

Primer name	Primer sequence (5'→3')
The primer used in two-sgRNA vector construction	
cpa-1sf	ATATATGGTCTCGATTGCAAGACTCAGAGGGATACGGTT
cpa-1f0	TGCAAGACTCAGAGGGATACGGTTTTAGAGCTAGAAATAGC
cpa-2r0	AACCCAAGACTCAGAGGGATACCAATCTCTTAGTCGACTCTAC
cpa-2sr	ATTATTGGTCTCGAAACCCAAGACTCAGAGGGATACCAA
The primer used for colony PCR and sequencing	
U626-IDF	TGTCCCAGGATTAGAATGATTAGGC
U629-IDR	AGCCCTCTTCTTTCGATCCATCAAC
Primers used in the present study for positive transgenic detection.	
CPA TOP	TCACTGAAGATGAAGATGGGAGTGGAG
CPA BOTIM	CGACGATCCCACTTCGCAATCTC
Cas9-F888	ATGGCTCCGAAGAAGAAGAGGAAG
Cas9-R888	GGCCAGGAGTTATCCAGGTCA
Primers used in the present study for transgenic mutation detection	
CPA TOP	TCACTGAAGATGAAGATGGGAGTGGAG
CPA BOTIM	CGACGATCCCACTTCGCAATCTC
Primers used in the present study for transgenic mutation detection based on SNP	
13550 F CMT	CTTACACTAGACGCTGAGAGAGATCTTGAT
13550 R CMT	CCCACACGTCTTCACCACTCAAG
Primers used in the present study for off-target Detection in BnaPPR	
ZUN21-F	GGAGTGAGTACGGTGTGCGAAATAACTATCATTCTTACG
ZUN21-R	GAGTTGGATGCTGGATGGCGGAGTCAAACCGGATTC
ZUN22-F	GGAGTGAGTACGGTGTGCAAACAATTATCATTCTTACG
ZUN22-R	GAGTTGGATGCTGGATGGGGTTTCAATTACGCGAGTC
ZUN23-F	GGAGTGAGTACGGTGTGCCAATCCATACTTTCTCTTG
ZUN23-R	GAGTTGGATGCTGGATGGCGAGAGAGACACAAGGTAG
ZUN24-F	GGAGTGAGTACGGTGTGCCACTACATATGCATATTCTAC
ZUN24-R	GAGTTGGATGCTGGATGGCATAAGTGAGAACAACCTTTG
ZUN25-F	GGAGTGAGTACGGTGTGCAAGAGTTCCCAACTATCATAT
ZUN25-R	GAGTTGGATGCTGGATGGAATATACGTTGTATCATACAA
Primers used in the present study positive detection of Overexpression plants	
35sf left	CATTTTCATTGGAGAGGACGTCGA
35 cpaRig.OE	CTCCACAGCTTCAGAGACTCGAC
Primers used in the present study for qRT-PCR	
cpaL.OE	GATTGGTACGTGAGCCTCGTATCC
cpaR.OE	CGTGTAGAGGTCATGCGCAATC
The primer used in cytoplasm identification	
MSS2-L	CTGAAGTTGAAAAAGGGAATGT
MSS2-R	TTGATCAAGACGAGGAGTAGCT

Primer name	Primer sequence (5'→3')
MSS4-L	AGTATTTTCGTTACCTTGGC
MSS4-R	GCTTGGTGGAAAAGATCGTA
MSS13-L	TAGCGCTATCTTTCGGCCCT
MSS13-R	ACCCGAAGGTCCTGGTCTCT
MSS14-L	GCTCGTTCGATTAAGCTCAA
MSS14-R	GAATTCCTCTTTCATTGCGG
The primer used in nap-CMS qRT-PCR	
nad5	GACGATCGATTATCTACCCA CTATCATTATGCCTTTGCAA
atp6	ACCAAGGATTGCCAAGCATT ATCCATCTTTGTTTCATGCTGC
Orf222	ATGATGGTGACCACATCTGCT GCAATCGTCCCTACCGTCGTA
Orf139	ATGAAAGAATCATTTTATGCT TATCGAAGTCCTCTCCTTCA
Ap3	TACCACCATCTGAAATGCGCG CATTTGCTGAGATACTGGATG

Table S1. The primer used in all studies.