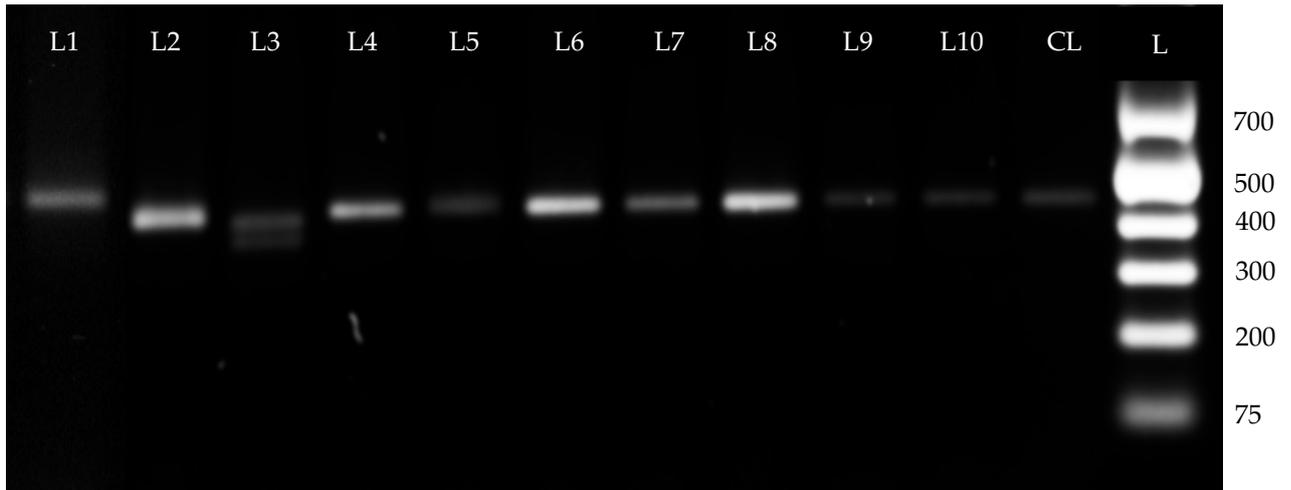


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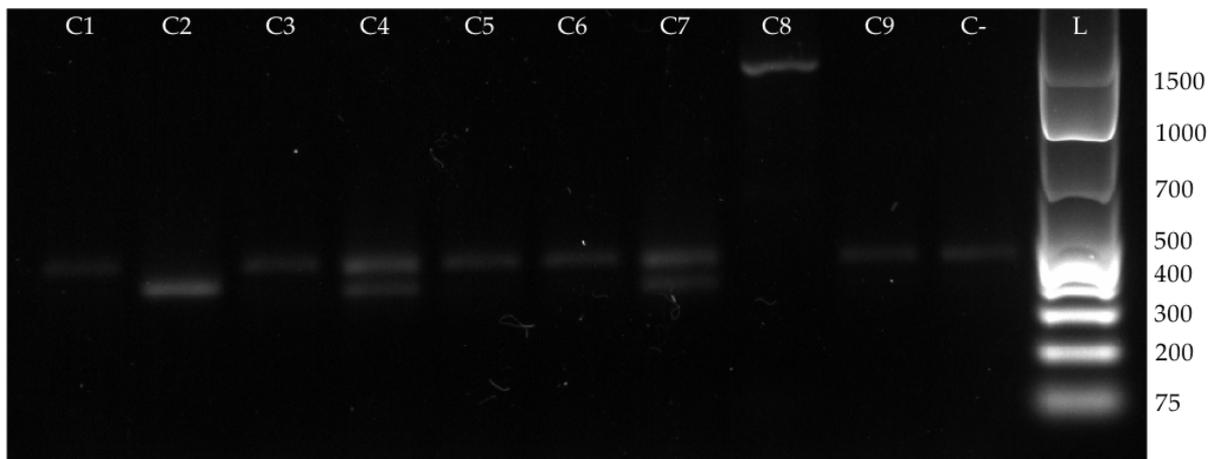
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 taaaggtaagttttaagtatataaaagtaggttgttataattgttaattggttaatgatt
 tttggattttgttttttaggattatgagttgcttgcacgtaagggagtggttgctga | E10
 ggcaagccttgtg**taa**gttaataattaatggatgaattttaaggagctcttacaagttca

Supplementary Figure S1. *CiPDS* DNA sequence, sgRNA target sites and the primer sites for PCR amplification of the fifth exon. The exons are depicted in red and introns in dark. Blue nucleotides indicate the target sites, bold red nucleotides indicate the PAM sequences. Nucleotides highlighted in blue indicate start and stop codons, respectively.



Supplementary Figure S2: DNA amplification of albino plant regenerated from hairy root lines by PCR with specific primer pair (E5-F and E5-R) surrounding the two target sites in the fifth exon of the *CiPDS*. L = Line, CL = Control Line (DNA from plant transformed with the wild type *A.rhizogenes* strain 15834), L = Ladder.



Supplementary Figure S3: Amplification by PCR of DNA extracted from albino plants regenerated from calli with specific primer pair (E5-F and E5-R) surrounding the two target sites in the fifth exon of the *CiPDS*. C = Callus, C- = DNA from plant regenerated from non-mutated callus, L = Ladder.

WT-KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGGQAYVEAQDGL...

L1.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**NVA***

L1.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

L2.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**RRGLKK***

L2.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LVA***

L3.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

L3.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LTGLC***

L4.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**VTGLC***

L4.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**HVA***

L5.1 (R) - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGGQAYVEAQDGL...

L5.2 (R) - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGG**TGLC***

L5.1 (P) - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**VTGLC***

L5.2 (P) - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**TWPEKVKFAIGLLPAMLGVDRLMLRPKMV***

L6.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM-TWPEKVKFAIGLLPAMLG**VTGLC***

L6.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

L7.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**RRGLKK***

L7.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LVA***

L8.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**DRMLMLRPKMV***

L8.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**ETGLC***

L9.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**RTGLC***

L9.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

L10.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

L10.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

Supplementary Figure S4: Predicted amino acid sequences of the CiPDS from the 10 albino hairy root lines and their shoots. Lx.x = Line x. allele x. For L5 (R) = Hairy root stage sequence, (P) = Plant stage sequence. In blue, the amino acids that differ from the wild type sequences. (-) shows the deleted amino acids and (*) shows the end of the amino acid sequence.

WT- KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGGQAYVEAQDGL...

C1.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**VTGLC***

C1.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**VTGLC***

C2.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**TGLC***

C2.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**TGLC***

C3.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

C3.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LYVA***

C4.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNN**DVA***

C4.2 - KPGEFSRFDFFPDVLPAPLNGIFAILR**NK** ----- -AYVEAQDGL...

C5.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**TGLC***

C5.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLG**ETGLC***

C6.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

C6.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGG**TGLC***

C7.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**RGLKK***

C7.2 - KPGEFSRFDFFPDVLPAPLNGIFAILR**NK** ----- -AYVEAQDGL...

C8.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

C8.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEM**LRGLKK***

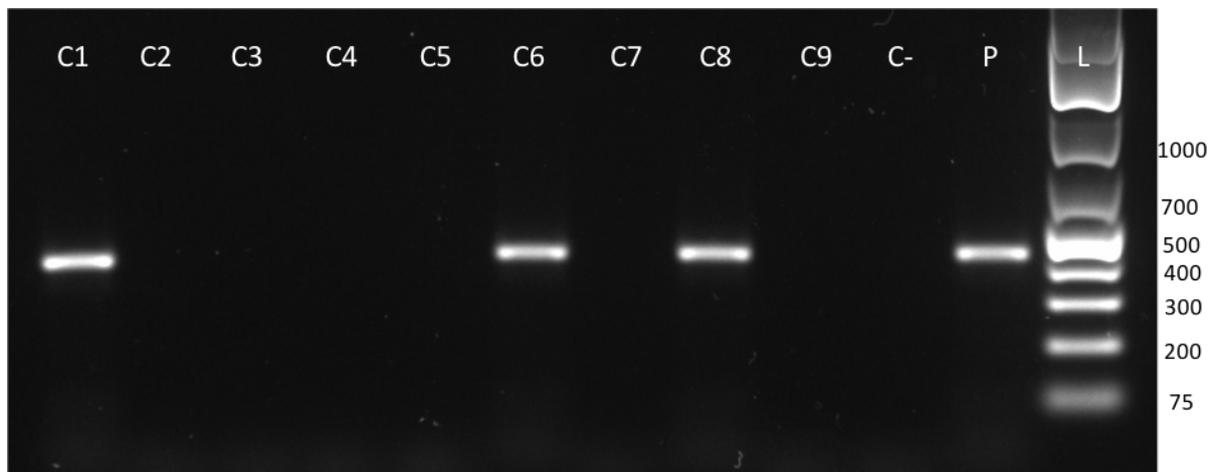
C9.1 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGG**TGLC***

C9.2 - KPGEFSRFDFFPDVLPAPLNGIFAILRNNEMLTWPEKVKFAIGLLPAMLGG**TGLC***

Supplementary Figure S5: Predicted amino acid sequence of the CiPDS from the 9 albino calli and their shoots. Cx.x = Callus x. allele x. In blue, the amino acids that differ from the wild type sequences, (-) shows the deleted amino acids and (*) shows the end of the amino acid sequence.

(TGAGTAGTCCAGATAAGGGAATTAGGGTTCTTATAGGGTTTCGCTCATGTGTTGAGCATATAAGAAACCTTAGTATGTATT
 TGTATTTGAAAATACTTCTATCAATAAAATTTCTAATTCCTAAAAACAAAATCCAGTGACGCGGCCGCATAAGGCCGCCATCTC
 GATGACGATGTTCTCAGGCTGTGACGACCCGTAAGCGGCTGGGTTGCTGCCGGCCCTGCAATGGCACTGGAACCCCAAGC
 CCGAGGAATCGGCGTGAGCGGTGCAAACCATCCGGCCCGGTACAAATCGGCGCGGCGCTGGGTGATGACCTGGTGGAGAA
 GTTGAAGGCCGCGCAGGCCGCCAGCGGCAACGCATCGAGGCAGAAGCACGCCCGGTGAATCGTGGCAAGCGGCCGCTGA
 TCGAATTGCGCAACCGGAACCCGTACATTGGGAA(nnn...nnn)TTCCAATGTACGTGCTATCCACAGGAAAGAGAAGTTTCG
 ACCTTTTTCCCTGCTAGGGCAATTTGCCCTAGCATCTGCTCCGTACATTAGGAACCGGCGGATGCTTCGCCCTCGATCAGGTTG
 CGGTAGCGCATGACTAGGATCGGGCCAGC)

Supplementary Figure S6: Partial sequence of the DNA fragments inserted at the target 2 site of the C8 mutant. The insert is composed of more than 6 fragments (depicted in red, black, green, purple or orange) of the pYLCRISPR-sgRNA1-sgRNA2. (nnn...nnn) is a part of the fragment, which was not sequenced.



Supplementary Figure S7: DNA amplification of albino plants regenerated from calli by PCR with specific primer pair (C9-F and C9-R) hybridizing in Cas9 gene. C = Callus, C- = DNA from plant regenerated from non-mutated callus, P = Plasmid pYLCRISPR-sgRNA1-sgRNA2 DNA, L = Ladder.

GGAATTCGGCTGGTCTCTCTCGAAAGAACCAACCTGTTTTCATAGCTCTGTTGACTGATAGAGTTTTGATA
 TGTTCAACTTTTCAGGTAAAACCAAGAGGATAGTAAAATTGAGGGTTAGCTTACAAATATTTATCGGTTCCCTA
 GCTATCGTAATGCCATGGCTTCCACATCGCTCATTGGAGCAACATCGCCATGCTTTATATAGCCTCGCCT
 CCAACCATTTATCGATGTCTTCgAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCG
 GCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAAGAAACAGCTATGACCATGATTACGC
 CAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCAAGCTCAATGGGTCTAGTCTGTAGATA
 CCATCACACTGGCGACCGCTCGAACATCAGTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTC
 TGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGTATCCCCCTGGCCAGTG
 CACGTCTGCTGTCAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGCATATCGGGGATGGAAGCTGGC
 GCATGATGACCACCGATATGGCCAGTGTGCCTGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCC
 ACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCCTGAATGG
 CGAATGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGCGGGTGAGCGTgGAAGACTAGTTTTAGAGCTA
 GAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCCGAGTCGGTGCCTTTTTT
 TGAGACCGACCGGATCCC

Supplementary Figure S8: Sequence of the cassette “CiU6-PlacZ ccdB-sgRNAscaffold” harboring by the plasmid pKanCiU6-1p-sgRNAscaffold. The CiU6-1p, the selectable marker PlacZ-*ccdB* and the sgRNA scaffold are written in blue, yellow and green respectively. In red is highlighted the *BbsI* recognition site which achieves a cleavage on the left side (shown in underlined and bold). Note that the G present in the 4 nucleotides of the cutting site, is the transcription initiator. Highlighted in blue is the *BbsI* recognition site which achieves a cleavage on the right side (shown in underlined and bold).

