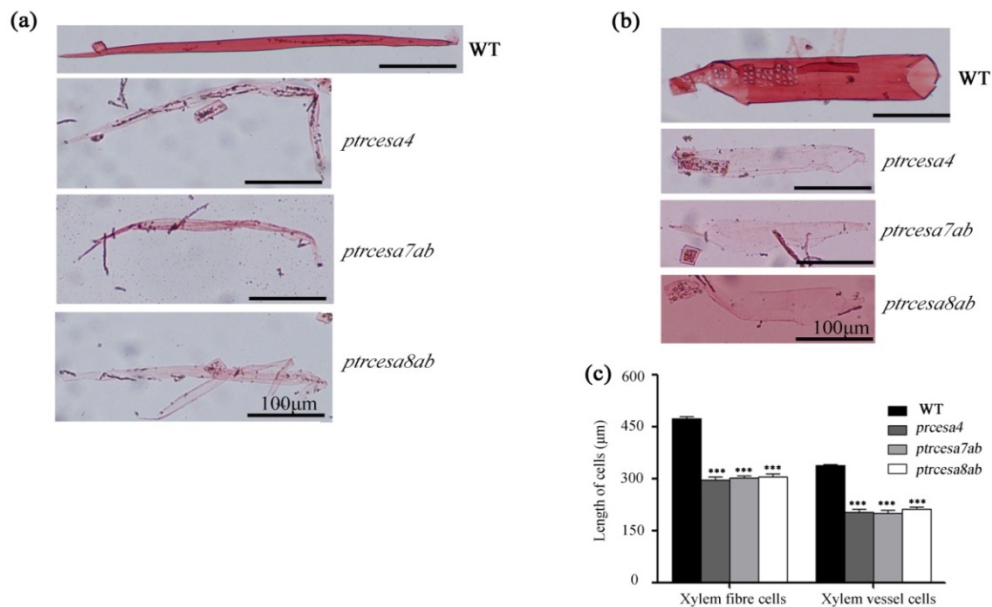
**Fig. S10 Observation of pith parenchyma in WT, *ptrcesa4*, *7a/b*, and *8a/b* mutants.** Scanning electron microscopy images of pith parenchyma in basal stem cross-sections of *ptrcesa* mutant and wild-type (WT) young trees. Upper and lower panels were from 2-month-old and 3-month-old *ptrcesa* mutant and WT trees, respectively. Scale bars: 20  $\mu$ m.



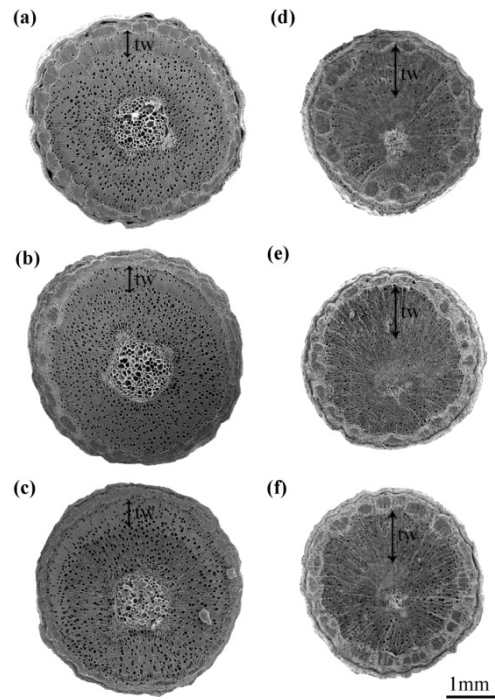
**Fig. S11 Wall thickness of xylem and phloem fibres in the basal stems of 6-month-old WT and *ptrcesa* mutants.** Xylem-I and -II indicated the developing and mature xylem, respectively. Values are means  $\pm$  SD of three replicates. Each replicate contained  $\sim$ 100 measurements. Asterisks denote a significant difference from WT by Student's *t*-test: \*\*\* $P < 0.001$ .



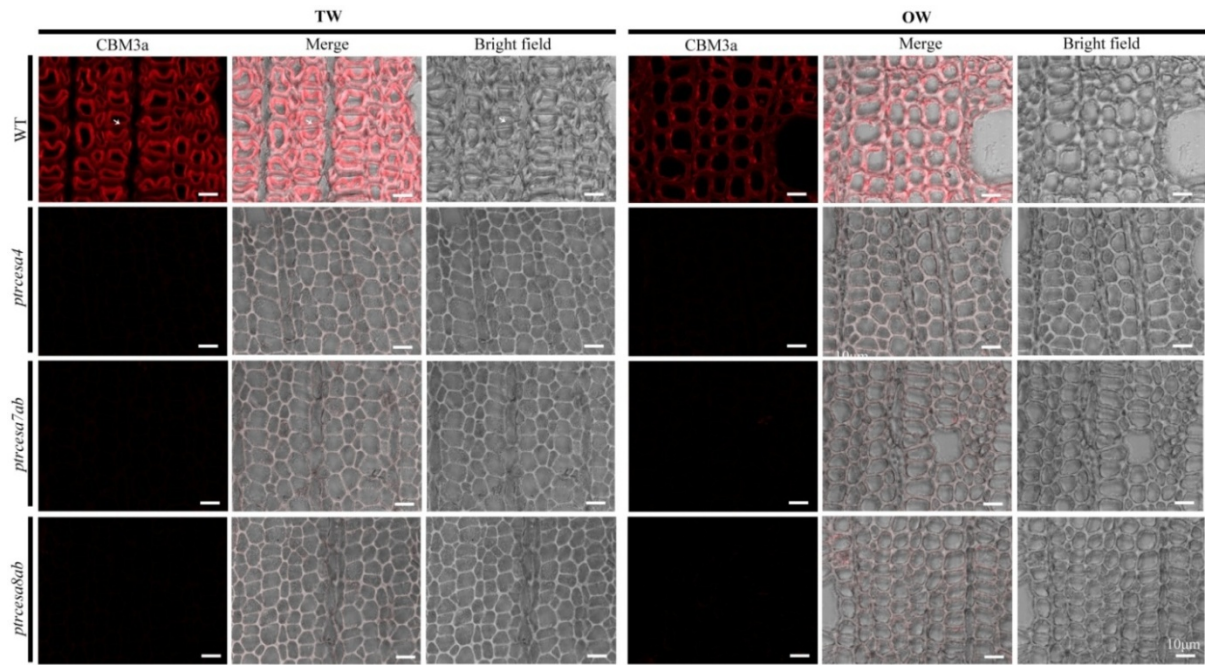
**Fig. S12 Microscopic analysis of the disaggregated xylem fibres and vessels in WT, *ptrcesa4*, *7a/b*, and *8a/b* mutants.** (a-b) Microscopic images of xylem fibers and vessels in the 20<sup>th</sup> stem internodes of the WT and *ptrcesa* mutants. Scale bars: 100  $\mu\text{m}$ . (c) The length of xylem fibers and vessels in WT and *ptrcesa* mutants. Values are means  $\pm$  SD of three replicates. Each replicate contained  $\sim$ 100 measurements. Asterisks denote significant difference from WT by Student's *t*-test: \*\*\* $P < 0.001$ .



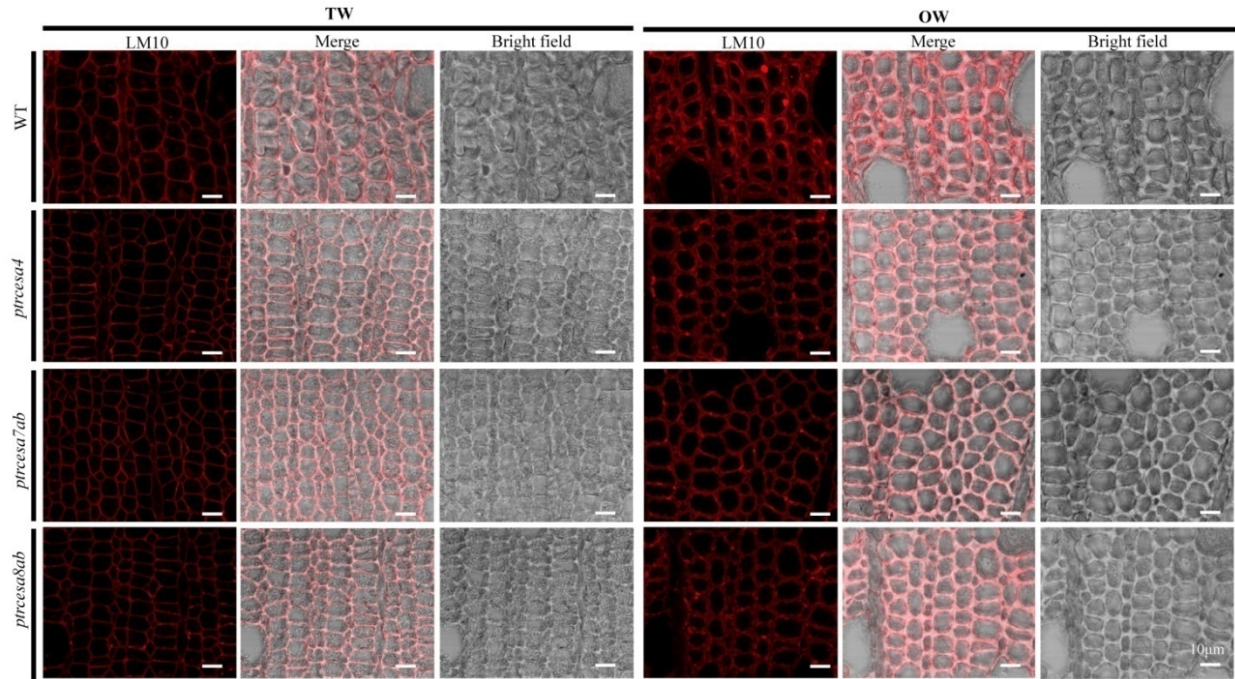
**Fig. S13 Induction of tension wood (TW) in WT and *ptrcesa* mutants under gravi-stimulation.** (a-f) WT, *ptrcesa8a*, *ptrcesa8b*, *ptrces8ab*, *ptrces7ab*, and *ptrcesa4* mutants straight grown for 4 months in a greenhouse were inclined by a 45° angle from the vertical direction to induce TW for 10 days. Scanning electron microscopy images were taken from the cross-sections of the 16<sup>th</sup> internode of each sample. Scale bars: 1mm.



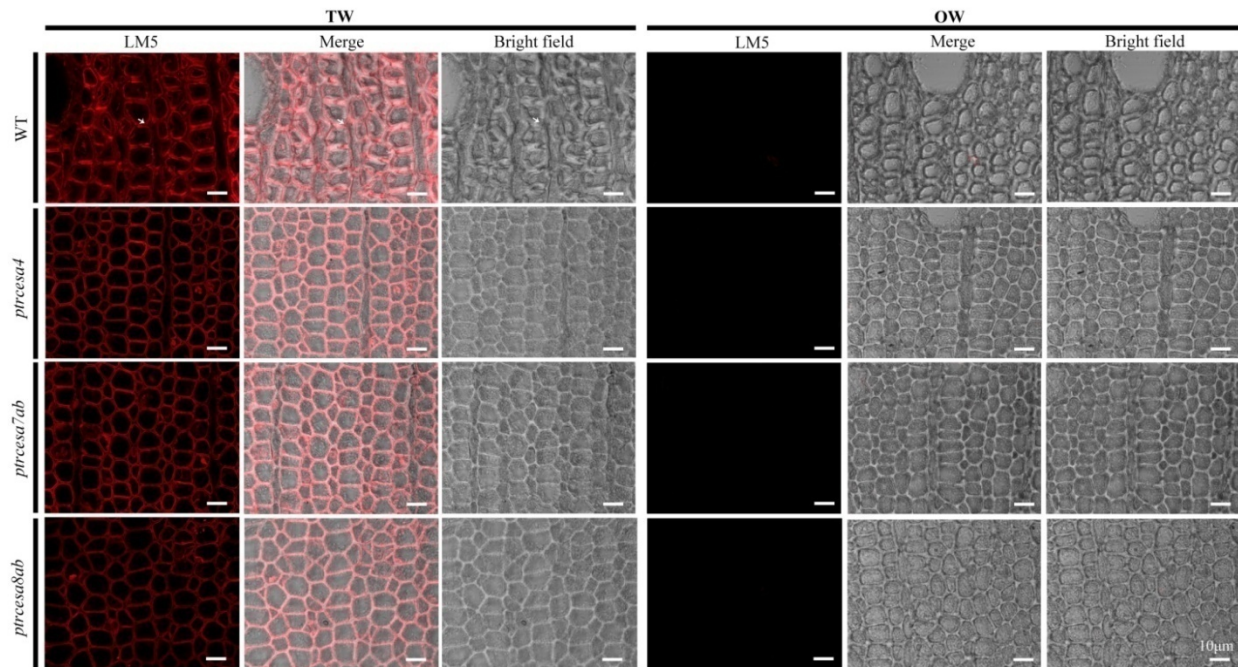
**Fig. S14 Immunolocalization of crystalline cellulose in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** The 8 $\mu$ m transverse cross-sections of the 16<sup>th</sup> internode from each sample were incubated with CBM3a-6 $\times$ His protein and anti-His antibody. CBM3a binds crystalline cellulose in plant cell wall. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. Arrowheads indicate G-layers of TW fibers in the WT. Scale bars: 10  $\mu$ m.



**Fig. S15 Immunolocalization of the xylan in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** The 8 $\mu$ m transverse cross-sections of the 16<sup>th</sup> internode from each sample were incubated with the LM10 antibody. LM10 binds the xylan in plant cell wall. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. Scale bars: 10  $\mu$ m.

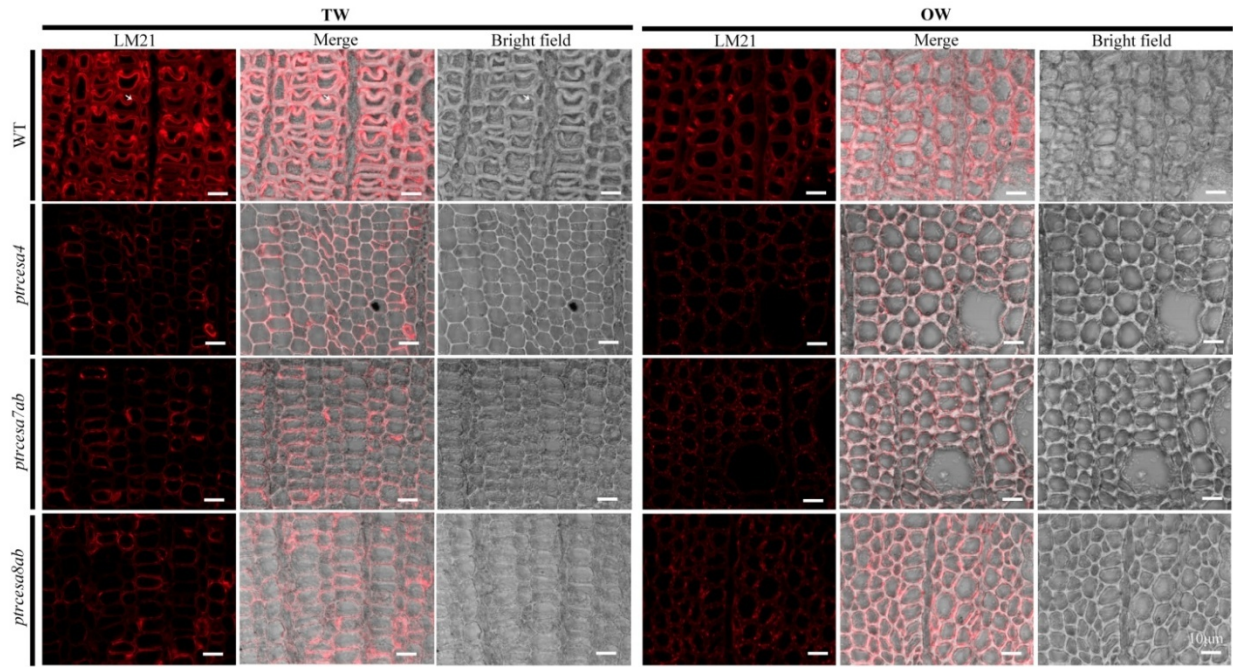


**Fig. S16 Immunolocalization of  $\beta$ -(1 $\rightarrow$ 4)-galactan in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** The 8 $\mu$ m transverse cross-sections of the 16<sup>th</sup> internode from each sample were incubated with the LM5 antibody. LM5 bind  $\beta$ -(1 $\rightarrow$ 4)-galactan in plant cell wall. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. The fibres of OW side showed no fluorescence in WT and mutants. Arrowheads indicate G-layers. Scale bars: 10  $\mu$ m.

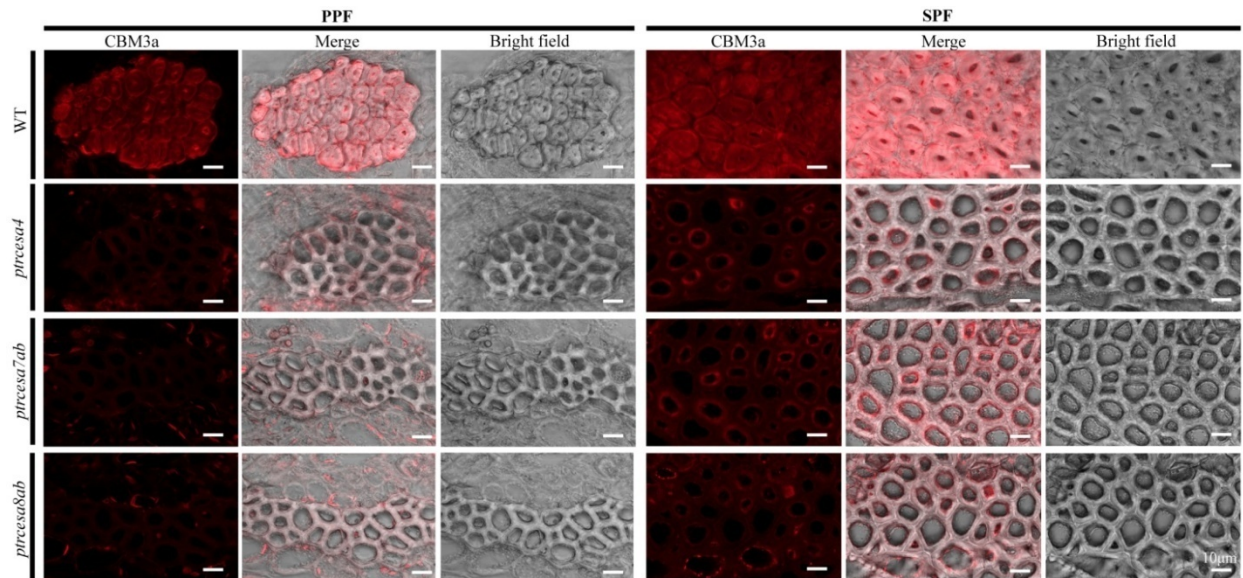




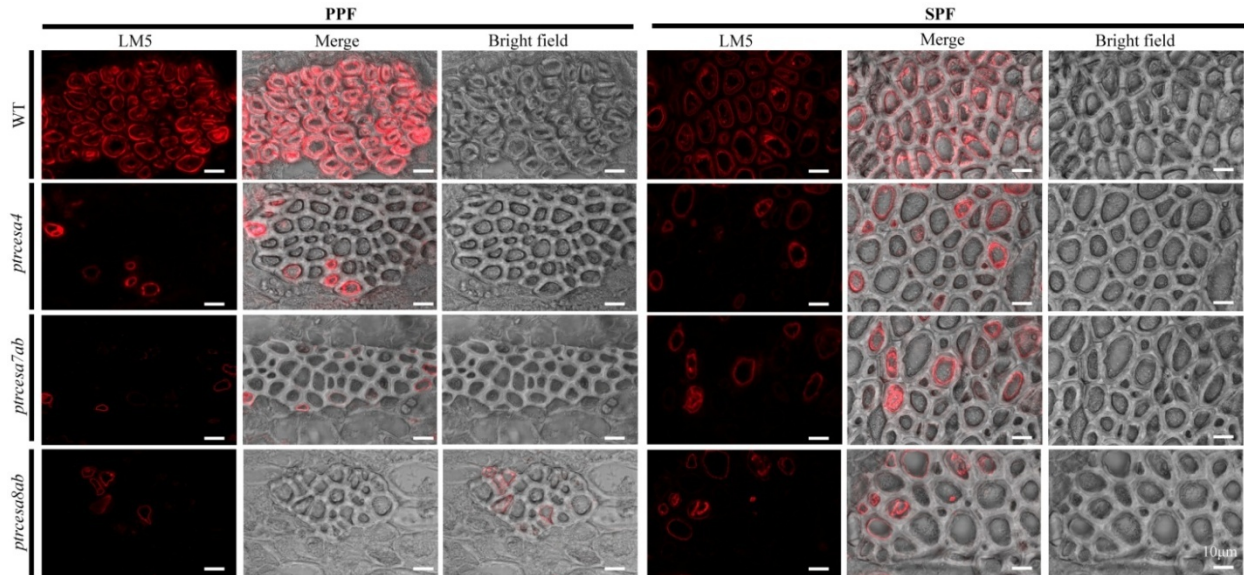
**Fig. S17 Immunolocalization of the mannan in tension and opposite wood (TW, OW) sides in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** The 8 $\mu$ m transverse cross-sections of the 16<sup>th</sup> internode from each sample were incubated with the LM21 antibody. LM21 binds the mannan in plant cell wall. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. Scale bars: 10  $\mu$ m.



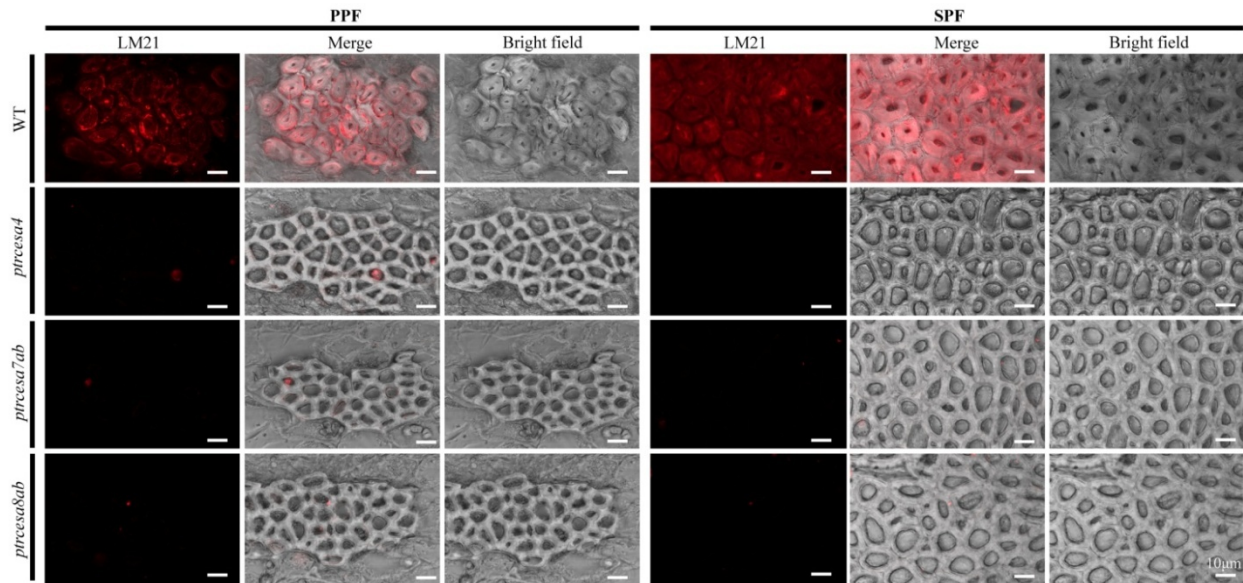
**Fig. S18 Immunolocalization of crystalline cellulose in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** Primary and secondary phloem fibers (PPF, SPF) in the 8 $\mu$ m transverse cross-sections of the 20<sup>th</sup> internode from each sample were incubated with CBM3a-6 $\times$ His protein and anti-His antibody. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. CBM3a binds crystalline cellulose in plant cell wall. Scale bars: 10  $\mu$ m.



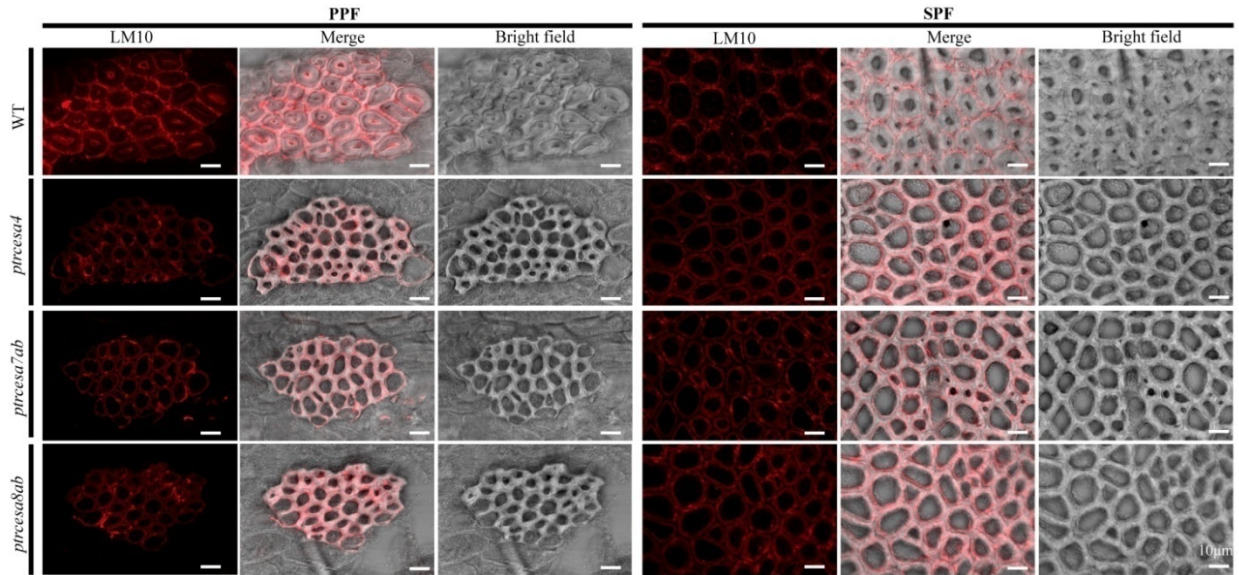
**Fig. S19 Immunolocalization of  $\beta$ -(1 $\rightarrow$ 4)-galactan in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** Primary and secondary phloem fibers (PPF, SPF) in the 8 $\mu$ m transverse cross-sections of the 20<sup>th</sup> internode from each sample were incubated with LM5 antibody. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. LM5 binds  $\beta$ -(1 $\rightarrow$ 4)-galactan in plant cell wall. Scale bars: 10  $\mu$ m.



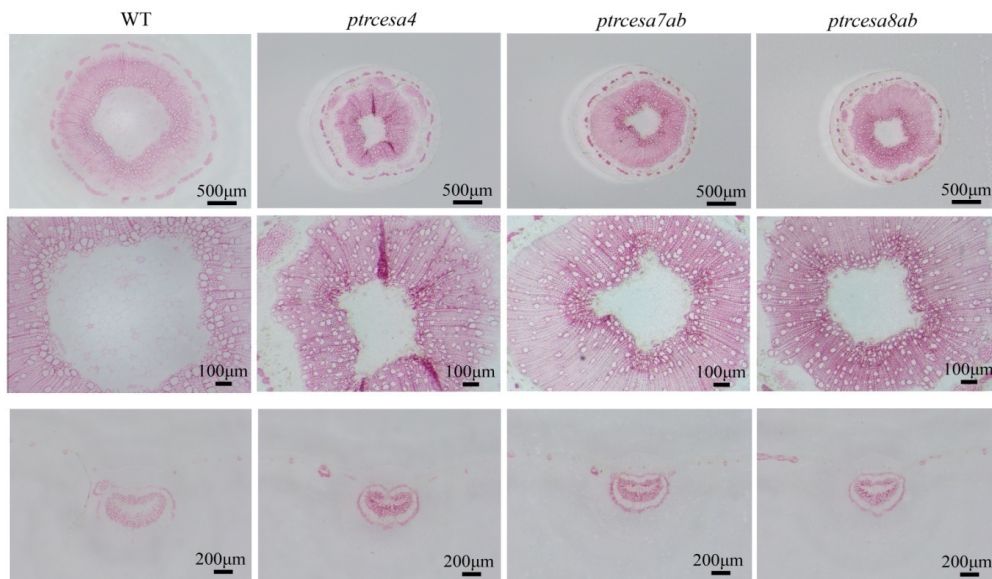
**Fig. S20 Immunolocalization of the mannan in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** Primary and secondary phloem fibers (PPF, SPF) in the 8  $\mu\text{m}$  transverse cross-sections of the 20<sup>th</sup> internode from each sample were incubated with LM5 antibody. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. LM21 binds the mannan in the plant cell wall. Scale bars: 10  $\mu\text{m}$ .



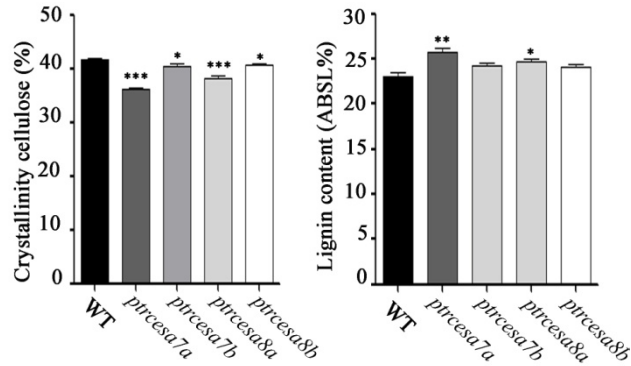
**Fig. S21 Immunolocalization of the xylan in phloem fibres of WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** Primary and secondary phloem fibers (PPF, SPF) in the 8  $\mu\text{m}$  transverse cross-sections of the 20<sup>th</sup> internode from each sample were incubated with LM5 antibody. Signals (red) were detected with Alexa Fluor 633 goat anti-rat IgG. LM10 binds the xylan in plant cell wall. Scale bars: 10  $\mu\text{m}$ .



**Fig. S22 Lignin phloroglucinol staining in WT, *ptrcesa4*, *7ab*, and *8ab* mutants.** (a-b) Cross-sections of the 10<sup>th</sup> stem internodes. (c) Cross-sections of the 8<sup>th</sup> leaf petioles (from apical bud) from WT and *ptrcesa* mutants. Scale bars: (a) 500  $\mu\text{m}$ ; (b) 200  $\mu\text{m}$ ; (c) 100  $\mu\text{m}$ .



**Fig. S23 Crystalline cellulose and lignin content in woods of WT, *ptrcesa7a*, *7b*, *8a*, and *8b* mutants.** Values are means  $\pm$  SD (n = 3). Asterisks denote significantly different from WT by Student's *t*-test: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.



**Methods S1 Analysis of putative Cas9/gRNA off-target sites.** Putative gRNA off-target sites were identified by a BLASTN search using a target sequence in the *P. trichocarpa* genome. The final off-target sites were selected according to the following criteria: two to three mismatches in the PAM-proximal region, four to five mismatches within the PAM-proximal region, or one to two mismatches in the PAM. Specific primers (Table S1) flanking the off-target sites were used to amplify the corresponding loci, and the PCR amplicons were sequenced as described above. Eventually, 8 potential off-target sites for gCHLI1-a and gCHLI1-b were analyzed in 6 arbitrarily chosen *ptrchli1* mutants, and 25 potential off-target sites were evaluated in *ptrcesa4-1#*, *4-2#* and *4-6#*, *ptrcesa7ab-3#*, *7ab-7#* and *7ab-13#*, and *ptrcesa8ab-1#*, *8ab-4#* and *8ab-8#*. These 9 knockout mutants were used for the subsequent phenotypic analysis.

**Methods S2 RNA extraction and RT-PCR analysis.** Total RNA was isolated using the pBIOZOL Plant Total RNA Extraction Reagent (Bio-Flux, China). First-strand cDNA synthesis was performed with 2  $\mu$ g RNA using the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, China) according to the manufacturer's procedure. Reverse transcription PCR (RT-PCR) was employed to analyze the expression of *PtrCesA4*, *7A*, *7B*, *8A* and *8B* genes in the *ptrcesa* mutants. RT-PCR analysis was performed for three biological replicates each sample. The *PtrActin2* gene was used as a reference control. The PCR conditions were set as follows: 3 min at 95°C; 28 cycles of 30 s at 95°C, 30 s at 62°C and 30 s at 72°C; and 7 min at 72°C.

**Methods S3 Scanning electron microscopy (SEM) of leaf epidermal cells.** Fresh 6<sup>th</sup> leaves below the terminal buds of 3-month-old mutant and wild-type young trees were immediately frozen in liquid nitrogen and stored until observation. These samples were laid on a copper sheet (1.5 cm × 2 cm), transferred to an SEM chamber and scanned to produce micrographs. These images were used to determine the shapes and sizes of the epidermal cells.

**Methods S4 Wood fibre and vessel cell length analysis.** The lengths of the wood fibres and vessel cells were determined according to the previously described method (Lautner *et al.*, 2007) with minor modifications. The 20<sup>th</sup> stem internodes of 6-month-old wild-type and mutant trees were peeled, cut into small pieces, and incubated in a maceration solution (10% HNO<sub>3</sub> and 10% CrO<sub>3</sub> in v/v, 1:1) for 2-4 h at 60 °C. Thereafter, each stem internode was rinsed lightly with water, and the libriform fibres and vessel cells were disaggregated into the water by oscillation. After staining with 0.1% acid magenta, images were taken under a light microscope (Olympus, BX43). The lengths of the wood fibres and vessel cells were measured using ImageJ software.

#### **References**

**Lautner S, Ehlting B, Windeisen E, Rennenberg H, Matyssek R, Fromm J. 2007.** Calcium nutrition has a significant influence on wood formation in poplar. *New Phytologist* **173**: 743-752.

**Table S1** Primers used in this study.

Purpose	Primer Name	Sequence (5'-3')
<b>Primers for CRISPR/Cas9 vector construction</b>		
<b>Cas9/gRNA-PtrCHLI1</b>	DT1-BsF/PtrCHLI1	ATATATGGTCTCGATTGTTACTTCCAGAAATTAAGGGTT
	DT1-F0/PtrCHLI1	TGTTACTTCCAGAAATTAAGGGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCHLI1	AACGAAAGTCCACCACTGTTAGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCHLI1	ATTATTGGTCTCTAAACGAAAGTCCACCACTGTTAGC
<b>Cas9/gRNA-PtrCHLI1/2-1</b>	DT1-BsF/PtrCHLI1/2-1	ATATATGGTCTCGATTGTTCTTGCTAAAGCTAATAGGTT
	DT1-F0/PtrCHLI1/2-1	TGTTCTTGCTAAAGCTAATAGGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCHLI1/2-1	AACAAGATTGGAGGTGTCATGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCHLI1/2-1	ATTATTGGTCTCTAAACAAGATTGGAGGTGTCATGC
<b>Cas9/gRNA-PtrCHLI1/2-2</b>	DT1-BsF/PtrCHLI1/2-2	ATATATGGTCTCGATTGTGCGATGGGTATTGAAGTCAGTT
	DT1-F0/PtrCHLI1/2-2	TGTCGATGGGTATTGAAGTCAGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCHLI1/2-2	AACTTTGCTGCTATAGTAGGGCCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCHLI1/2-2	ATTATTGGTCTCTAAACTTTGCTGCTATAGTAGGGCC
<b>Cas9/gRNA-PtrCesA4-1</b>	DT1-BsF/PtrCesA4-1	ATATATGGTCTCGATTGATACGATGCTTATGCATTGGTT
	DT1-F0/PtrCesA4-1	TGATACGATGCTTATGCATTGGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA4-1	AACATTCGTCGGCCTTTTCGTCATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA4-1	ATTATTGGTCTCTAAACATTCGTCGGCCTTTTCGTC
<b>Cas9/gRNA-PtrCesA4-2</b>	DT1-BsF/PtrCesA4-2	ATATATGGTCTCGATTGATCTGTGTTAGAAGTTCGAGTT
	DT1-F0/PtrCesA4-2	TGATCTGTGTTAGAAGTTCGAGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA4-2	AACCCTCAAGTCCCTTGGAGGACAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA4-2	ATTATTGGTCTCTAAACCCTCAAGTCCCTTGGAGGAC
<b>Cas9/gRNA-PtrCesA7A-1</b>	DT1-BsF/PtrCesA7A-1	ATATATGGTCTCGATTGTATTATTGCAGGTTAGACGGTT
	DT1-F0/PtrCesA7A-1	TGTATTATTGCAGGTTAGACGGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA7A-1	AACTATTGGATCTCATGGAGAGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA7A-1	ATTATTGGTCTCTAAACTATTGGATCTCATGGAGAGC
<b>Cas9/gRNA-PtrCesA7A-2</b>	DT1-BsF/PtrCesA7A-2	ATATATGGTCTCGATTGACAGGAATGGATAACGAGAGTT
	DT1-F0/PtrCesA7A-2	TGACAGGAATGGATAACGAGAGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA7A-2	AACTGACACTGAAGGAAATGAGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA7A-2	ATTATTGGTCTCTAAACTGACACTGAAGGAAATGAGC
<b>Cas9/gRNA-PtrCesA7B-1</b>	DT1-BsF/PtrCesA7B-1	ATATATGGTCTCGATTGCAAGATGACGATGCAGAAGGTT
	DT1-F0/PtrCesA7B-1	TGCAAGATGACGATGCAGAAGGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA7B-1	AACTTGGATCTCATGGAGAACAACAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA7B-1	ATTATTGGTCTCTAAACTTGGATCTCATGGAGAACAC
<b>Cas9/gRNA-PtrCesA7B-2</b>	DT1-BsF/PtrCesA7B-2	ATATATGGTCTCGATTGCCCAATATGCTTGCTCCAGGTT
	DT1-F0/PtrCesA7B-2	TGCCCAATATGCTTGCTCCAGGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA7B-2	AACGCAAGATCAATCCGTATAGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA7B-2	ATTATTGGTCTCTAAACGCAAGATCAATCCGTATAGC
<b>Cas9/gRNA-PtrCesA8A-1</b>	DT1-BsF/PtrCesA8A-1	ATATATGGTCTCGATTGTGTTTCGAGTATGAGATCAGTT
	DT1-F0/PtrCesA8A-1	TGTGTTTCGAGTATGAGATCAGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA8A-1	AACGGTGGGACATGATGCAAATCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA8A-1	ATTATTGGTCTCTAAACGGTGGGACATGATGCAAATC
<b>Cas9/gRNA-PtrCesA8A-2</b>	DT1-BsF/PtrCesA8A-2	ATATATGGTCTCGATTGGGAAATGAACTACCTCGACGTT
	DT1-F0/PtrCesA8A-2	TGGGAAATGAACTACCTCGACGTTTATAGAGCTAGAAATAGC
	DT2-R0/PtrCesA8A-2	AACGAGCTCGTGACATAGAGGGCAATCTCTTAGTCGACTCTAC



	DT2-BsR/PtrCesA8A-2	ATTATTGGTCTCTAAACGAGCTCGTGACATAGAGGGC
<b>Cas9/gRNA-PtrCesA8B-1</b>	DT1-BsF/PtrCesA8B-1	ATATATGGTCTCGATTGGGGCCGAAAGTTTGCTGGTT
	DT1-F0/PtrCesA8B-1	TGGGGCCGAAAGTTTGCTGGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtrCesA8B-1	AACGAAAATCCCTTCCATTTGCCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA8B-1	ATTATTGGTCTCTAAACGAAAATCCCTTCCATTTGCC
<b>Cas9/gRNA-PtrCesA8B-2</b>	DT1-BsF/PtrCesA8B-2	ATATATGGTCTCGATTGGTGAGCCTTCTCAGCTTGCGTT
	DT1-F0/PtrCesA8B-2	TGGTGAGCCTTCTCAGCTTGCGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtrCesA8B-2	AACGCTTTCTCCAGAGAACTTGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA8B-2	ATTATTGGTCTCTAAACGCTTTCTCCAGAGAACTTGC
<b>Cas9/gRNA-PtrCesA7A/B-1</b>	DT1-BsF/PtrCesA7A/B-1	ATATATGGTCTCGATTGCGCAAAGAAAGAGGGAGGGGTT
	DT1-F0/PtrCesA7A/B-1	TGCGCAAAGAAAGAGGGAGGGGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtrCesA7A/B-1	AACTATCCAGTTTCTGAACCTGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA7A/B-1	ATTATTGGTCTCTAAACTATCCAGTTTCTGAACCTGC
<b>Cas9/gRNA-PtrCesA7A/B-2</b>	DT1-BsF/PtrCesA7A/B-2	ATATATGGTCTCGATTGTTTGTGACTTCAACTTTAAGTT
	DT1-F0/PtrCesA7A/B-2	TGTTTGTGACTTCAACTTTAAGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtrCesA7A/B-2	AACCTTCTCGAGTCCAGCGGCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA7A/B-2	ATTATTGGTCTCTAAACCTTCTCGAGTCCAGCGGC
<b>Cas9/gRNA-PtrCesA8A/B-1</b>	DT1-BsF/PtrCesA8A/B-1	ATATATGGTCTCGATTGGTCCGCCGGAACAGCAGAGTT
	DT1-F0/PtrCesA8A/B-1	TGGTCCGCCGGAACAGCAGAGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtrCesA8A/B-1	AACCTACAGAATAACAAATCCTCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA8A/B-1	ATTATTGGTCTCTAAACCTACAGAATAACAAATCCTC
<b>Cas9/gRNA-PtrCesA8A/B-2</b>	DT1-BsF/PtrCesA8A/B-2	ATATATGGTCTCGATTGAACTGGTTGTGTTTTCAATGTT
	DT1-F0/PtrCesA8A/B-2	TGAACTGGTTGTGTTTTCAATGTTTTAGAGCTAGAAATAGC
	DT2-R0/PtrCesA8A/B-2	AACCAAGTAGGTCGAGATGTATCAATCTCTTAGTCGACTCTAC
	DT2-BsR/PtrCesA8A/B-2	ATTATTGGTCTCTAAACCAAGTAGGTCGAGATGTATC
<b>Primers used to identify Cas9-gRNAs targeted sites</b>		
<b>gCHLI1-a/b-identify</b>	gCHLI1-a/b-identify-F	GCCACTAAACAATGCATCATTTTC
	gCHLI1-a/b-identify-R	TCCTAGCTGAGGAAATTTGTTGC
<b>gCHLI1/2-a/b/c/d-identify</b>	gCHLI1/2-a/b/c/d-identify-F	TCGACCCTTCTCCAAACCTTCCA
	gCHLI1/2-a/b/c/d-identify-R	CCCTAGCTGAGGCAATTTGTTGC
<b>gCesA4-1/2-identify</b>	gCesA4-1/2-identify-F	ATGGCTGGCCTTGTCACGGGCAG
	gCesA4-1/2-identify-R	TGTCGACAGGATAATCAACGGAT
<b>gCesA4-3/4-identify</b>	gCesA4-3/4-identify-F	GGTGAGTTGAAAGATTACTGAAT
	gCesA4-3/4-identify-R	CTAGCACTCCACCCACATTGTT
<b>gCesA7A-a/b-identify</b>	gCesA7A-a/b-identify-F	TCATTCTCTCGATCTTATCTTT
	gCesA7A-a/b-identify-R	GGTTCTACACTAACTACAAACAA
<b>gCesA7A-c/d-identify</b>	gCesA7A-c/d-identify-F	TTGTTTGTAGTTAGTGTAGAACC
	gCesA7A-c/d-identify-R	ACTTCTAAGCAACAAGCAAACCTT
<b>gCesA7B-a/b/c/d-identify</b>	gCesA7B-a/b/c/d-identify-F	GCATGGGATTCTCAAGCTGATTT
	gCesA7B-a/b/c/d-identify-R	TATAGATGTCGATGCGAATTACG
<b>gCesA8A-a/b-identify</b>	gCesA8A-a/b-identify-F	ATGATGGAATCTGGGGCTCCT
	gCesA8A-a/b-identify-R	TGCATCATTAACCATTAACATT
<b>gCesA8A-c/d-identify</b>	gCesA8A-c/d-identify-F	TCAAGAACATTAAGGTCTTGGGT
	gCesA8A-c/d-identify-R	TATGATGATGCCAAGCCTTACTG
<b>gCesA8B-a/b-identify</b>	gCesA8B-a/b-identify-F	ATGATGGAATCTGGGGCTCCT
	gCesA8B-a/b-identify-R	AAACTTCATGGATTAAACTAGAA
<b>gCesA8B-c/d-identify</b>	gCesA8B-c/d-identify-F	CTATAGGAAGCAAAAAATCTCAT

	gCesA8B-c/d-identify-R	GGACTGCTAATAAATATTGAAGT
gCesA7A-1/2-identify	gCesA7A-1/2-identify-F	TCATTCTCTTCGATCTTATCTTT
	gCesA7A-1/2-identify-R	GGTTCTACACTAACTACAAACAA
gCesA7A-3/4-identify	gCesA7A-3/4-identify-F	CTGGACTAAAACAAACCCATGCT
	gCesA7A-3/4-identify-R	ACTTCTAAGCAACAAGCAAACCT
gCesA7B-1/2-identify	gCesA7B-1/2-identify-F	GCATGGGATTCTCAAGCTGATTT
	gCesA7B-1/2-identify-R	TATAGATGTCGATGCGAATTACG
gCesA7B-3/4-identify	gCesA7B-3/4-identify-F	CTGGCTTACCCCGTGTGTATGCT
	gCesA7B-3/4-identify-R	AAATACATTTTCAGAGCTACTGGA
gCesA8A-1/2-identify	gCesA8A-1/2-identify-F	AATGTTAATGGTTAATGATGCA
	gCesA8A-1/2-identify-R	TTATAATGCAGCATAGTGAATAT
gCesA8A-3/4-identify	gCesA8A-3/4-identify-F	TCAAGAACATTAAGGTCTTGGGT
	gCesA8A-3/4-identify-R	AAATGTCATGGAAGCCCACATTG
gCesA8B-1/2-identify	gCesA8B-1/2-identify-F	TTCTAGTTAATCCATGAAGTTT
	gCesA8B-1/2-identify-R	ATGAGATTTTTTGCTTCCTATAG
gCesA8B-3/4-identify	gCesA8B-3/4-identify-F	ACTTCAATATTTATTAGCAGTCC
	gCesA8B-3/4-identify-R	AATGGGTAATGGGTAGCCTTG
<b>Primers used to identify off-targets</b>		
gCHLI1a-off-1	gCHLI1-a-off-1-F	TCGACCCTTCTCCAAACCTTCCA
	gCHLI1-a-off-1-R	CCCTAGCTGAGGCAATTTGTTGC
gCHLI1a-off-2	gCHLI1-a-off-2-F	CCACATTCTTAAGCATATTAATA
	gCHLI1-a-off-2-R	TCACTTATATTCCTATCTACTC
gCHLI1a-off-3	gCHLI1-a-off-3-F	TTGTTCTAAGCGCGACCGTAC
	gCHLI1-a-off-3-R	CAGAAGTGAAGTGAAGATGAG
gCHLI1a-off-4	gCHLI1-a-off-4-F	GCTTAGCTAGATGAATCGTTGT
	gCHLI1-a-off-4-R	CATTGCAAATCTTGGAGACATG
gCHLI1a-off-5	gCHLI1-a-off-5-F	GCTGTACTCTTCATCGGAAGT
	gCHLI1-a-off-5-R	ATACTGTGTTTTCAACTTGATCC
gCHLI1b-off-1	gCHLI1-b-off-1-F	TCGACCCTTCTCCAAACCTTCCA
	gCHLI1-b-off-1-R	CCCTAGCTGAGGCAATTTGTTGC
gCHLI1b-off-2	gCHLI1-b-off-2-F	CCGTTCTACGATATTTATCGG
	gCHLI1-b-off-2-R	CTCACGAACATGATACACTGAT
gCHLI1b-off-3	gCHLI1-b-off-3-F	GGCTAACGTCTCCTTCTGCA
	gCHLI1-b-off-3-R	GTTCCTCTCTATATCCACACG
gCesA4-1-off-1	gCesA4-1-off-1-F	GAACCTGAGCTCTGTGCACTA
	gCesA4-1-off-1-R	GAGACAATGTCAACAAGTCTC
gCesA4-1-off-2	gCesA4-1-off-2-F	CTTGTATTGATCCCATGGTTGT
	gCesA4-1-off-2-R	AAGAAGTTACATCTTCGCAAGG
gCesA4-3-off-1	gCesA4-3-off-1-F	GCAGGCAAGGCTTGATGATATTG
	gCesA4-3-off-1-R	CCGGATTCTACAATCTTCATGTG
gCesA4-3-off-2	gCesA4-3-off-2-F	GTGTTTAGCATTGTGATAGCGG
	gCesA4-3-off-2-R	CAAGGTCTCTCGTTGAGAATTC
gCesA4-4-off-1	gCesA4-4-off-1-F	GTAATCATGTAGAGTGCAATGG
	gCesA4-4-off-1-R	GCTTGACACACGTAGAACATC
gCesA4-4-off-2	gCesA4-4-off-2-F	GAACTTCTCGGCAAACCAAG
	gCesA4-4-off-2-R	GCAAGTGAAGTGACAGGTT
gCesA7A/B-1-off-1	gCesA7A/B-1-off-1-F	GGATTGAGCAATGAGATTTC

	gCesA7A/B-1-off-1-R	TGTAAGTGTTCATCCACTAGC
<b>gCesA7A/B-1-off-2</b>	gCesA7A/B-1-off-2-F	CCTTCTTCTCAACAGCGATT
	gCesA7A/B-1-off-2-R	AGACGATGGAGGAGATGCTT
<b>gCesA7A/B-1-off-3</b>	gCesA7A/B-1-off-3-F	GGTCGTGTCAGTTAGGCTGG
	gCesA7A/B-1-off-3-R	CAAGGCCAAATGTATTGCAATC
<b>gCesA7A/B-1-off-4</b>	gCesA7A/B-1-off-4-F	GCTTGCATCCTCTGGCTATA
	gCesA7A/B-1-off-4-R	CAATTAGGCGTTCATCTCCT
<b>gCesA7A/B-1-off-5</b>	gCesA7A/B-1-off-5-F	GTACCTTGATTTGAATCTGCA
	gCesA7A/B-1-off-5-R	TCCAAGAAATTCCTTGAGTGC
<b>gCesA7A/B-1-off-6</b>	gCesA7A/B-1-off-6-F	GCTGTGCGATCTCTCAATCAAC
	gCesA7A/B-1-off-6-R	GTCATAGCTGCTGCTGATTCT
<b>gCesA7A/B-2-off-1</b>	gCesA7A/B-2-off-1-F	GATATGCCTCATTTCAATCCATG
	gCesA7A/B-2-off-1-R	CTAATAGGCCTTGTTGACATCAC
<b>gCesA7A/B-2-off-2</b>	gCesA7A/B-2-off-2-F	GCAAACATTATGGTGACGAC
	gCesA7A/B-2-off-2-R	GCTGCCTTTGGTCATAACAAT
<b>gCesA7A/B-3-off-1</b>	gCesA7A/B-3-off-1-F	GAAAGTACAACCTCCTTGCTA
	gCesA7A/B-3-off-1-R	CTTGTTGCTGCTTCATTCCT
<b>gCesA7A/B-3-off-2</b>	gCesA7A/B-3-off-2-F	GTTCATCATGCCAAATGAGGTA
	gCesA7A/B-3-off-2-R	TGCACAGTGAATTTGAAATTACC
<b>gCesA7A/B-3-off-3</b>	gCesA7A/B-3-off-3-F	GTTCATCATGCCAATTGAGGTA
	gCesA7A/B-3-off-3-R	GCATAATCTAGAGACCAAGAAC
<b>gCesA7A/B-3-off-4</b>	gCesA7A/B-3-off-4-F	ATAGCTATCCGTTTGCCTGTGA
	gCesA7A/B-3-off-4-R	GAGAATTCTAAGGTATTTAGAC
<b>gCesA7A/B-4-off-1</b>	gCesA7A/B-4-off-1-F	CATGCTAACACAAGAAGAAGG
	gCesA7A/B-4-off-1-R	CATCAACACGAAGGCTGTTG
<b>gCesA8A/B-2-off-1</b>	gCesA8A/B-2-off-1-F	CTAGGTGCTAACTACTCA
	gCesA8A/B-2-off-1-R	GACGGTTCTCCTTCATTGTC
<b>gCesA8A/B-2-off-2</b>	gCesA8A/B-2-off-2-F	GAAGCCATTGGCTGTGATTG
	gCesA8A/B-2-off-2-R	CCGTTTTAGTCGCAACATAC
<b>gCesA8A/B-3-off-1</b>	gCesA8A/B-3-off-1-F	GCACGAAGATTCCAATTGGA
	gCesA8A/B-3-off-1-R	GTACAAGTAGTGGCAATGTGC
<b>gCesA8A/B-3-off-2</b>	gCesA8A/B-3-off-2-F	GAAGTTAGGACACATGTCAA
	gCesA8A/B-3-off-2-R	CTACCATGCTCTCGTTACGA
<b>gCesA8A/B-3-off-3</b>	gCesA8A/B-3-off-3-F	GATGTTTCTATGGACGTCTA
	gCesA8A/B-3-off-3-R	GATATCATTCCAATCAGAGCAA
<b>gCesA8A/B-3-off-4</b>	gCesA8A/B-3-off-4-F	GACTCTGGTTGATGTGAATACT
	gCesA8A/B-3-off-4-R	GCTACTAAGCTTCAACTATACA
<b>Primers used to identify transgenic plants</b>		
<b><i>zCas</i></b>	zCas-F	TGAGAACATCGTCATTGAGATGG
	zCas-R	TCAGCTTGTCATTCTCATCGTAC
<b><i>Hyg</i></b>	Hyg-F	GAGCTTGTGCGATCGACAGAT
	Hyg-R	CATATGCGCGATTGCTGATC
<b><i>PtrActin2</i></b>	Actin2-F	AACATGGGATTGTTAGCAACTGG
	Actin2-R	TCCATCACCAGAATCCAGCACA
<b>Primers used for RT-PCR</b>		
<b>PtrCesA4</b>	PtrCesA4-F	GACGATGCCGAGTTTGGAGAGC
	PtrCesA4-R	CAACTGTGCAAAAGTGGGATAACCT

<b>PtrCesA7A</b>	PtrCesA7A-F	GGAATTCAGGGTCCGGTGTACG
	PtrCesA7A-R	AATAGACCTCCATCCACGGCAA
<b>PtrCesA7B</b>	PtrCesA7B -F	AAGGCAAGCTTTGTATGGCTATGAC
	PtrCesA7B -R	AATAGACCTCCAGCCACGACAA
<b>PtrCesA8A</b>	PtrCesA8A -F	TTGTTGGTGTGTTGCGGGATT
	PtrCesA8A -R	CATCTCGCAGTTCATGTA ACTCAACTACT
<b>PtrCesA8B</b>	PtrCesA8B-F	CTGGCATCGATACGAACTTCAC
	PtrCesA8B-R	TCTTGAGAGAACTACAACGAGGA

**Table S2** The Cas9/gRNA-induced target site mutations in single *PtrCHL11* gene of *Populus trichocarpa*.

Gene: Potri.004G053400 ( <i>PtrCHL11</i> )						
Vector: Cas9/gRNA-CHL11						
gRNA: gCHL11-a (TTTACTTCCAGAAATTAAGGTGG); gCHL11-b (CCGAAAAGTCCACCACTGTTAGG)						
Target sequences: CHL11-a, TTTACTTCCAGAAATTAAGGTGG; CHL11-b, CCGAAAAGTCCACCACTGTTAGG						
Line (#)	CHL11-a TTTACTTCCAGAAATTAAGGTGG	Mutation types (Number of plasmids containing the cloned PCR products)		CHL11-b CCGAAAAGTCCACCACTGTTAGG	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrchl1-1#</i>	... ----- AAGGTGGTTGCT	-44 (×25)	homozygous	AGGGACCGGAA ----- ...	-44 (×25)	homozygous
<i>ptrchl1-2#</i>	GTTGATTTACTTCCAGAAATT - AGGTGGTTGCT GTTGATTTACTTCCAGAAATTAAGGTGGTTGCT	-1 (×16) 0 (×9)	heterozygous	AGGGACC ----- CACCACTGTTAGGTCCAT AGGGACCGGAAAAGTCCACCACTGTTAGGTCCAT	-8 (×16) 0 (×9)	heterozygous
<i>ptrchl1-3#</i>	GTTGATTTACTTCCAGAAATTAAGGTGGTTGCT ... ----- AGGTGGTTGCT ... ----- AAGGTGGTTGCT ... ----- AGGTGGTTGCT	0 (×13) -55 (×7) -44 (×2) -42 (×3)	chimeric	AGGGACCGGAA a AGTCCACCACTGTTAGGTCCAT A ----- ... AGGGACCGGAA ----- ... AGGGACCGGAAAT ----- ...	+1 (×13) -55 (×7) -44 (×2) -42 (×3)	chimeric
<i>ptrchl1-4#</i>	... ----- TGACCCCTATAACTCAG ... ----- AGGTGGTTGCT	-66 (×11) -22 (×14)	biallelic	AGG- ----- ... AGGGACCGGAA - GTCCACCACTGTTAGGTCCAT	-66 (×11) -1 (×14)	biallelic
<i>ptrchl1-5#</i>	... ----- GGTGACCCCTATA ... ----- GGTGACCCCTATA	-57 (×17) -31 (×8)	biallelic	AGGGACCGGA ----- ... AGGGACCGGAA a AGTCCACCACTGTTAGGTCCAT	-57 (×17) +1 (×8)	biallelic
<i>ptrchl1-6#</i>	... ----- AAGGTGGTTGCT GTTGATTTACTTCCAGAAATTAAGGTGGTTGCT	-44 (×10) 0 (×15)	heterozygous	AGGGACCGGAA ----- ... AGGGACCGGAAAAGTCCACCACTGTTAGGTCCAT	-44 (×10) 0 (×15)	heterozygous
<i>ptrchl1-7#</i>	GTTGATTTACTTCCAGAAATT - AGGTGGTTGCT ... ----- AAGGTGGTTGCT	-1 (×14) -44 (×11)	biallelic	AGGGACCGGAA - GTCCACCACTGTTAGGTCCAT AGGGACCGGAA ----- ...	-1 (×14) -44 (×11)	biallelic
<i>ptrchl1-8#</i>	... ----- AAGGTGGTTGCT GTTGATTTACTTCCAGAAATTAAGGTGGTTGCT	-44 (×16) 0 (×9)	heterozygous	AGGGACCGGAA ----- ... AGGGACCGGAAAAGTCCACCACTGTTAGGTCCAT	-44 (×16) 0 (×9)	heterozygous
<i>ptrchl1-9#</i>	... ----- AAGGTGGTTGCT GTTGATTTACTTCCAGAAATTAAGGTGGTTGCT	-44 (×10) 0 (×15)	heterozygous	AGGGACCGGAA ----- ... AGGGACCGGAAAAGTCCACCACTGTTAGGTCCAT	-44 (×10) 0 (×15)	heterozygous
<i>ptrchl1-10#</i>	GTTGATTTACTTCCAGAAATT - AGGTGGTTGCT	-1 (×25)	homozygous	AGGGACCGGAA - GTCCACCACTGTTAGGTCCAT AGGGACCGG ----- AGGTCCAT	-1 (×13) -16 (×12)	biallelic
<i>ptrchl1-11#</i>	... ----- AGGTGGTTGCT ... ----- GATCCAGAGTCGA	-45 (×8) -83 (×17)	biallelic	AGGGACCGGAA ----- ... AGGGACCGGAA ----- ...	-45 (×8) -83 (×17)	biallelic
<i>ptrchl1-12#</i>	... ----- AGGTGGTTGCT GTTGATTTAT ----- AGGTGGTTGCT	-45 (×15) -12 (×10)	biallelic	AGGGACCGGAA ----- ... AGGGACCGGAA - GTCCACCACTGTTAGGTCCAT	-45 (×15) -1 (×10)	biallelic
<i>ptrchl1-13#</i>	... ----- AGGTGGTTGCT ... ----- AAGGTGGTTGCT	-45 (×3) -44 (×12)	chimeric	AGGGACCGGAA ----- ... AGGGACCGGAA ----- ...	-45 (×3) -44 (×12)	chimeric

	... -----AGG <u>TGG</u> TTGCT GTTGATTACTTCCAGAAATTAAGG <u>TGG</u> TTGCT	-42 (×6) 0 (×4)		AGGG <u>ACCG</u> GAAATT ----- ... AGGG <u>ACCG</u> GAAAGTCCACCACTGTTAGGTCCAT	-42 (×6) 0 (×4)	
<i>ptrchl1-14#</i>	... ----- GATCCAGAGTCGA	-83 (×25)	homozygous	AGGG <u>ACCG</u> GAA ----- ...	-83 (×25)	homozygous
<i>ptrchl1-15#</i>	... ----- AGG <u>TGG</u> TTGCT	-45 (×25)	homozygous	AGGG <u>ACCG</u> GAA ----- ...	-45 (×25)	homozygous
<i>ptrchl1-16#</i>	GTTGATTACTTCCAGAAATTA a AGG <u>TGG</u> TTGCT GTTGATTACTTCCAGAAATT - AGG <u>TGG</u> TTGCT	+1 (×13) -1 (×12)	biallelic	AGGG <u>ACCG</u> GAA - GTCCACCACTGTTAGGTCCAT AGGG <u>ACCG</u> GAA a AGTCCACCACTGTTAGGTCCAT	-1 (×13) +1 (×12)	biallelic
<i>ptrchl1-17#</i>	... ----- CCTATAACTCAGATGA GTTGATTACTTCCAGAAATTAAGG <u>TGG</u> TTGCT	-67 (×15) 0 (×10)	heterozygous	AGGG <u>ACC</u> ----- ... AGGG <u>ACCG</u> GAAAGTCCACCACTGTTAGGTCCAT	-67 (×15) 0 (×10)	heterozygous
<i>ptrchl1-18#</i>	... -----TGACCCCTATAACTCAG ----- AGG <u>TGG</u> TTGCT	-66 (×12) -22 (×13)	biallelic	AGG----- ... AGGG <u>ACCG</u> GAA - GTCCACCACTGTTAGGTCCAT	-66 (×12) -1 (×13)	biallelic

**Table S3** The Cas9/gRNA-targeted mutations in both *PtrCHL11* and 2 genes of *Populus trichocarpa*.

Gene: Potri.004G053400 ( <i>PtrCHL11</i> ) / Potri.011G063400 ( <i>PtrCHL12</i> )						
Vector: Cas9/gRNA-CHL11/2-1						
gRNA: gCHL11/2-a (CTTCTTGCTAAAGCTAATAGAGG); gCHL11/2-b (CCCAAGATTGGAGGTGTCATGAT)						
Target sequences: CHL11-a, CTTCTTGCTAAAGCTAATAGAGG; CHL11-b, CCCAAGATTGGAGGTGTCATGAT						
CHL12-a, CTTCTTGCTAAAGCTAATAGAGG; CHL12-b, CCCAAGATTGGAGGTGTCATGAT						
Line (#)	CHL11/2-a CHL11, CTTCTTGCTAAAGCTAATAGAGG CHL12, CTTCTTGCTAAAGCTAATAGAGG	Mutation types (Number of plasmids containing the cloned PCR products)		CHL11/2-b CHL11, CCCAAGATTGGAGGTGTCATGAT CHL12, CCCAAGATTGGAGGTGTCATGAT	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrchli1/2-1#-CHL11-CHL12</i>	... TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA ...	-319 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGC --- TAGAGGGATTC CGGGTCTTCTTGCTAAAGCTA - TAGAGGGATTC	-3 (×16) -1 (×9)	biallelic	TTGATCCC ----- GAGGTGTCATGATCATGG TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-7 (×16) -1 (×9)	biallelic
<i>ptrchli1/2-2#-CHL11-CHL12</i>	CGGGTCTTCTTGCTAAAGC --- TAGAGGCATTC	-3 (×25)	homozygous	TTGATCCC ----- GAGGTGTCATGATCATGG TTGAT ----- GTCATGATCATGG	-7 (×13) -15 (×12)	biallelic
	TTGTGGGACAATT ----- TCGTCATTCTTAC CGGGTCTTCTTGCTAAAGC --- TAGAGGGATTC	-356 (×19) -9 (×6)	biallelic	TTGATCCCAAG a ATTGGAGGTGTCATGATCATGG TTGATCCCAAG ---- GAGGTGTCATGATCATGG	+1 (×19) -4 (×6)	biallelic
<i>ptrchli1/2-3#-CHL11-CHL12</i>	... TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA -----	-319 (×25)	homozygous
	... TAGAGGCATTC	-321 (×25)	homozygous	TTGATCCCAA -----	-321 (×25)	homozygous
<i>ptrchli1/2-4#-CHL11-CHL12</i>	... TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA -----	-319 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGC --- TAGAGGGATTC CGGGTCTTCTTGCTAAAGCTA - TAGAGGGATTC	-3 (×14) -1 (×7)	chimeric	TTGATCCC ----- GAGGTGTCATGATCATGG TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-7 (×14) -1 (×7)	chimeric
	CGGGTCTTCTTGCTAAAGCTAATAGAGGGATTC	0 (×4)		TTGATCCCAAGATTGGAGGTGTCATGATCATGG	0 (×4)	
<i>ptrchli1/2-5#-CHL11-CHL12</i>	... TAGAGGCATTC GCTCTACCGAGG ----- TTAATCTTTTGA CGGGTCTTCTTGCTAAAGCTAATAGAGGGATTC	-319 (×7) -66 (×5) 0 (×13)	chimeric	TTGATCCCAAGA ----- TTGATCCCAAGATTGGAGGTGTCATGATCATGG	-319 (×7) 0 (×18)	heterozygous
	... TAGAGGCATTC CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC	-319 (×18) +1 (×7)	biallelic	TTGATCCCAAGA ----- TTGATCCCAAGA --- GAGGTGTCATGATCATGG	-319 (×18) -3 (×7)	biallelic
<i>ptrchli1/2-6#-CHL11-CHL12</i>	... TAGAGGCATTC	-319 (×17)	biallelic	TTGATCCCAAGA -----	-319 (×17)	biallelic
	CGGGTCT ----- TAGAGGCATTC	-15 (×8)		TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×8)	
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC TGGTGACCCCTATAA ... ^43 ATTC (^43:aagatcaacataaagattaacttcatcaactctcaccgaggg)	+1 (×15) -217+43 (×10)	biallelic	TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×25)	homozygous
<i>ptrchli1/2-7#-CHL11-CHL12</i>	... TAGAGGCATTC	-319 (×16)	biallelic	TTGATCCCAAGA -----	-319 (×16)	biallelic
	CGGGTCT ----- TAGAGGCATTC	-15 (×9)		TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×9)	
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC	+1 (×16)	heterozygous	TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×19)	biallelic

	CGGGTCTTCTTGCTAAAGCTAATAGAGGGATTC	0 (×9)		TTGATCC - - - - - TTGGAGGTGTCATGATCATGG	-5 (×6)	
<i>ptrchli1/2-8#-CHLI1</i> <i>-CHLI2</i>	... - - - - - TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA - - - - - ...	-319 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC CGGGTCTTCTTGCTAAAGCTAATAGAGGGATTC	+1 (×5) 0 (×20)	heterozygous	TTGATCC - - - - - TTGGAGGTGTCATGATCATGG	-5 (×25)	homozygous
<i>ptrchli1/2-9#-CHLI1</i> <i>-CHLI2</i>	... - - - - - TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA - - - - - ...	-319 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC CGGGTCTTCTTGCTAAAGC - - ATAGAGGGATTC	+1 (×13) -2 (×12)	biallelic	TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×25)	homozygous
<i>ptrchli1/2-10#-CHLI1</i> <i>-CHLI2</i>	... - - - - - TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA - - - - - ...	-319 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC	+1 (×14)	chimeric	TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGCTA - TAGAGGGATTC CGGGTCTTCTTGCTAAAGCTAATAGAGGGATTC	-1 (×5) 0 (×6)				
<i>ptrchli1/2-11#-CHLI1</i> <i>-CHLI2</i>	... - - - - - TAGAGGCATTC	-319 (×9)	biallelic	TTGATCCCAAGA - - - - - ...	-319 (×9)	biallelic
	CGGGTCT - - - - - TAGAGGCATTC	-15 (×16)		TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×16)	
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC	+1 (×25)	homozygous	TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×25)	homozygous
<i>ptrchli1/2-12#-CHLI1</i> <i>-CHLI2</i>	... - - - - - TAGAGGCATTC	-319 (×25)	homozygous	TTGATCCCAAGA - - - - - ...	-319 (×25)	homozygous
	CGGGTCTTCTTGCTAAAGCTAA a TAGAGGGATTC	+1 (×14)	biallelic	TTGATCCCAAG - TTGGAGGTGTCATGATCATGG	-1 (×14)	biallelic
	CGGGTCTTCTTGCTAAAGCTA - TAGAGGGATTC	-1 (×11)		TTGATCCCAAG a ATTGGAGGTGTCATGATCATGG	+1 (×11)	
<b>Vector:</b> Cas9/gRNA-CHLI1/2-2 <b>gRNA:</b> gCHLI1/2-c (GTCGATGGGTATTGAAGTCAGGG); gCHLI1/2-d (CCATTTGCTGCTATAGTAGGGCA) <b>Target sequences:</b> CHLI1-c, GTCGATGGGTATTGAAGTCAGGG; CHLI1-d, CCATTTGCTGCTATAGTAGGGCA CHLI2-c, GTCCATGGGTATTGCAGTCAGGG; CHLI2-d, CCATTTGCTGCAATAGTAGGGCA						
Line (#)	CHLI1/2-c CHLI1, GTCGATGGGTATTGAAGTCAGGG CHLI2, GTCCATGGGTATTGAAGTCAGGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCHLI1/2-d CHLI1, CCATTTGCTGCTATAGTAGGGCA CHLI2, CCATTTGCTGCAATAGTAGGGCA	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrchli1/2-13#-CHLI1</i> <i>-CHLI2</i>	CCAGAGTCGATGGGTATTGAA - TCAGGGAGAGT	-1 (×25)	homozygous	TATATCCATTTGCTGCTATAGTAGGGCAAGATG TATATCCATT - GCTGCTATAGTAGGGCAAGATG	0 (×22) -1 (×3)	heterozygous
	CCAGAGTCCATGGGTATTGA - - - CAGGGAGAGT CCAGAGTCCATGGGTATTGA - - - - - AGAGT	-3 (×15) -8 (×10)	biallelic	TGTATCCATTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
<i>ptrchli1/2-14#-CHLI1</i> <i>-CHLI2</i>	CCAGAGTCGATGGGTATTGAA - TCAGGGAGAGT ... - - - - - AAGTCAGGGAGAGT	-1 (×13) -159 (×12)	biallelic	TATATCCATTTGCTGCTATAGTAGGGCAAGATG	0 (×25)	WT
	CCAGAGTCCATGGGTATTGAAGTCAGGGAGAGT	0 (×25)	WT	TGTATCCATTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
<i>ptrchli1/2-15#-CHLI1</i> <i>-CHLI2</i>	CCAGAGTCGATGGGTATTGAA - TCAGGGAGAGT ... - - - - - AAGTCAGGGAGAGT	-1 (×9) -159 (×16)	biallelic	TATATCCATTTGCTGCTATAGTAGGGCAAGATG	0 (×25)	WT
	- - - - - CCATGGGTATTGAAGTCAGGGAGAGT CCAGAGTCCATGGGTATTGAAGTCAGGGAGAGT	-7 (×14) 0 (×11)	heterozygous	TGTATCCATTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
	CCAGAGTCGATGGGTATTGAA - TCAGGGAGAGT CCAGAGTCGATGGGTATTGAAGTCAGGGAGAGT	-1 (×21) 0 (×4)	heterozygous	TATATCCATTT - - TGCTATAGTAGGGCAAGATG TATATCCATTTGCTGCTATAGTAGGGCAAGATG	-2 (×13) 0 (×12)	heterozygous



<b>-CHLI2</b>	CCAGAGTCC ----- TCTGTCAGGGAGAGT CCAGAGTCCATGGGTATTGAAGTCAGGGAGAGT	-9 (×14) 0 (×11)	heterozygous	TGTAT <u>CCA</u> TTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
<b><i>ptrchli1/2-17#</i> -CHLI1 -CHLI2</b>	CCAGAGTTCGATGGGTATTGA -- TCAGGGAGAGT ... ----- TCAGGGAGAGT	-2 (×9) -210 (×16)	biallelic	TATAT <u>CCA</u> TT - GCTGCTATAGTAGGGCAAGATG TATAT <u>CCA</u> TTTG ----- ...	-1 (×9) -210 (×16)	biallelic
	CCAGAGTCCATGGGTATT ---- TCAGGGAGAGT CCAGAGTCCATGGGTATTGAA - TCAGGGAGAGT CCAGAGTCCATGGGTATTGA ----- GT	-4 (×18) -1 (×5) -11 (×2)	chimeric	TGTAT <u>CCA</u> TTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
<b><i>ptrchli1/2-18#</i> -CHLI1 -CHLI2</b>	... ----- TCAGGGAGAGT	-211 (×25)	homozygous	TATAT <u>CCA</u> TTT ----- ...	-211 (×25)	homozygous
	CCAGAGTCCATGGGTATTGA -- TCAGGGAGAGT ----- TCAGGGAGAGT	-2 (×13) -24 (×12)	biallelic	TGTAT <u>CCA</u> TTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
<b><i>ptrchli1/2-19#</i> -CHLI1 -CHLI2</b>	... ----- TCAGGGAGAGT	-210 (×10)	biallelic	TATAT <u>CCA</u> TTTG ----- ...	-210 (×10)	biallelic
	CCAGAGTTCGATGGGTATTGA -- TCAGGGAGAGT	-2 (×15)		TATAT <u>CCA</u> TT - GCTGCTATAGTAGGGCAAGATG	-1 (×15)	
	CCAGAGTCCATGGGTATT ---- TCAGGGAGAGT CCAGAGTCCATGGGTATTGA ----- GT	-4 (×9) -11 (×16)	biallelic	TGTAT <u>CCA</u> TTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT
<b><i>ptrchli1/2-20#</i> -CHLI1 -CHLI2</b>	... ----- TCAGGGAGAGT	-210 (×11)	biallelic	TATAT <u>CCA</u> TTTG ----- ...	-210 (×11)	biallelic
	CCAGAGTTCGATGGGTATTGA -- TCAGGGAGAGT	-2 (×14)		TATAT <u>CCA</u> TTT t GCTGCTATAGTAGGGCAAGATG	+1 (×14)	
	CCAGAGTCCATGGGTATTGA -- TCAGGGAGAGT CCAGAGTCCATGGGTATTGAA - TCAGGGAGAGT	-2 (×9) -1 (×16)	biallelic	TGTAT <u>CCA</u> TTTGCTGCAATAGTAGGGCAAGATG	0 (×25)	WT

**Table S4** Analysis of potential off-target sites among the Cas9/gRNA-induced *ptrchlI* mutants.

On/Off targets	Sequence name	Bases position of gRNAs																			PAM			Target gene or genomic position	Off-target Editing (Y/N)	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22			23
On	<b>gCHLI1-a</b>	T	T	T	A	C	T	T	C	C	A	G	A	A	A	T	T	A	A	G	G	T	G	G	CHLI1	-
Off	gCHLI1a-off-1	T	T	T	A	C	T	T	C	C	T	G	A	A	A	T	T	A	A	G	G	T	A	G	CHLI2	No
	gCHLI1a-off-2	T	T	T	A	C	T	T	G	C	A	T	A	A	A	T	T	A	A	G	G	T	G	G	Chr09 (+4335300)	No
	gCHLI1a-off-3	C	T	T	A	C	C	A	C	C	A	G	A	A	A	T	T	A	A	A	G	T	G	G	Chr13 (-9790031)	No
	gCHLI1a-off-4	G	T	C	A	C	T	T	T	C	A	G	A	A	A	T	T	A	A	G	G	A	G	G	Potri.006G228600	No
	gCHLI1a-off-5	A	C	T	C	C	C	T	C	C	A	G	A	A	A	T	T	A	T	G	G	T	G	G	Chr12 (-2022439)	No
On	<b>gCHLI1-b</b>	C	C	T	A	A	C	A	G	T	G	G	T	G	G	A	C	T	T	T	C	C	G	G	CHLI1	-
Off	gCHLI1b-off-1	C	C	T	A	A	C	A	G	T	C	G	T	G	G	A	C	T	T	C	C	C	C	G	CHLI2	No
	gCHLI1b-off-2	A	T	T	A	G	A	A	T	T	G	G	T	G	G	A	C	T	T	T	C	C	G	G	Potri.018G015300	No
	gCHLI1b-off-3	G	T	A	A	A	C	C	G	T	G	G	T	G	G	A	C	T	T	T	C	T	G	A	Potri.013G004900	No

Nucleotides in green shading are the same with the designed gRNAs target sites and mismatched nucleotides are without shading. The PAM regions are highlighted in red shading. Potential off-target sites in protein coding gene regions are annotated with gene ID and the others located in noncoding regions are detailed with position information on chromosomes.

**Number of plasmids containing the cloned PCR products**

**gCHLI1a-off-1**

WT TTTACTTCCTGAAATTAAGGTAG (×10)  
*ptrchlI*-1# TTTACTTCCTGAAATTAAGGTAG (×15)  
*ptrchlI*-4# TTTACTTCCTGAAATTAAGGTAG (×15)  
*ptrchlI*-5# TTTACTTCCTGAAATTAAGGTAG (×15)  
*ptrchlI*-11# TTTACTTCCTGAAATTAAGGTAG (×15)  
*ptrchlI*-14# TTTACTTCCTGAAATTAAGGTAG (×15)  
*ptrchlI*-15# TTTACTTCCTGAAATTAAGGTAG (×15)

**gCHLI1a-off-2**

WT TTTACTTGCATAAATTAAGGTGG (×10)  
*ptrchlI*-1# TTTACTTGCATAAATTAAGGTGG (×15)  
*ptrchlI*-8# TTTACTTGCATAAATTAAGGTGG (×15)  
*ptrchlI*-11# TTTACTTGCATAAATTAAGGTGG (×15)  
*ptrchlI*-12# TTTACTTGCATAAATTAAGGTGG (×15)  
*ptrchlI*-15# TTTACTTGCATAAATTAAGGTGG (×15)  
*ptrchlI*-16# TTTACTTGCATAAATTAAGGTGG (×15)

**gCHLI1a-off-3**

WT CTTACCACCAGAAATTAAGTGG (×10)  
*ptrchli1-1#* CTTACCACCAGAAATTAAGTGG (×15)  
*ptrchli1-2#* CTTACCACCAGAAATTAAGTGG (×15)  
*ptrchli1-7#* CTTACCACCAGAAATTAAGTGG (×15)  
*ptrchli1-10#* CTTACCACCAGAAATTAAGTGG (×15)  
*ptrchli1-16#* CTTACCACCAGAAATTAAGTGG (×15)  
*ptrchli1-18#* CTTACCACCAGAAATTAAGTGG (×15)

**gCHLI1a-off-4**

WT GTCACTTTCAGAAATTAAGGAGG (×10)  
*ptrchli1-3#* GTCACTTTCAGAAATTAAGGAGG (×15)  
*ptrchli1-4#* GTCACTTTCAGAAATTAAGGAGG (×15)  
*ptrchli1-5#* GTCACTTTCAGAAATTAAGGAGG (×15)  
*ptrchli1-7#* GTCACTTTCAGAAATTAAGGAGG (×15)  
*ptrchli1-9#* GTCACTTTCAGAAATTAAGGAGG (×15)  
*ptrchli1-10#* GTCACTTTCAGAAATTAAGGAGG (×15)

**gCHLI1a-off-5**

WT ACTCCCTCCAGAAATTATGGTGG (×10)  
*ptrchli1-3#* ACTCCCTCCAGAAATTATGGTGG (×15)  
*ptrchli1-4#* ACTCCCTCCAGAAATTATGGTGG (×15)  
*ptrchli1-5#* ACTCCCTCCAGAAATTATGGTGG (×15)  
*ptrchli1-11#* ACTCCCTCCAGAAATTATGGTGG (×15)  
*ptrchli1-12#* ACTCCCTCCAGAAATTATGGTGG (×15)  
*ptrchli1-14#* ACTCCCTCCAGAAATTATGGTGG (×15)

**gCHLI1b-off-1**

WT CGGGGAAGTCCACGACTGTTAGG (×10)  
*ptrchli1-1#* CGGGGAAGTCCACGACTGTTAGG (×15)  
*ptrchli1-4#* CGGGGAAGTCCACGACTGTTAGG (×15)  
*ptrchli1-5#* CGGGGAAGTCCACGACTGTTAGG (×15)  
*ptrchli1-11#* CGGGGAAGTCCACGACTGTTAGG (×15)  
*ptrchli1-14#* CGGGGAAGTCCACGACTGTTAGG (×15)  
*ptrchli1-15#* CGGGGAAGTCCACGACTGTTAGG (×15)

**gCHLI1b-off-2**

WT CCGGAAAGTCCACCAATTCTAAT (×10)  
*ptrchli1-1#* CCGGAAAGTCCACCAATTCTAAT (×15)  
*ptrchli1-11#* CCGGAAAGTCCACCAATTCTAAT (×15)  
*ptrchli1-12#* CCGGAAAGTCCACCAATTCTAAT (×15)  
*ptrchli1-15#* CCGGAAAGTCCACCAATTCTAAT (×15)  
*ptrchli1-16#* CCGGAAAGTCCACCAATTCTAAT (×15)  
*ptrchli1-17#* CCGGAAAGTCCACCAATTCTAAT (×15)

**gCHL1b-off-3**

WT TCAGAAAGTCCACCACGGTTTAC (×10)  
*ptrchli1-4#* TCAGAAAGTCCACCACGGTTTAC (×15)  
*ptrchli1-5#* TCAGAAAGTCCACCACGGTTTAC (×15)  
*ptrchli1-7#* TCAGAAAGTCCACCACGGTTTAC (×15)  
*ptrchli1-11#* TCAGAAAGTCCACCACGGTTTAC (×15)  
*ptrchli1-12#* TCAGAAAGTCCACCACGGTTTAC (×15)  
*ptrchli1-14#* TCAGAAAGTCCACCACGGTTTAC (×15)

**Table S5** The Cas9/gRNA-targeted mutations in *PtrCesA4*, *7A*, *7B*, *7A/B*, *8A*, *8B* and *8A/B* genes of *Populus trichocarpa*.

<b>Gene: Potri.002G257900 (<i>PtrCesA4</i>)</b>						
<b>Vector:</b> Cas9/gRNA-PtrCesA4-1 <b>gRNA:</b> gCesA4-1 (CATACGATGCTTATGCATTG <u>TGG</u> ); gCesA4-2 ( <u>CCC</u> ATTCGTCCGGCCTTTTCGTC) <b>Target sequences:</b> CesA4-1, CATACGATGCTTATGCATTG <u>TGG</u> ; CesA4-2, <u>CCC</u> ATTCGTCCGGCCTTTTCGTC						
Line (#)	gCesA4-1 CATACGATGCTTATGCATTG <u>TGG</u>	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA4-2 <u>CCC</u> ATTCGTCCGGCCTTTTCGTC	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa4-1#</i>	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×14) 0 (×11)	heterozygous	TGCAC <u>CCC</u> ATTCGT --- GCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT a CGTCCGGCCTTTTCGTCAGCAG	-3 (×14) +1 (×11)	biallelic
<i>ptrcesa4-2#</i>	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×17) 0 (×8)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×15) +1 (×10)	biallelic
<i>ptrcesa4-3#</i>	----- GT <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCA a TTG <u>TGG</u> CCTTAT	-127 (×16) +1 (×9)	biallelic	TGCAC <u>CCC</u> ATT ----- TCGTCAGCAG TGCAC <u>CCC</u> ATT a CGTCCGGCCTTTTCGTCAGCAG	-12 (×16) +1 (×9)	biallelic
<i>ptrcesa4-4#</i>	TCCAGCATAACGATGCTTATGCA a TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	+1 (×11) 0 (×14)	heterozygous	TGCAC <u>CCC</u> ATT ----- GGCCTTTTCGTCAGCAG	-5 (×25)	homozygous
<b>Vector:</b> Cas9/gRNA-PtrCesA4-2 <b>gRNA:</b> gCesA4-3 (CATCTGTGTTAGAACTTCGAT <u>TGG</u> ); gCesA4-4 ( <u>CCT</u> CCTCAAGGTCCTTGGAGGAG) <b>Target sequences:</b> CesA4-3, CATCTGTGTTAGAACTTCGAT <u>TGG</u> ; CesA4-4, <u>CCT</u> CCTCAAGGTCCTTGGAGGAG						
Line (#)	gCesA4-3 CATCTGTGTTAGAACTTCGAT <u>TGG</u>	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA4-4 <u>CCC</u> ATTCGTCCGGCCTTTTCGTC	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa4-5#</i>	AGCAACATCTGTGTTAGAA --- CGAT <u>TGG</u> AGTGG AGCAACATCTGTGTTAGAAC -- CGAT <u>TGG</u> AGTGG	-3 (×13) -2 (×12)	biallelic	CAAGG <u>CCT</u> CCTCAAGGTCCTTGGAGGAGTAGAC	0 (×25)	WT
<i>ptrcesa4-6#</i>	AGCAACATCTGTGTTAGAACT - CGAT <u>TGG</u> AGTGG AGCAACATCTGTGTTAGAACT -----	-1 (×12) -127 (×13)	biallelic	CAAGG <u>CCT</u> CCT - AAGGTCCTTGGAGGAGTAGAC ----- AACTTCACTGTTA	-1 (×12) -127 (×13)	biallelic
<b>Gene: Potri.006G181900 (<i>PtrCesA7A</i>)</b>						
<b>Vector:</b> Cas9/gRNA-PtrCesA7A-1 <b>gRNA:</b> gCesA7A-a (CTATTATTGCAGGTTAGACG <u>AGG</u> ); gCesA7A-b ( <u>CC</u> CATTGGATCTCATGGAGAGC) <b>Target sequences:</b> CesA7A-a, CTATTATTGCAGGTTAGACG <u>AGG</u> ; CesA7A-b, <u>CC</u> CATTGGATCTCATGGAGAGC						

Line (#)	<b>gCesA7A-a</b> CTATTATTGCAGGTTAGACG <u>AGG</u>	<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)		<b>gCesA7A-b</b> <u>CCCTATTGGATCTCATGGAGAGC</u>	<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7A-1#</i>	GTGAACTATTATTGCAGGTTA - ACG <u>AGG</u> CAAGA	-1 (×25)	homozygous	GAGTT <u>CCCTAT</u> - GGATCTCATGGAGAGCAGATG	-1 (×25)	homozygous
<i>ptrcesa7A-2#</i>	GTGAACTATTATTGCAGGTT - - ACG <u>AGG</u> CAAGA GTGAACTATTATTGCAGGTTAG g ACG <u>AGG</u> CAAGA	+1 (×15) -2 (×10)	biallelic	GAGTT <u>CCCTAT</u> tGGATCTCATGGAGAGCAGAT GAGTT <u>CCCTAT</u> - - - - CTCATGGAGAGCAGATG	+1 (×10) -5 (×15)	biallelic
<b>Vector:</b> Cas9/gRNA-PtrCesA7A-2 <b>gRNA:</b> gCesA7A-c (GACAGGAATGGATAACGAGA <u>AGG</u> ); gCesA7A-d ( <u>CCAT</u> GACACTGAAGGAAATGAGC) <b>Target sequences:</b> CesA7A-c, GACAGGAATGGATAACGAGA <u>AGG</u> ; CesA7A-d, <u>CCAT</u> GACACTGAAGGAAATGAGC						
Line (#)	<b>gCesA7A-c</b> GACAGGAATGGATAACGAGA <u>AGG</u>	<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)		<b>gCesA7A-d</b> <u>CCAT</u> GACACTGAAGGAAATGAGC	<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7A-3#</i>	(inverted 834 ) AGA <u>AGG</u> AGCTA	inversion (×25)	homozygous	GGAGG <u>CCAT</u> GTA (inverted 834 ) GGAGG <u>CCAT</u> - - (-2+inverted 834 )	inversion (×17) inversion (×8)	biallelic
<i>ptrcesa7A-4#</i>	(inverted 834 ) AGA <u>AGG</u> AGCTA	inversion (×25)	homozygous	GGAGG <u>CCAT</u> GTA (inverted 834 )	inversion (×25)	homozygous
<i>ptrcesa7A-5#</i>	TGCTGGACAGGAATGGATAAC - AGA <u>AGG</u> AGCTA TGCTGGACAGGAATGGATAACgtAGA <u>AGG</u> AGCTA	-1 (×19) +1 (×6)	biallelic	GGAGG <u>CCAT</u> GACACTGAAGGAAATGAGCTCCCT GGAGG <u>CCAT</u> GAACTGAAGGAAATGAGCTCCCT	+1 (×19) 0 (×6)	heterozygous
<i>ptrcesa7A-6#</i>	TGCTGGACAGGAATGGATAAC - - GA <u>AGG</u> AGCTA	-2 (×25)	homozygous	GGAGG <u>CCAT</u> GAgCACTGAAGGAAATGAGCTCCCT GGAGG <u>CCAT</u> GACACTGAAGGAAATGAGCTCCCT	+1 (×8) 0 (×17)	heterozygous
<i>ptrcesa7A-7#</i>	TGCTGGACAGGAATGGATAAC - AGA <u>AGG</u> AGCTA	-1 (×25)	homozygous	GGAGG <u>CCAT</u> GTA - ACTGAAGGAAATGAGCTCCCT	-1 (×25)	homozygous
<b>Gene:</b> Potri.018G103900 ( <i>PtrCesA7B</i> )						
<b>Vector:</b> Cas9/gRNA-PtrCesA7B-1 <b>gRNA:</b> gCesA7B-a (ACAAGATGACGATGCAGAAG <u>CGG</u> ); gCesA7B-b ( <u>CCATT</u> GGATCTCATGGAGAACAG) <b>Target sequences:</b> CesA7B-a, ACAAGATGACGATGCAGAAG <u>CGG</u> ; CesA7B-b, <u>CCATT</u> GGATCTCATGGAGAACAG						
Line (#)	<b>gCesA7B-a</b> ACAAGATGACGATGCAGAAG <u>CGG</u>	<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)		<b>gCesA7B-b</b> <u>CCATT</u> GGATCTCATGGAGAACAG	<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7B-1#</i>	CCTGAACAAGATGACGATGCAG - AG <u>CGG</u> CCATG CCTGAACAAGATGACGATGCAGAAG <u>CGG</u> CCATG	+1 (×16) 0 (×9)	heterozygous	GTTCT <u>CCATT</u> G t GATCTCATGGAGAACAGATGCT GTTCT <u>CCATT</u> G - ATCTCATGGAGAACAGATGCT	+1 (×19) -1 (×6)	biallelic
<i>ptrcesa7B-2#</i>	CCTGAACAAG - - - - - AAG <u>CGG</u> CCATG CCTGAACAAGATGACGAT - - - - AAG <u>CGG</u> CCATG	-12 (×10) -4(×25)	biallelic	GTTCT <u>CCATT</u> G - ATCTCATGGAGAACAGATGCT GTTCT <u>CCATT</u> G t GATCTCATGGAGAACAGATGCT	-1 (×11)	biallelic

					+1 (×14)	
<b>Vector:</b> Cas9/gRNA-PtrCesA7B-2 <b>gRNA:</b> gCesA7B-c (GCCCAATATGCTTGCTCCAGTGG); gCesA7B-d ( <u>CCAGCAAGATCAATCCGTATAGA</u> ) <b>Target sequences:</b> CesA7B-c, GCCCAATATGCTTGCTCCAGTGG; CesA7B-d, <u>CCAGCAAGATCAATCCGTATAGA</u>						
Line (#)	gCesA7B-c GCCCAATATGCTTGCTCCAGTGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA7B-d <u>CCAGCAAGATCAATCCGTATAGA</u>	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7B-3#</i>	(inverted 407 ) CAGTGGATATC GGCGAGCCCAATATGCTTGCTC † CAGTGGATATC	inversion (×15) +1 (×10)	biallelic	TGCATCCAGCA (inverted 407 ) TGCATCCAGCA - GATCAATCCGTATAGAATGGT	inversion (×15) -1 (×10)	biallelic
<i>ptrcesa7B-4#</i>	(inverted 407 ) CAGTGGATATC	inversion (×25)	homozygous	TGCATCCAGCA (inverted 407 )	inversion (×25)	homozygous
<i>ptrcesa7B-5#</i>	(inverted 407 ) CAGTGGATATC GGCGAGCCCAATATGCTTGC - - CAGTGGATATC	inversion (×11) -2 (×14)	biallelic	TGCATCCAGCA (inverted 407 ) TGCATCCAGCA - GATCAATCCGTATAGAATGGT	inversion (×11) -1 (×14)	biallelic
<i>ptrcesa7B-6#</i>	(inverted 407 ) CAGTGGATATC GGCGAGCCCAATATGCTTGC - - CAGTGGATATC	inversion (×16) -2 (×9)	biallelic	TGCATCCAGCA (inverted 407 ) TGCATCCAGCA - GATCAATCCGTATAGAATGGT	inversion (×16) -1 (×9)	biallelic
<i>ptrcesa7B-7#</i>	GGCGAGCCCAATATGC - - - - - CAGTGGATATC GGCGAGCCCAATATGCTTGCTC CAGTGGATATC	-6 (×18) 0 (×7)	heterozygous	TGCATCCAGCAA - GATCAATCCGTATAGAATGGT TGCATCCAGCAAGATCAATCCGTATAGAATGGT	-1 (×18) 0 (×7)	heterozygous
<i>ptrcesa7B-8#</i>	(inverted 407 ) CAGTGGATATC GGCGAGCCCAATATGCTTGC - - CAGTGGATATC	inversion (×13) -2 (×12)	biallelic	TGCATCCAGCA (inverted 407 ) TGCATCCAGCA - GATCAATCCGTATAGAATGGT	inversion (×13) -1 (×12)	biallelic
<b>Gene:</b> Potri.011G069600 ( <i>PtrCesA8A</i> )						
<b>Vector:</b> Cas9/gRNA-PtrCesA8A-1 <b>gRNA:</b> gCesA8A-a (TTGTTTCGAGTATGAGATCAAGG); gCesA8A-b ( <u>CCAGGTGGGACATGATGCAAATG</u> ) <b>Target sequences:</b> CesA8A-a, TTGTTTCGAGTATGAGATCAAGG; CesA8A-b, <u>CCAGGTGGGACATGATGCAAATG</u>						
Line (#)	gCesA8A-a TTGTTTCGAGTATGAGATCAAGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8A-b <u>CCAGGTGGGACATGATGCAAATG</u>	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa8A-1#</i>	... - - - - - ATCAAGGAGGGG	-82 (×25)	homozygous	GGTGACCAGGT - - - - -	-82 (×25)	homozygous
<i>ptrcesa8A-2#</i>	... - - - - - ATCAAGGAGGGG AAGTCTTGTTTCGAGTATGAGAcTCAAGGAGGGG	-82 (×19) +1 (×6)	biallelic	GGTGACCAGGT - - - - - GGTGACCAGGT - - GACATGATGCAAATGGAGAC	-82 (×19) -2 (×6)	biallelic

<b>Vector:</b> Cas9/gRNA-PtrCesA8A-2 <b>gRNA:</b> gCesA8A-c (GGGAAATGAACTACCTCGACTGG); gCesA8A-d (CCGGAGCTCGTGACATAGAGGGA) <b>Target sequences:</b> CesA8A-c, GGGAAATGAACTACCTCGACTGG; CesA8A-d, CCGGAGCTCGTGACATAGAGGGA						
Line (#)	gCesA8A-c GGGAAATGAACTACCTCGACTGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8A-d CCGGAGCTCGTGACATAGAGGGA	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa8A-3#</i>	ATAGAGGGAAATGAACTACCT -- ACTGGTATAT	-2 (×25)	homozygous	AAACACCGGAGa TCGTGACATAGAGGGAAATGA	+1 (×25)	homozygous
<i>ptrcesa8A-4#</i>	ATAGAGGGAAATGAACTACCTC - ACTGGTATAT ATAGAGGGAAATGAACTACCT -- ACTGGTATAT	-1 (×9) -2 (×16)	biallelic	AAACACCGGAGg TCGTGACATAGAGGGAAATGA AAACACCGGAGaCTCGTGACATAGAGGGAAATGA	+1 (×9) +1 (×16)	biallelic
<i>ptrcesa8A-5#</i>	ATAGAGGGAAATGAACTACCTCGACTGGTATAT	0 (×25)	WT	AAACACCGGAG ----- AGGGAAATGA	-12 (×25)	homozygous
<i>ptrcesa8A-6#</i>	ATAGAGGGAAATGAACTA ----- ACTGGTATAT	-5 (×25)	homozygous	... ----- -CGTGACATAGAGGGAAATGA	-16 (×25)	homozygous
<i>ptrcesa8A-7#</i>	... ----- GACTGGTATAT	-30 (×25)	homozygous	AAACACCGGAG ----- ...	-30 (×25)	homozygous
<b>Gene:</b> Potri.004G059600 ( <i>PtrCesA8B</i> )						
<b>Vector:</b> Cas9/gRNA-PtrCesA8B-1 <b>gRNA:</b> gCesA8B-a (AGGGCCGGAAAGTTTGCTTGCGG); gCesA8B-b (CCTGAAAATCCCTTCCATTTGCT) <b>Target sequences:</b> CesA8B-a, AGGGCCGGAAAGTTTGCTTGCGG; CesA8B-b, CCTGAAAATCCCTTCCATTTGCT						
Line (#)	gCesA8B-a AGGGCCGGAAAGTTTGCTTGCGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8B-b CCTGAAAATCCCTTCCATTTGCT	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa8B-1#</i>	CAAAGAGGGCCGGAAAGTTTGC ----- ...	-172 (×25)	homozygous	... ----- -AATCCCTTCCATTTGCTTGTTA	-172 (×25)	homozygous
<i>ptrcesa8B-2#</i>	CAAAGAGGGCCGGAAAGTTTGC t TTGCGGTGCGG	+1 (×25)	homozygous	ATATTCCTIGAA - ATCCCTTCCATTTGCTTGTTA	-1 (×25)	homozygous
<i>ptrcesa8B-3#</i>	CAAAGAGGGCCGGAAAGTTTGC a TTGCGGTGCGG CAAAGAGGGCCGGAAAGTTTGCTTGCGGTGCGG	+1 (×22) 0 (×3)	heterozygous	ATATTCCTIGAA t AATCCCTTCCATTTGCTTGTTA ATATTCCTIGAA -- TCCCTTCCATTTGCTTGTTA	+1 (×13) -2 (×12)	heterozygous
<b>Vector:</b> Cas9/gRNA-PtrCesA8B-2 <b>gRNA:</b> gCesA8B-c (GGTGAGCCTTCTCAGCTTGCTGG); gCesA8B-d (CCTGCTTTCTCCAGAGAAGCTTGC) <b>Target sequences:</b> CesA8B-c, GGTGAGCCTTCTCAGCTTGCTGG; CesA8B-d, CCTGCTTTCTCCAGAGAAGCTTGC						
Line (#)	gCesA8B-c GGTGAGCCTTCTCAGCTTGCTGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8B-d CCTGCTTTCTCCAGAGAAGCTTGC	Mutation types (Number of plasmids containing the cloned PCR products)	



<i>ptrcesa8B-4#</i>	GAGAGGGTGAGCCTTCTC - - - - TGCTGGTGTGG GAGAGGGTGAGCCTTCTCAGCTTGCTGGTGTGG	-4 (×19) 0 (×6)	heterozygous	TTATGCCTGCTTTCTCCAGAGAACTTGCTGGAT	0 (×25)	WT
<i>ptrcesa8B-5#</i>	GAGAGGGTGAGCCTTCTCAGC - TGCTGGTGTGG	-1 (×25)	homozygous	TTATGCCTGCT - - - - CCAGAGAACTTGCTGGAT	-4 (×25)	homozygous
<b>Gene:</b> Potri.006G181900 ( <i>PtrCesA7A</i> )/Potri.018G103900 ( <i>PtrCesA7B</i> )						
<b>Vector:</b> Cas9/gRNA-PtrCesA7A/B-1 <b>gRNA:</b> gCesA7A/B-1 (ACGCAAAGAAAGAGGGAGGGTGG); gCesA7A/B-2 (CCATATCCAGTTTCTGAACCTGG) <b>Target sequences:</b> CesA7A-1, ACGCAAAGAAAGAGGGAGGGTGG; CesA7A-2, CCATATCCAGTTTCTGAACCTGG CesA7B-1, ACGAAAAGAAAGAGGGAGGGTGG; CesA7B-2, CCATATCCAGTTTCTGAACCTGG						
Line (#)	gCesA7A/B-1 CesA7A, ACGCAAAGAAAGAGGGAGGGTGG CesA7B, ACGAAAAGAAAGAGGGAGGGTGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA7A/B-2 CesA7A, CCATATCCAGTTTCTGAACCTGG CesA7B, CCATATCCAGTTTCTGAACCTGG	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7ab-1#-7A</i>  -7B	... ----- AGGGTGGAAAGA	-138 (×25)	homozygous	TGCACCCATAT - - - - - ...	-138 (×25)	homozygous
	ATGGGACGAAAAGAAAGAGG - - GGGTGGAAAGA	-2 (×25)	homozygous	TGCACCA - - - - CCAGTTTCTGAACCTGGTATTT	-4 (×25)	homozygous
<i>ptrcesa7ab-2#-7A</i>  -7B	... ----- AAAGA	-39 (×17)	biallelic	TGCACCCATA - CCAGTTTCTGAACCTGGTATTT	-1 (×10)	chimera
	... ----- GGGTGGAAAGA	-139 (×8)		TGCACCCATAT - - - - - ...	-139 (×8)	
	... ----- GGTGGAAAGA	-133 (×25)	homozygous	TGCACCCATATCCAGTTTCTGAACCTGGTATTT	0 (×7)	homozygous
<i>ptrcesa7ab-3#-7A</i>  -7B	... ----- TGCCTTCTCTC	-645 (×11)	biallelic	TGGATCTCATGGA - - - - - ...	-645 (×11)	biallelic
	... ----- AGGGTGGAAAGA	-315 (×14)		GTCAGATCAAGAC - - - - - ...	-315 (×14)	
	... ----- GGGTGGAAAGA ATGGGACGAAAAGAAAGAGGGAaGGGTGGAAAG GA	-129 (×13) +1 (×12)	biallelic	TGCACCCATATC - - - - - ...	-129 (×13)	biallelic
	+1 (×12)	TGCACCCATAT c CCAGTTTCTGAACCTGGTATTT		+1 (×12)		
<i>ptrcesa7ab-4#-7A</i>  -7B	TGGGACGCAAAGAAAGAGGGAaGGGTGGAAAG	+1 (×25)	homozygous	TGCACCCATATC^276CAGTTTCTGAACCTGGTATT	+276 (×25)	homozygous
	TGGGACGAAAAGAAAGAGGG - GGGTGGAAAG TGGGACGAAAAGAAAGAG - - - - -	-1 (×11) -15 (×14)	biallelic	TGCACCCAT - - - - - TCTGAACCTGGTATTT	-8 (×11)	biallelic
	-15 (×14)	TGCACCC - - - - - CAGTTTCTGAACCTGGTATTT		-5 (×14)		
<b>Vector:</b> Cas9/gRNA-PtrCesA7A/B-2 <b>gRNA:</b> gCesA7A/B-3 (TTTTGTGACTTCAACTTTAATGG); gCesA7A/B-4 (CCTCCTTCCTCGAGTCCAGCGGC) <b>Target sequences:</b> CesA7A-3, TTTTGTGACTTCAACTTTAATGG; CesA7A-4, CCTCCTTCCTCGAGTCCAGCGGC CesA7B-3, TTTTGTGACTTCAACTTTAATGG; CesA7B-4, CCTCCTTCCTCGAGTCCAGCGGC						
Line (#)	gCesA7A/B-3 CesA7A, TTTTGTGACTTCAACTTTAATGG CesA7B, TTTTGTGACTTCAACTTTAATGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA7A/B-4 CesA7A, CCTCCTTCCTCGAGTCCAGCGGC CesA7B, CCTCCTTCCTCGAGTCCAGCGGC	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7ab-5#-7A</i>	GCAATTTTGTGACTTCAACTTTAATGGAAAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC	-4 (×16)	biallelic

<b>-7B</b>				GCGTACC --- TTCCTCGAGTCCAGCGGCTCTGC	-3 (×9)	
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCT t TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×15) -1 (×10)	biallelic
<i>ptrcesa7ab-6#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT - CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC GCGTACCTCCTTCCTCGAGTCCAGCGGCTCTGC	-1 (×13) -7 (×9) 0 (×3)	chimeric
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCT a TCCTCGAGTCCGGCAGCTCTGC GT ----- CCTCGAGTCCGGCAGCTCTGC GTGTACCTCCTTCCTCGAGTCCGGCAGCTCTGC	+1 (×9) -10 (×12) 0 (×4)	chimeric
<i>ptrcesa7ab-7#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCTT a CCTCGAGTCCAGCGGCTCTGC	+1 (×25)	homozygous
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCT ---- CGAGTCCGGCAGCTCTGC	-4 (×25)	homozygous
<i>ptrcesa7ab-8#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCTT a CCTCGAGTCCAGCGGCTCTGC	+1 (×25)	homozygous
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCT ---- CGAGTCCGGCAGCTCTGC GTGTACC <sup>14</sup> TCC <sup>4</sup> TTCCTCGAGTCCGGCAGCTCT GC ( <sup>14</sup> : aagctattagctc <sup>4</sup> : acca)	-4 (×12) +18 (×13)	biallelic
<i>ptrcesa7ab-9#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCTT a CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC	+1 (×11) -7 (×6)	biallelic
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCT t TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCTTCCTCGAGTCCGGCAGCTCTGC	+1 (×18) 0 (×7)	heterozygous
<i>ptrcesa7ab-10#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT - CCTCGAGTCCAGCGGCTCTGC GCGTACCTCC --- CTCGAGTCCAGCGGCTCTGC	-1 (×13) -3 (×12)	biallelic
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCT a TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×14) -1 (×11)	biallelic
<i>ptrcesa7ab-11#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCC -- CCTCGAGTCCAGCGGCTCTGC GCGTACCTCCTT t CCTCGAGTCCAGCGGCTCTGC	-2 (×22) +1 (×3)	biallelic
	GCAATTTTTGTGACTTC ----- ...	-33 (×14)	heterozygous	... ----- CTCGAGTCCGGCAGCTCTGC	-33 (×14)	biallelic
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×11)		GTGTACCTC ----- TTC	-21 (×11)	
<i>ptrcesa7ab-12#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT ---- CGAGTCCAGCGGCTCTGC GCGT ----- CCTCGAGTCCAGCGGCTCTGC	-4 (×16) -8 (×9)	biallelic
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC --- TTCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	-3 (×18) -1 (×7)	biallelic
<i>ptrcesa7ab-13#-7A</i> <b>-7B</b>	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT - CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC	-1 (×12) -7 (×13)	biallelic
	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GT ----- CCTCGAGTCCGGCAGCTCTGC	-10 (×25)	homozygous
<b>Gene:</b> Potri.011G069600 ( <i>PtrCesA8A</i> )/ Potri.004G059600 ( <i>PtrCesA8B</i> )						

<b>Vector:</b> Cas9/gRNA-PtrCesA8A/B-1 <b>gRNA:</b> gCesA8A/B-1 (AGTTCCGCCGGAACAGCAGATGG); gCesA8A/B-2 (CCACTACAGAATAACAAATCCTG) <b>Target sequences:</b> CesA8A-1, AGTTCCGCCGGAACAGCAGATGG; CesA8A-2, CCACTACAGAATAACAAATCCTG CesA8B-1, AGTTCCGCCGGAACAGCAGATGG; CesA8B-2, CCACTACAGAATAACAAATCCTG						
Line (#)	gCesA8A/B-1 CesA8A-1, AGTTCCGCCGGAACAGCAGATGG CesA8B-1, AGTTCCGCCGGAACAGCAGATGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8A/B-2 CesA8A-2, CCACTACAGAATAACAAATCCTG CesA8B-2, CCACTACAGAATAACAAATCCTG	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa8ab-1#-8A</i> <b>-8B</b>	GCTCAAGTTCCGCCGGAACA --- GATGGAAGAG	-3 (×23)	biallelic	TTCTTCCACTA ----- TAACAAATCCTGTTGAT	-5 (×23)	biallelic
	GCTCAAGTTCCGCCGGA ----- GATGGAAGAG	-5 (×2)		TTCTTCCACTA a CAGAATAACAAATCCTGTTGAT	+1 (×2)	
	GCTCAAGTTCCGCCGGA ----- GAAGACTAAGAT GCTCAAGTTCCGCCG ----- ATGGAAGAT	-4 (×13) -9 (×12)	biallelic	TTCTTCCACTA t CAGAATAACAAATCCTGTCGAT TTCTTCCACTA a CAGAATAACAAATCCTGTCGAT	+1 (×11) +1 (×14)	biallelic
<i>ptrcesa8ab-2#-8A</i> <b>-8B</b>	GCTCAAGTTCCGCCGGAACAGC a AGATGGAAG	+1 (×15)		heterozygous	TTCTTCCACTA - AGAATAACAAATCCTGTTGAT	
	GCTCAAGTTCCGCCGGAACAGCAGATGGAAGA	0 (×10)	TTCTTCCACTACAGAATAACAAATCCTGTTGAT		0 (×15)	
	... ----- AGATGGAAGAT	-279 (×25)	homozygous	TTCTTCCACTA t CAGAATAACAAATCCTGTCGAT	+1 (×25)	homozygous
<i>ptrcesa8ab-3#-8A</i> <b>-8B</b>	GCTCAAGTTCCGCCGGAACA --- GATGGAAGAG	-3 (×18)	biallelic	TTCTTCCACTACAGAATAACAAATCCTGTTGAT	0 (×25)	WT
	GCTCAAGTTCCGCCGGA ----- GATGGAAGAG	-5 (×7)		biallelic	TTCTTCCACTAC a AGAATAACAAATCCTGTCGAT TTCTTCCACTA a CAGAATAACAAATCCTGTCGAT TTCTTCCACTA - AGAATAACAAATCCTGTCGAT TTCTTCCACTACAGAATAACAAATCCTGTCGAT	
	GCTCAAGTTCCGCCGGA ----- GAAGACTAAGAT GCTCAAGTTCCGCCG ----- ATGGAAGAT	-4 (×13) -9 (×12)				
<b>Vector:</b> Cas9/gRNA-PtrCesA8A/B-2 <b>gRNA:</b> gCesA8A/B-3 (GAACTGGTTGTGTTTTCAATAGG); gCesA8A/B-4 (CCACAAGTAGGTCGAGATGTATG) <b>Target sequences:</b> CesA8A-3, GAACTGGTTGTGTTTTCAATAGG; CesA8A-4, CCACAAGTAGGTCGAGATGTATG CesA8B-3, GAACTGGTTGTGTTTTCAATAGG; CesA8B-4, CCACAAGTAGGTCGAGATGTATG						
Line#	gCesA8A/B-3 CesA8A-3, GAACTGGTTGTGTTTTCAATAGG CesA8B-3, GAACTGGTTGTGTTTTCAATAGG	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8A/B-4 CesA8A-4, CCACAAGTAGGTCGAGATGTATG CesA8B-4, CCACAAGTAGGTCGAGATGTATG	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa8ab-4#-8A</i> <b>-8B</b>	TGTGGGAACTGGTTGTGTTTTC t AATAGGCAAG	+1 (×25)	homozygous	TGGATCCACA g GTAGGTCGAGATGTATGCTAT TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×3) 0 (×22)	heterozygous
	TGTGGGAACTGGTTGTGTTTTC a AATAGGCAAG	+1 (×23)	biallelic	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
	TGTGGGAACTGGTTGTGTTTTC t AATAGGCAAG	+1 (×2)				
<i>ptrcesa8ab-5#-8A</i> <b>-8B</b>	(inverted 687) CAATAGGCAAGC	inversion (×13)	biallelic	TGGATCCACAAG (inverted 687) TGGATCCACAAGTAGGTCGAGATGTATGCTATG	inversion (×13) 0 (×12)	heterozygous
	... ----- GGCAAGC	-28 (×12)				
	TGTGGGAACTGGTGA ----- AAAGGCAAGC TGTGGGAACTGGTTGTG ----- GGCAAGC	-7 (×21) -9 (×4)	biallelic	TGGATCCACA g GTAGGTCGAGATGTATGCTAT TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×4) 0 (×21)	heterozygous

<i>ptrcesa8ab-6#-8A</i> <b>-8B</b>	TGTGGGAAGCTGGTTGTGTTTTTC t AAT <u>AGG</u> CAAG	+1 (×18)	biallelic	TGGAT <u>CC</u> CAA a GTAGGTCGAGATGTATGCTAT	+1 (×22)	heterozygous
	TGTGGGAAGCTGGT ----- ...	-31 (×7)		TGGAT <u>CC</u> CAAGTAGGTCGAGATGTATGCTATG	0 (×3)	
	TGTGGGAAGCTGGTTGTGTTTTTC a AAT <u>AGG</u> CAAG	+1 (×15)	chimeric	TGGAT <u>CC</u> CAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
	TGTGGGAAGCTGGTTGTGTTTTTCaaAAT <u>AGG</u> CAAG	+2 (×2)				
	TGTGGGAAGCTGGTTGTGTA --- AAT <u>AGG</u> CAAGC	-3 (×8)				
<i>ptrcesa8ab-7#-8A</i> <b>-8B</b>	... ----- AAT <u>AGG</u> CAAGC	-688 (×25)	homozygous	TGGAT <u>CC</u> CAAG ----- ...	-688 (×25)	homozygous
	TGTGGGAAGCTGGTTGTGTTTTTCtatAAT <u>AGG</u> CAAG	+3 (×14)	biallelic	TGGAT <u>CC</u> CAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
TGTGGGAAGCTGGTT ^19^ TTTTGAAT <u>AGG</u> CAAGC	+19 (×11)					
	(^19^: aacatgaggtaacaacga)					
<i>ptrcesa8ab-8#-8A</i> <b>-8B</b>	TGTGGGAAGCTGGTTGTG ----- AAT <u>AGG</u> CAAGC	-5 (×25)	homozygous	TGGAT <u>CC</u> CAA - TAGGTCGAGATGTATGCTATG	-1 (×5)	heterozygous
				TGGAT <u>CC</u> CAAGTAGGTCGAGATGTATGCTATG	0 (×20)	
	TGTGGGAAGCTGGTTGTGT ----- AAT <u>AGG</u> CAAGC	-4 (×25)	homozygous	TGGAT <u>CC</u> CAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT

**Table S6** Analysis of potential off-target sites among the Cas9/gRNA-induced *ptrcesa* mutants.

On/Off targets	gRNA name	Bases position of gRNAs																			PAM			Target gene or genomic position	Off-target Editing (Yes/No)	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22			23
On	<b>gCesA4-1</b>	C	A	T	A	C	G	A	T	G	C	T	T	A	T	G	C	A	T	T	G	T	G	G	<b>PtrCesA4</b>	-
Off	gCesA4-1-off-1	G	T	G	A	G	G	A	G	G	C	T	T	A	T	G	C	A	T	T	G	T	G	G	Potri.019G000800	No
	gCesA4-1-off-2	T	G	A	G	G	G	A	T	G	C	T	T	A	T	G	C	T	T	T	G	T	G	G	Potri.006G052600	No
On	<b>gCesA4-2</b>	G	A	C	G	A	A	A	A	G	G	C	C	G	G	A	C	G	A	A	T	G	G	G	<b>PtrCesA4</b>	-
Off	-	NO off-target homologous sequences																			-			-	-	
On	<b>gCesA4-3</b>	C	A	T	C	T	G	T	G	T	T	A	G	A	A	C	T	T	C	G	A	T	G	G	<b>PtrCesA4</b>	-
Off	gCesA4-3-off-1	A	A	G	A	T	G	T	T	T	G	A	G	A	A	C	T	T	C	G	A	T	G	G	Potri.001G144100	No
	gCesA4-3-off-2	T	T	A	A	G	C	T	G	T	T	A	G	A	A	C	T	T	C	G	A	C	G	G	Chr12 (+9333611)	No
On	<b>gCesA4-4</b>	C	T	C	C	T	C	C	A	A	G	G	A	C	C	T	T	G	A	G	G	A	G	G	<b>PtrCesA4</b>	-
Off	gCesA4-4-off-1	T	C	T	G	T	C	C	G	A	G	G	A	C	C	T	T	G	A	G	G	T	G	G	Potri.014G014400	No
	gCesA4-4-off-2	T	C	C	C	T	C	A	A	A	G	A	A	C	C	T	T	G	A	G	G	A	G	T	Chr19 (-6105951)	No
On	<b>gCesA7A/B-1</b>	A	C	G	C	A	A	A	G	A	A	A	G	A	G	G	G	A	G	G	G	T	G	G	<b>PtrCesA7A</b>	-
		A	C	G	A	A	A	A	G	A	A	A	G	A	G	G	G	A	G	G	G	T	G	G	<b>PtrCesA7B</b>	-
Off	gCesA7A/B-1-off-1	A	C	A	A	A	A	A	A	A	A	G	A	G	G	G	A	G	G	G	A	G	G	Potri.004G210200	No	
	gCesA7A/B-1-off-2	A	G	A	G	A	A	A	G	A	A	A	G	A	G	G	G	A	G	G	G	A	G	G	Potri.001G314000	No
	gCesA7A/B-1-off-3	G	A	G	A	A	G	A	G	A	A	A	G	A	G	G	G	A	G	G	G	A	G	G	Potri.011G084300	No
	gCesA7A/B-1-off-4	A	A	A	A	A	G	A	G	A	A	A	G	A	G	G	G	A	G	G	G	A	G	G	Chr01(-21435525)	No
	gCesA7A/B-1-off-5	A	T	G	A	G	A	A	G	A	A	A	G	A	G	G	G	A	G	G	G	A	G	G	Potri.003G046500	No
	gCesA7A/B-1-off-6	A	C	G	C	A	A	G	G	A	G	A	G	A	G	G	G	A	G	G	G	T	G	A	Chr18 (-6513265)	No
On	<b>gCesA7A/B-2</b>	C	C	A	G	G	T	T	C	A	G	A	A	A	C	T	G	G	A	T	A	T	G	G	<b>PtrCesA7A</b>	-
		C	C	A	G	G	T	T	C	A	G	A	A	A	C	T	G	G	A	T	A	T	G	G	<b>PtrCesA7B</b>	-
Off	gCesA7A/B-2-off-1	T	G	A	T	G	T	T	C	T	T	A	A	A	C	T	G	G	A	T	A	T	G	G	Chr17 (+506091)	No
	gCesA7A/B-2-off-2	T	G	C	A	G	A	T	C	A	G	A	A	A	C	T	G	G	A	T	A	G	G	G	Potri.008G190100	No
On	<b>gCesA7A/B-3</b>	T	T	T	T	G	T	G	A	C	T	T	C	A	A	C	T	T	T	A	A	T	G	G	<b>PtrCesA7A</b>	-
		T	T	T	T	G	T	G	A	C	T	T	C	A	A	C	T	T	T	A	A	T	G	G	<b>PtrCesA7B</b>	-

Off	gCesA7A/B-3-off-1	T	G	T	T	C	T	G	A	C	T	T	C	A	A	C	T	T	T	A	A	A	G	G	Chr05(-12012362)	No
	gCesA7A/B-3-off-2	A	C	G	T	G	T	C	A	C	T	T	A	A	A	C	T	T	T	A	A	T	G	G	Scaffold_696	No
	gCesA7A/B-3-off-3	A	C	A	C	G	T	C	A	C	T	T	A	A	A	C	T	T	T	A	A	T	G	G	Chr12 (-5722266)	No
	gCesA7A/B-3-off-4	A	T	C	A	A	T	G	A	G	T	T	C	A	A	C	T	T	T	A	A	T	G	G	Chr12 (-5867397)	No
On	gCesA7A/B-4	G	C	C	G	C	T	G	G	A	C	T	C	G	A	G	G	A	A	G	G	A	G	G	PtrCesA7A	-
		G	C	T	G	C	C	G	G	A	C	T	C	G	A	G	G	A	A	G	G	A	G	G	PtrCesA7B	-
Off	gCesA7A/B-4-off-1	A	G	A	C	A	T	G	G	A	C	T	C	G	A	G	G	A	A	G	G	A	G	G	Potri.014G036100	No
On	gCesA8A/B-1	A	G	T	T	C	C	G	C	C	G	G	A	A	C	A	G	C	A	G	A	T	G	G	PtrCesA8A	-
		A	G	T	T	C	C	G	C	C	G	G	A	A	C	A	G	C	A	G	A	T	G	G	PtrCesA8B	-
Off	-	NO off-target homologous sequences																				-	-	-		
On	gCesA8A/B-2	C	A	G	G	A	T	T	T	G	T	T	A	T	T	C	T	G	T	A	G	T	G	G	PtrCesA8A	-
		C	A	G	G	A	T	T	T	G	T	T	A	T	T	C	T	G	T	A	G	T	G	G	PtrCesA8B	-
Off	gCesA8A/B-2-off-1	C	A	G	G	A	T	T	T	G	T	T	A	T	T	C	G	G	T	A	G	T	G	C	Potri.001G266400	No
	gCesA8A/B-2-off-2	G	G	T	C	A	T	T	T	G	G	A	A	T	T	C	T	G	T	A	G	T	G	G	Potri.001G456800	No
On	gCesA8A/B-3	G	A	A	C	T	G	G	T	T	G	T	G	T	T	T	C	A	A	T	A	G	G	PtrCesA8A	-	
		G	A	A	C	T	G	G	T	T	G	T	G	T	T	T	C	A	A	T	A	G	G	PtrCesA8B	-	
Off	gCesA8A/B-3-off-1	G	A	A	C	T	G	G	T	T	G	T	C	T	A	T	T	C	A	T	T	A	G	G	Chr06 (+1623375)	No
	gCesA8A/B-3-off-2	A	A	C	C	T	G	G	T	T	G	T	G	T	T	G	C	A	T	T	A	G	G	Chr17(-10225639)	No	
	gCesA8A/B-3-off-3	T	A	T	G	T	G	G	T	T	G	T	G	T	T	T	C	C	A	A	A	G	G	Potri.011G116200	No	
	gCesA8A/B-3-off-4	G	A	G	C	T	G	G	T	T	G	T	G	T	T	T	A	A	A	A	A	G	A	Potri.004G147800	No	
On	gCesA8A/B-4	C	A	T	A	C	A	T	C	T	C	G	A	C	C	T	A	C	T	T	G	T	G	G	PtrCesA8A	-
		C	A	T	A	C	A	T	C	T	C	G	A	C	C	T	A	C	T	T	G	T	G	G	PtrCesA8B	-
Off	-	NO off-target homologous sequences																				-	-	-		

Nucleotides in green shading are the same with the designed gRNAs target sites and mismatched nucleotides are without shading. The PAM regions are highlighted in red shading. Potential off-target sites in protein coding gene regions are annotated with gene ID and the others located in noncoding regions are detailed with position information on chromosomes.

**Number of plasmids containing  
the cloned PCR products**

**gCesA4-1-off-1**

WT **GTGAGGAG**GCTTATGCATTGTGG (×10)  
*ptrcesa4-1*# GTGAGGAGGCTTATGCATTGTGG (×15)  
*ptrcesa4-4*# GTGAGGAGGCTTATGCATTGTGG (×15)

**gCesA4-1-off-2**

WT **TGAGG**GATGCTTATGCT**TTGTGG** (×10)  
*ptrcesa4-1#* TGAGGGATGCTTATGCTTT**GTGG** (×15)  
*ptrcesa4-4#* TGAGGGATGCTTATGCTTT**GTGG** (×15)

**gCesA4-3-off-1**

WT **AAGA**TGT**TTG**AGAACTTCGAT**GG** (×10)  
*ptrcesa4-6#* AAGATGTTT**GAGA**ACTTCGAT**GG** (×15)

**gCesA4-3-off-2**

WT **TTAAGC**TGTTAGAACTTCG**ACGG** (×10)  
*ptrcesa4-6#* TTAAGCTGTTAGAACTTCG**ACGG** (×15)

**gCesA4-4-off-1**

WT **CCACCTCAAGGTCCTCGGACAGA** (×10)  
*ptrcesa4-6#* **CCACCTCAAGGTCCTCGGACAGA** (×15)

**gCesA4-4-off-2**

WT **ACTCCTCAAGGTCTTTGAGGGA** (×10)  
*ptrcesa4-6#* **ACTCCTCAAGGTCTTTGAGGGA** (×15)

**gCesA7A/B-1-off-1**

WT AC**AA**AAA**A**AAAGAGGGAGGG**AGG** (×10)  
*ptrcesa7ab-3#* AC**AAAAAAAAA**AGAGGGAGGG**AGG** (×15)

**gCesA7A/B-1-off-2**

WT A**GAG**AAAGAAAGAGGGAGGG**AGG** (×10)  
*ptrcesa7ab-3#* A**GAG**AAAGAAAGAGGGAGGG**AGG** (×15)

**gCesA7A/B-1-off-3**

WT **GAGA**A**G**AGAAAGAGGGAGGG**AGG** (×10)  
*ptrcesa7ab-3#* **GAGA**A**G**AGAAAGAGGGAGGG**AGG** (×15)

**gCesA7A/B-1-off-4**

WT **AAAA**A**G**AGAAAGAGGGAGGG**AGG** (×10)  
*ptrcesa7ab-3#* **AAAA**A**G**AGAAAGAGGGAGGG**AGG** (×15)

**gCesA7A/B-1-off-5**

WT A**TGAG**AAGAAAGAGGGAGGG**AGG** (×10)  
*ptrcesa7ab-3#* A**TGAG**AAGAAAGAGGGAGGG**AGG** (×15)

**gCesA7A/B-1-off-6**

WT ACGCA**AGGAG**AGAGGGAGGG**TGA** (×6)

*ptrcesa7ab-3#* ATGCAAGGAGAGAGGGAGGGTGA (×4) SNP  
ACGCAAGGAGAGAGGGAGGGTGA (×7)  
ATGCAAGGAGAGAGGGAGGGTGA (×8)

**gCesA7A/B-2-off-1**

WT CCATATCCAGTTTAAAGAACATCA (×10)  
*ptrcesa7ab-3#* CCATATCCAGTTTAAGAACATCA (×15)

**gCesA7A/B-2-off-2**

WT CCTATCCAGTTTCTGATCTGCA (×10)  
*ptrcesa7ab-3#* CCTATCCAGTTTCTGATCTGCA (×15)

**gCesA7A/B-3-off-1**

WT TGTTCTGACTTCAACTTTAAAGG (×10)  
*ptrcesa7ab-7#* TGTTCTGACTTCAACTTTAAAGG (×15)  
*ptrcesa7ab-13#* TGTTCTGACTTCAACTTTAAAGG (×15)

**gCesA7A/B-3-off-2**

WT ACGTGTCACTTAAACTTTAATGG (×10)  
*ptrcesa7ab-7#* ACGTGTCACTTAAACTTTAATGG (×15)  
*ptrcesa7ab-13#* ACGTGTCACTTAAACTTTAATGG (×15)

**gCesA7A/B-3-off-3**

WT ACACGTCACTTAAACTTTAATGG (×10)  
*ptrcesa7ab-7#* ACACGTCACTTAAACTTTAATGG (×15)  
*ptrcesa7ab-13#* ACACGTCACTTAAACTTTAATGG (×15)

**gCesA7A/B-3-off-4**

WT ATCAAATGAGTTCAACTTTAATGG (×10)  
*ptrcesa7ab-7#* ATCAAATGAGTTCAACTTTAATGG (×15)  
*ptrcesa7ab-13#* ATCAAATGAGTTCAACTTTAATGG (×15)

**gCesA7A/B-4-off-1**

WT CCTCCTTCCTCGAGTCCA TGTCT (×10)  
*ptrcesa7ab-7#* CCTCCTTCCTCGAGTCCATGTCT (×15)  
*ptrcesa7ab-13#* CCTCCTTCCTCGAGTCCATGTCT (×15)

**gCesA8A/B-2-off-1**

WT GCACTACCGAATAACAAATCCTG (×10)  
*ptrcesa8ab-1#* GCACTACCGAATAACAAATCCTG (×15)

**gCesA8A/B-2-off-2**



WT CCACTACAGAATT**TC**CAAAT**GACC** (×10)  
*ptrcesa8ab-1#* CCACTACAGAATTCCAAATGACC (×15)

**gCesA8A/B-3-off-1**

WT GAACTGGTTGT**CT**ATT**CA**T**T**AGG (×5)  
GAACTGGTTGT**CT**ATT**CA**T**C**AGG (×5) SNP  
*ptrcesa8ab-4#* GAACTGGTTGTCTATT**CA**T**T**AGG (×7)  
GAACTGGTTGTCTATT**CA**T**C**AGG (×8)  
*ptrcesa8ab-8#* GAACTGGTTGTCTATT**CA**T**T**AGG (×8)  
GAACTGGTTGTCTATT**CA**T**C**AGG (×7)

**gCesA8A/B-3-off-2**

WT **AA**CCTGGTTGTGTTT**G**CA**T**TAGG (×10)  
*ptrcesa8ab-4#* AACCTGGTTGTGTTTGCATTAGG (×15)  
*ptrcesa8ab-8#* AACCTGGTTGTGTTTGCATTAGG (×15)

**gCesA8A/B-3-off-3**

WT **TAT**GTGGTTGTGTTT**CCAA**AGG (×5)  
**TAT**GTG**ATT**GTGTTT**CCAA**ACG (×5) SNP  
*ptrcesa8ab-4#* TATGTGGTTGTGTTTCCAAAGG (×7)  
TATGTG**ATT**GTGTTTCCAA**ACG** (×8)  
*ptrcesa8ab-8#* TATGTGGTTGTGTTTCCAAAGG (×6)  
TATGTG**ATT**GTGTTTCCAA**ACG** (×9)

**gCesA8A/B-3-off-4**

WT GAG**G**CTGGTTGTGTTT**AAAA**AGA (×10)  
*ptrcesa8ab-4#* GAGCTGGTTGTGTTTAAAAAGA (×15)  
*ptrcesa8ab-8#* GAGCTGGTTGTGTTTAAAAAGA (×15)

**Table S7** Inheritance of the Cas9/gRNA-induced mutations in progenies of *ptrcesa4*, *7a/b* and *8a/b* mutants through asexual propagation methods. The progenies did change in the edited sites of *PtrCesA4*, *7A/B* or *8A/B*, in which was labeled with empty box and brilliant yellow color.

Line (#)		gCesA4-1 Target seq: CATACGATGCTTATGCATTG <u>TGG</u>	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA4-2 Target seq: <u>CCC</u> ATTCGTCGGCCTTTTCGTC	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa4-2#</i>		TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×17) 0 (×8)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×15) +1 (×10)	biallelic
Apical bud	T0	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×7) 0 (×18)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×11) +1 (×14)	biallelic
Axillary bud	T1-1	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×11) 0 (×14)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×11) +1 (×14)	biallelic
	T1-2	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×13) 0 (×12)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×15) +1 (×10)	biallelic
	T2-1	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×16) 0 (×9)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×12) +1 (×13)	biallelic
	T2-2	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×17) 0 (×8)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×10) +1 (×15)	biallelic
Shoot regeneration	T1-1	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT	-1 (×14) 0 (×9) -1 (×2)	chimeric	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×12) +1 (×13)	biallelic
	T1-2	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×10) 0 (×15)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t - - - - GCCTTTTCGTC AGCAG	+1 (×10) +1.5 (×15)	biallelic
	T1-3	TCCAGCATAACGATGCTTATGC - TTG <u>TGG</u> CCTTAT TCCAGCATAACGATGCTTATGCATTG <u>TGG</u> CCTTAT	-1 (×18) 0 (×7)	heterozygous	TGCAC <u>CCC</u> ATT c CGTCCGGCCTTTTCGTCAGCAG TGCAC <u>CCC</u> ATT t CGTCCGGCCTTTTCGTCAGCAG	+1 (×13) +1 (×12)	biallelic
Line (#)		gCesA4-3 Target seq: CATCTGTGTTAGAACTTCGAT <u>TGG</u>	Mutation types (Number of plasmids containing the cloned PCR products)		gCesA4-4 Target seq: CCTCAAGGTCCTTGGAGGAG	Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa4-5#</i>		AGCAACATCTGTGTTAGAA - - - CGAT <u>TGG</u> AGTG AGCAACATCTGTGTTAGAAC - - CGAT <u>TGG</u> AGTG	-3 (×13) -2 (×12)	biallelic	CAAGG <u>CCT</u> CCTCAAGGTCCTTGGAGGAGTAGAC	0 (×25)	WT
Apical bud	T0	AGCAACATCTGTGTTAGAA - - - CGAT <u>TGG</u> AGTG AGCAACATCTGTGTTAGAAC - - CGAT <u>TGG</u> AGTG	-3 (×15) -2 (×10)	biallelic	CAAGG <u>CCT</u> CCTCAAGGTCCTTGGAGGAGTAGAC	0 (×25)	WT
Axillary bud	T1-1	AGCAACATCTGTGTTAGAA - - - CGAT <u>TGG</u> AGTG AGCAACATCTGTGTTAGAAC - - CGAT <u>TGG</u> AGTG	-3 (×11) -2 (×14)	biallelic	CAAGG <u>CCT</u> CCTCAAGGTCCTTGGAGGAGTAGAC	0 (×25)	WT

	T1-2	AGCAACATCTGTGTTAGAA --- CGATGGAGTG AGCAACATCTGTGTTAGAAC -- CGATGGAGTG	-3 (×9) -2 (×16)	biallelic	CAAGGCCTCCTCAAGGTCCTTGAGGAGTAGAC	0 (×25)	WT
	T2-1	AGCAACATCTGTGTTAGAA --- CGATGGAGTG AGCAACATCTGTGTTAGAAC -- CGATGGAGTG	-3 (×10) -2 (×15)	biallelic	CAAGGCCTCCTCAAGGTCCTTGAGGAGTAGAC	0 (×25)	WT
	T2-2	AGCAACATCTGTGTTAGAA --- CGATGGAGTG AGCAACATCTGTGTTAGAAC -- CGATGGAGTG	-3 (×12) -2 (×13)	biallelic	CAAGGCCTCCTCAAGGTCCTTGAGGAGTAGAC	0 (×25)	WT
Shoot regeneration	T1-1	AGCAACATCTGTGTTAGAA --- CGATGGAGTG AGCAACATCTGTGTTAGAAC -- CGATGGAGTG	-3 (×12) -2 (×13)	biallelic	CAAGGCCTCCTCAAGGTCCTTGAGGAGTAGAC	0 (×25)	WT
	T1-2	AGCAACATCTGTGTTAGAA --- CGATGGAGTG AGCAACATCTGTGTTAGAAC -- CGATGGAGTG	-3 (×16) -2 (×9)	biallelic	CAAGGCCTCCTCAAGGTCCTTGAGGAGTAGAC	0 (×25)	WT
	T1-3	AGCAACATCTGTGTTAGAA --- CGATGGAGTG AGCAACATCTGTGTTAGAAC -- CGATGGAGTG	-3 (×17) -2 (×8)	biallelic	CAAGGCCTCCTCAAGGTCCTTGAGGAGTAGAC	0 (×25)	WT
Line (#)	gCesA7A/B-3 CesA7A, TTTGTGACTTCAACTTTAATGG CesA7B, TTTGTGACTTCAACTTTAATGG		Mutation types (Number of plasmids containing the cloned PCR products)	gCesA7A/B-4 CesA7A, CCTCCTCCTCGAGTCCAGCGGC CesA7B, CCTCCTCCTCGAGTCCGCAGC		Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrcesa7ab-5#-7A-7B</i>		GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC GCGTACC - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×16) -3 (×9)	biallelic
		GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GTGTACCTCCT † TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×15) -1 (×10)	biallelic
Apical bud	T0-7A-7B	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC GCGTACC - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×9) -3 (×16)	biallelic
		GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GTGTACCTCCT † TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×11) -1 (×14)	biallelic
Axillary bud	T1-1-7A	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC GCGTACC - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×14) -3 (×11)	biallelic
	-7B	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GTGTACCTCCT † TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×13) -1 (×12)	biallelic
	T1-2-7A	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC GCGTACC - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×13) -3 (×12)	biallelic
	-7B	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GTGTACCTCCT † TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×12) -1 (×13)	biallelic
	T2-1-7A	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC GCGTACC - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×10) -3 (×15)	biallelic
	-7B	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GTGTACCTCCT † TCCTCGAGTCCGGCAGCTCTGC GTGTACCTCCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×14) -1 (×11)	biallelic
	T2-2-7A	GCAATTTTGTGACTTCAACTTTAATGG AAGAA	0 (×25)	WT	GCGTACCTCCT - - - - CGAGTCCAGCGGCTCTGC GCGTACC - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×16) -3 (×9)	biallelic

	-7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCT † TCCTCGAGTCCGGCAGCTCTGC GTGTAC <u>CT</u> CCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×12) -1 (×13)	biallelic
<b>Shoot regeneration</b>	T1-1-7A-7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT - - - - CGAGTCCAGCGGCTCTGC GCGTAC <u>C</u> - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×10) -3 (×15)	biallelic
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCT † TCCTCGAGTCCGGCAGCTCTGC GTGTAC <u>CT</u> CCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×13) -1 (×12)	biallelic
	T1-2-7A-7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT - - - - CGAGTCCAGCGGCTCTGC GCGTAC <u>C</u> - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×14) -3 (×11)	biallelic
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCT † TCCTCGAGTCCGGCAGCTCTGC GTGTAC <u>CT</u> CCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×12) -1 (×13)	biallelic
	T1-3-7A-7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT - - - - CGAGTCCAGCGGCTCTGC GCGTAC <u>C</u> - - - TTCCTCGAGTCCAGCGGCTCTGC	-4 (×14) -3 (×11)	biallelic
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCT † TCCTCGAGTCCGGCAGCTCTGC GTGTAC <u>CT</u> CCT - CCTCGAGTCCGGCAGCTCTGC	+1 (×12) -1 (×13)	biallelic
<b>Line#</b>		<b>gCesA7A/B-3</b> CesA7A, TTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u> CesA7B, TTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>		<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)	<b>gCesA7A/B-4</b> CesA7A, <u>CTC</u> CTTCCTCGAGTCCAGCGGC CesA7B, <u>CTC</u> CTTCCTCGAGTCCGGCAGC		<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)
<i>ptrcesa7ab-6#-7A-7B</i>		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT - CCTCGAGTCCAGCGGCTCTG GCGTAC <u>CT</u> C - - - - - GAGTCCAGCGGCTCTG GCGTAC <u>CT</u> CCTCCTCGAGTCCAGCGGCTCTG	-1 (×13) -7 (×9) 0 (×3)	chimeric
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCTa TCCTCGAGTCCGGCAGCTCTG GT - - - - - CCTCGAGTCCGGCAGCTCTG GTGTAC <u>CT</u> CCTCCTCGAGTCCGGCAGCTCTG	+1 (×9) -10 (×12) 0 (×4)	chimeric
<b>Apical bud</b>	T0-7A-7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT - CCTCGAGTCCAGCGGCTCTG GCGTAC <u>CT</u> C - - - - - GAGTCCAGCGGCTCTG GCGTAC <u>CT</u> CCTCCTCGAGTCCAGCGGCTCTG	-1 (×12) -7 (×8) 0 (×5)	chimeric
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCTa TCCTCGAGTCCGGCAGCTCTG GT - - - - - CCTCGAGTCCGGCAGCTCTG GTGTAC <u>CT</u> CCTCCTCGAGTCCGGCAGCTCTG	+1 (×9) -10 (×10) 0 (×6)	chimeric
<b>Axillary bud</b>	T1-1-7A-7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT- CCTCGAGTCCAGCGGCTCTGC GCGTAC <u>CT</u> C - - - - - GAGTCCAGCGGCTCTGC GCGTAC <u>CT</u> CCTCCTCGAGTCCAGCGGCTCTGC	-1 (×13) -7 (×8) 0 (×4)	chimeric
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTAC <u>CT</u> CCTa TCCTCGAGTCCGGCAGCTCTG GT - - - - - CCTCGAGTCCGGCAGCTCTG GTGTAC <u>CT</u> CCTCCTCGAGTCCGGCAGCTCTG	+1 (×11) -10 (×10) 0 (×4)	chimeric
	T1-2-	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTAC <u>CT</u> CCT - CCTCGAGTCCAGCGGCTCTG	-1 (×10)	chimeric

	7A -7B				GCGTACCTC ----- GAGTCCAGCGGCTCTGC GCGTACCTCCTTCCTCGAGTCCAGCGGCTCTGC	-7 (×9) 0 (×6)		
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCTaTCCTCGAGTCCGGCAGCTCTG GT ----- CCTCGAGTCCGGCAGCTCTGC GTGTACCTCCTTCCTCGAGTCCGGCAGCTCTGC	+1 (×13) -10 (×8) 0 (×4)	chimeric	
	T2-1- 7A -7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT- CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC GCGTACCTCCTTCCTCGAGTCCAGCGGCTCTGC	-1 (×10) -7 (×10) 0 (×5)	chimeric	
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCTa TCCTCGAGTCCGGCAGCTCTG GT ----- CCTCGAGTCCGGCAGCTCTGC GTGTACCTCCTTCCTCGAGTCCGGCAGCTCTGC	+1 (×11) -10 (×9) 0 (×5)	chimeric	
	T2-2- 7A -7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT- CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC GCGTACCTCCTTCCTCGAGTCCAGCGGCTCTGC	-1 (×12) -7 (×9) 0 (×4)	chimeric	
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCTa TCCTCGAGTCCGGCAGCTCTG GT ----- CCTCGAGTCCGGCAGCTCTGC GTGTACCTCCTTCCTCGAGTCCGGCAGCTCTGC	+1 (×11) -10 (×10) 0 (×4)	chimeric	
Shoot regeneration	T1-1- 7A -7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u> GCAATTTTTGTGACTTCAA ----- ...]	0 (×22) -244 (×3)	heterozygou s	GCGTACCTCCT- CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC ... ----- TTTACGGTTCAAT]	-1 (×12) -7 (×10) -244 (×3)	chimeric	
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCTaTCCTCGAGTCCGGCAGCTCTG GT ----- CCTCGAGTCCGGCAGCTCTGC	+1 (×11) -10 (×14)	biallelic	
	T1-2- 7A -7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT- CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC	-1 (×14) -7 (×11)	biallelic	
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCTa TCCTCGAGTCCGGCAGCTCTG GT ----- CCTCGAGTCCGGCAGCTCTGC	+1 (×12) -10 (×13)	biallelic	
	T1-3- 7A -7B	GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GCGTACCTCCT- CCTCGAGTCCAGCGGCTCTGC GCGTACCTC ----- GAGTCCAGCGGCTCTGC	-1 (×15) -7 (×10)	biallelic	
		GCAATTTTTGTGACTTCAACTTTAAT <u>TGGAAGAA</u>	0 (×25)	WT	GTGTACCTCCTaTCCTCGAGTCCGGCAGCTCTG GT ----- CCTCGAGTCCGGCAGCTCTGC	+1 (×11) -10 (×14)	biallelic	
Line (#)	gCesA8A/B-1 CesA8A-1, AGTTCCGCCGGAACAGCAGAT <u>TGG</u> CesA8B-1, AGTTCCGCCGGAACAGCAGAT <u>TGG</u>		Mutation types (Number of plasmids containing the cloned PCR products)		gCesA8A/B-2 CesA8A-2, <u>CCACTACAGAATAACAAATCCTG</u> CesA8B-2, <u>CCACTACAGAATAACAAATCCTG</u>		Mutation types (Number of plasmids containing the cloned PCR products)	
<i>ptrces8ab-3#-8A-8B</i>	GCTCAAGTCCGCCGGAACA --- GAT <u>TGGAAGA</u>		-3 (×18)	biallelic	TTCTT <u>CCACTACAGAATAACAAATCCTG</u> TGAT	0 (×25)	WT	
	GCTCAAGTCCGCCGGA --- GAT <u>TGGAAGA</u>		-5 (×7)					
	GCTCAAGTCCGCCGGA --- GAAGACTAAGA		-4 (×13)	biallelic		TTCTT <u>CCACTACaAGAATAACAAATCCTG</u> TGCA	+1 (×10)	chimeric

		GCTCAAGTTCGCGCG ----- ATGGAAGA	-9 (×12)		TTCTT <u>CC</u> ACTAaCAGAATAACAAATCCTGTGCGA TTCTT <u>CC</u> ACTA- AGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTGCGAT	+1 (×2) -1 (×10) 0 (×3)	
Apical bud	T0-8A -8B	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×19)	biallelic	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×25)	WT
		GCTCAAGTTCGCGCGGAA ----- GATGGAAGA	-5 (×6)				
	GCTCAAGTTCGCGCGGA ---- GAAGACTAAGA GCTCAAGTTCGCGCG ----- ATGGAAGA	-4 (×14) -9 (×11)	biallelic	TTCTT <u>CC</u> ACTACaAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTAaCAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTA-AGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTGCGAT	+1 (×10) +1 (×2) -1 (×9) 0 (×4)	chimeric	
Axillary bud	T1-1- 8A -8B	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×18)	biallelic	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×25)	WT
		GCTCAAGTTCGCGCGGAA ----- GATGGAAGA	-5 (×7)				
	GCTCAAGTTCGCGCGGA ---- GAAGACTAAGA GCTCAAGTTCGCGCG ----- ATGGAAGA	-4 (×14) -9 (×11)	biallelic	TTCTT <u>CC</u> ACTACaAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTAaCAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTA-AGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTGCGAT	+1 (×10) +1 (×4) -1 (×9) 0 (×2)	chimeric	
	T1-2- 8A -8B	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×15)	biallelic	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×24)	WT
		GCTCAAGTTCGCGCGGAA ----- GATGGAAGA	-5 (×10)				
	GCTCAAGTTCGCGCGGA ---- GAAGACTAAGA GCTCAAGTTCGCGCG ----- ATGGAAGA	-4 (×9) -9 (×16)	biallelic	TTCTT <u>CC</u> ACTACaAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTAaCAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTA-AGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTGCGAT	+1 (×10) +1 (×2) -1 (×11) 0 (×2)	chimeric	
	T2-1- 8A -8B	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×15)	biallelic	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×20)	heterozygous
		GCTCAAGTTCGCGCGGAA ----- GATGGAAGA	-5 (×10)		TTCTT <u>CC</u> ACTA --- AATAACAAATCCTGTTGAT	-3 (×5)	
	GCTCAAGTTCGCGCGGA ---- GAAGACTAAGA GCTCAAGTTCGCGCG ----- ATGGAAGA	-4 (×15) -9 (×10)	biallelic	TTCTT <u>CC</u> ACTACaAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTAaCAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTA-AGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTGCGAT	+1 (×8) +1 (×3) -1 (×11) 0 (×3)	chimeric	
	T2-2- 8A -8B	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×14)	biallelic	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×25)	WT
GCTCAAGTTCGCGCGGAA ----- GATGGAAGA		-5 (×11)					
GCTCAAGTTCGCGCGGA ---- GAAGACTAAGA GCTCAAGTTCGCGCG ----- ATGGAAGA	-4 (×10) -9 (×15)	biallelic	TTCTT <u>CC</u> ACTACaAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTAaCAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTA-AGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTGCGAT	+1 (×9) +1 (×1) -1 (×12) 0 (×3)	chimeric		
Shoot regeneration	T1-1- 8A -8B	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×25)	homozygous	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×23)	heterozygous
		GCTCAAGTTCGCGCGGAA ----- GATGGAAGA	-5 (×10)		TTCTT <u>CC</u> CAC ----- AACAAATCCTGTTGAT	-8 (×2)	
	GCTCAAGTTCGCGCGGA ---- GAAGACTAAGA GCTCAAGTTCGCGCG ----- ATGGAAGA	-4 (×12) -9 (×13)	biallelic	TTCTT <u>CC</u> ACTAC aAGAATAACAAATCCTGTGCGAT TTCTT <u>CC</u> ACTA - AGAATAACAAATCCTGTGCGAT	+1 (×12) -1 (×13)	biallelic	
T1-2-	GCTCAAGTTCGCGCGGAACA --- GATGGAAGA	-3 (×25)	homozygous	TTCTT <u>CC</u> ACTACAGAATAACAAATCCTGTTGAT	0 (×25)	WT	

	8A -8B	GCTCAAGTCCGCCGGA ---- GAAGACTAAGA GCTCAAGTCCGCCG ----- ATGGAAGA	-4 (×13) -9 (×12)	biallelic	TTCTTCCACTACaAGAATAACAAATCCTGTGCGAT TTCTTCCACTA- AGAATAACAAATCCTGTGCGAT	+1 (×9) -1 (×16)	biallelic
	T1-3- 8A -8B	GCTCAAGTCCGCCGGAACA --- GATGGAAGA GCTCAAGTCCGCCGGAA ---- GATGGAAGA	-3 (×20) -5 (×5)	biallelic	TTCTTCCACTACAGAATAACAAATCCTGTTGAT TTCTTCCACTA t CAGAATAACAAATCCTGTTGAT	0 (×22) +1 (×2)	heterozygous
		GCTCAAGTCCGCCGGA ---- GAAGACTAAGA GCTCAAGTCCGCCG ----- ATGGAAGA	-4 (×12) -9 (×13)	biallelic	TTCTTCCACTACaAGAATAACAAATCCTGTGCGAT TTCTTCCACTA- AGAATAACAAATCCTGTGCGAT	+1 (×9) -1 (×16)	biallelic
<b>Line (#)</b>		<b>gCesA8A/B-3</b> CesA8A-3, GAACTGGTTGTGTTTTCAATAGG CesA8B-3, GAACTGGTTGTGTTTTCAATAGG		<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)	<b>gCesA8A/B-4</b> CesA8A-4, CCACAAGTAGGTCGAGATGTATG CesA8B-4, CCACAAGTAGGTCGAGATGTATG		<b>Mutation types</b> (Number of plasmids containing the cloned PCR products)
<i>ptrcesA8ab-6#</i>	8A -8B	TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT ----- ...	+1 (×18) -31 (×7)	biallelic	TGGATCCACAAaGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×22) 0 (×3)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaA ATAGGCAAG TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×15) +2 (×2) -3 (×8)	chimeric	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
<b>Apical bud</b>	T0-8A -8B	TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT ----- ...	+1 (×14) -31 (×11)	biallelic	TGGATCCACAAaGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×17) 0 (×8)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaA ATAGGCAAG TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×13) +2 (×2) -3 (×10)	chimeric	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
		TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×12) -31 (×13) +1 (×17) +2 (×1) -3 (×7)	biallelic chimeric	TGGATCCACAAaGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×13) 0 (×12) 0 (×25)	heterozygous WT
<b>Axillary bud</b>	T1-1- 8A -8B	TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGT ----- ...	+1 (×12) -31 (×13)	biallelic	TGGATCCACAAaGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×13) 0 (×12)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaA ATAGGCAAG TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×17) +2 (×1) -3 (×7)	chimeric	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
	T1-2- 8A -8B	TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT ----- ...	+1 (×12) -31 (×13)	biallelic	TGGATCCACAAaGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×15) 0 (×10)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaA ATAGGCAAG TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×18) +2 (×1) -3 (×6)	chimeric	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
		TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT ----- ...	+1 (×10) -31 (×15)	biallelic	TGGATCCACAAaGTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×16) 0 (×9)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCaAATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaA ATAGGCAAG TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×20) +2 (×1) -3 (×5)	chimeric	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
	T2-1- 8A -8B	TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT ----- ...	+1 (×15) -31 (×10)	biallelic	TGGATCCACAA a GTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×16) 0 (×9)	heterozygous

		TGTGGGAAGTGGTTGTGTTTTCa AATAGGCAAG TGTGGGAAGTGGTTGTGTTTTCaaAATAGGCAA G TGTGGGAAGTGGTTGTGTA --- AATAGGCAAG	+1 (×17) +2 (×1) -3 (×7)	chimeric	TGGATCCACAAGTAGGTCGAGATGTATGCTATG	0 (×25)	WT
Shoot regeneration	T1-1- 8A -8B	TGTGGGAAGTGGTTGTGTTTTCt AATAGGCAAG TGTGGGAAGTGGT -----...	+1 (×11) -31 (×14)	biallelic	TGGATCCACAA a GTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×15) 0 (×10)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCa AATAGGCAAG	+1 (×25)	homozygous	TGGATCCACAAGTAGGTCGAGATGTATGCTATG TGGATCCACAA c GTAGGTCGAGATGTATGCTATG	0 (×23) +1 (×2)	heterozygous
	T1-2- 8A -8B	TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT -----...	+1 (×20) -31 (×5)	biallelic	TGGATCCACAA a GTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×16) 0 (×9)	heterozygous
		TGTGGGAAGTGGTTGTGTA --- AATAGGCAAGC ..... AATAGGCAAGC	-3 (×3) -689 (×22)	biallelic	TGGATCCACAAGTAGGTCGAGATGTATGCTATG TGGATCCACAAA -----...	0 (×3) -689 (×22)	heterozygous
	T1-3- 8A -8B	TGTGGGAAGTGGTTGTGTTTTC t AATAGGCAAG TGTGGGAAGTGGT -----...	+1 (×9) -31 (×16)	biallelic	TGGATCCACAA a GTAGGTCGAGATGTATGCTATG TGGATCCACAAGTAGGTCGAGATGTATGCTATG	+1 (×9) 0 (×16)	heterozygous
		TGTGGGAAGTGGTTGTGTTTTCa AATAGGCAAG ..... AATAGGCAAGC	+1 (×2) -689 (×23)	biallelic	TGGATCCACAAGTAGGTCGAGATGTATGCTATG TGGATCCACAAA -----...	0 (×2) -689 (×23)	heterozygous



**Table S8** Inheritance of the Cas9/gRNA-induced mutations in the progeny of the *ptrcesa* lines.

<i>ptrcesa</i> line#	Genotype	Apical bud	Axillary bud				Shoot regeneration		
		T0	T1-1	T1-2	T2-1	T2-2	T1-1	T1-2	T1-3
<i>ptrcesa4-2#</i>	heterozygous/biallelic	N/N	N/N	N/N	N/N	N/N	Y/N	N/Y	N/N
<i>ptrcesa4-5#</i>	biallelic/WT	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
<i>ptrcesa7ab-5#-7A</i>	WT/biallelic	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
<i>-5#-7B</i>	WT/biallelic	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
<i>ptrcesa7ab-6#-7A</i>	WT/chimeric	N/N	N/N	N/N	N/N	N/N	Y/Y	N/Y	N/Y
<i>-6#-7B</i>	WT/chimeric	N/N	N/N	N/N	N/N	N/N	N/Y	N/Y	N/Y
<i>ptrcesa8ab-3#-8A</i>	biallelic/WT	N/N	N/N	N/N	N/Y	N/N	Y/Y	Y/N	N/Y
<i>-3#-8B</i>	biallelic/chimeric	N/N	N/N	N/N	N/N	N/N	N/Y	N/Y	N/Y
<i>ptrcesa8ab-6#-8A</i>	biallelic/heterozygous	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
<i>-6#-8B</i>	chimeric/WT	N/N	N/N	N/N	N/N	N/N	Y/Y	Y/Y	Y/Y

Biallelic, two alleles of the gene were edited and the different mutations were induced.  
Chimeric, more than three different allelic mutations in a target editing site of the gene.