Fine Mapping and Candidate Gene Validation of Tomato Gene *Carpelloid Stamen and Parthenocarpy* (*csp*)

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supplementary Figures and Tables

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Figure S1. The sequence of the *copia* LTR retrotransposon in the *csp* mutant. The red letters indicate *TAP3* gene sequences.

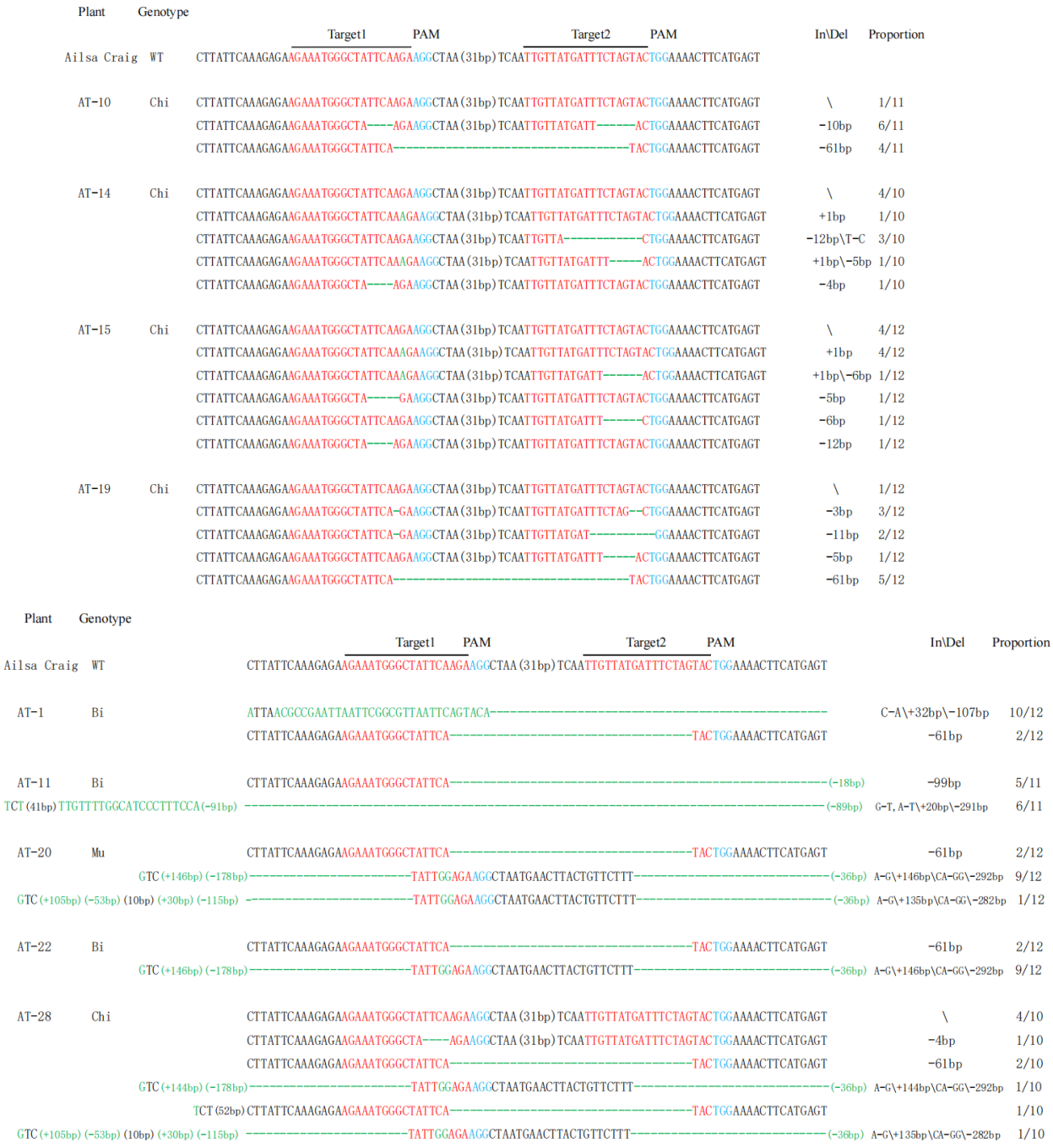

Figure S2. Sequence variations of the *TAP3* gene-edited T0 generation plants. *tap3*^{cr} mutant alleles identified from three T0 plants. WT represents the wild type, Ho represents the homozygous mutation, Mu represents the multi-allele mutation, and He represents the heterozygous mutation. The red letters represent the target sequence of the *TAP3* gene, the blue letters indicate the protospacer-adjacent motif (PAM), the green letter indicates the insertion sequence, and the green hyphens mark the deletion sequences. The number on the right indicates the number of bases inserted or deleted.

Figure S3. An allelism test between the *csp* mutant and *TAP3* gene-edited mutant *tap3*^{cr4}. (a) Agarose gel electrophoresis of PCR fragments amplified from *csp*, *tap3*^{cr4} and the individualities of their allelism test population. (b) *csp*/*tap3*^{cr4} flower; (c) *csp*/*tap3*^{cr4} flower; the white bar is 1 cm. (d) *csp*/*tap3*^{cr4} anther cone; (e) *csp*/*tap3*^{cr4} anther cone; (f) *csp*/*tap3*^{cr4} stamens; (g) *csp*/*tap3*^{cr4} stamens; the white bar is 0.5 cm. (h) *csp*/*tap3*^{cr4} pollen; (i) *csp*/*tap3*^{cr4} pollen; the red bar is 50 μm. (j) *csp*/*tap3*^{cr4} fruit ; (k) *csp*/*tap3*^{cr4} fruit; the white bar is 1 cm.

Table S1 General information of InDel markers for mapping *CSP*

Primer Name	Position (SL4.0ch04)	Forward Primer (5'→3')	Reverse Primer (5'→3')
HP847	61,157,566	GAGTTGGAGGAACGTGTGTC	CCTCATGTGTTACCATTTCC
HP613	61,656,951	AACAAATCTTCTCTTCAGCCA	CCTTTCTGAGGCACATTAGG
HP615	61,797,919	GCCAGCCTTAAATATTCCAC	CAGAAAGGCAACAATGAAGAG
HP617	62,480,644	TGTTTTAGTTGCTCATAGGATG	CCTACCAACACCACACAATC
HP619	62,948,718	GAAATTCTGCTCCCAAATGC	CTTGAAAAGTATAATCCAGGG
LP3	63,000,047	AGAGAGATTTGCACTCAGTTAGAC	CGGCCTTAAGCAGAGACGGATTC
LP5	63,025,077	CCTGCCTGCATGTACCTCCAGC	AGCCTGCATGAATCCTCGTTAGC
LP6	63,030,842	GCAATGTACTCATAGTCCTGACA	AGGGATACAAGAGCGGAGTTAC
LP8	63,065,003	GGCTAAACTTACTGAGCTTCCTCG	ACTGACTCGAACCAACGTAATCA
LP9	63,097,942	GTCCAAACACACATTTACGACGTC	CCTCTTAATTTCGTGTCCAATTGC
HP621	63,346,786	AGCCCTATTATCTGTAATGC	ACTAAGCTCTTCCTATGTGC
HP3423	63,715,686	CACCTAACTTCGACCCTGCT	GCATTGATGTCCTGGTAAAGAG
HP4453	64,026,342	GCCATGGCCATCCATTCTAC	GTTTTCCCCATTATGACATTTC

Table S2 General information of primers for amplification and sequencing of *TAP3* gene

Primer Name	Forward Primer (5'→3')	Reverse Primer (5'→3')
LS1	GCCACATGGTTGAAGTACATTCC	CTCATGAAGTTTTCCAGTACTAG
LS2	AGTTAACTTGACCTTCTAGGGT	AGCTTCCTTAGCTGCTCTTGC
LS3	ATCCAAGATCTCGATCGATCC	TCGTCTTTGTGAGCTTGTTCAC
LS4	TGTCGAGTGAAATCTAAGAAGGT	AGGTATTAACAAGCTTAAGCACT
LS5	AGGTGATTGGCAATCAGATTGA	ATCATGTATTGTTGGGGCAGAG
LS6	GTGCAAGATACATATTGTGTGGC	AGGTTAGGTACGAATTCGATCG

Table S3 The primers for sequencing the *copia* LTR retrotransposon in the *csp* mutant

Primer name	Primer sequence (5'→3')
M13F	GTAAAACGACGGCCAGT
7B-2	GTAGCTGAGCGGATGAATCAAACA
7B-3	GAGATGGAGTCCCTACACAA
7B-4	ACATCTCCATAGGTGTCTTGC
7B-5	CCTCGTTCAATGTGCAGAAGCTACC
7B-6	GTTTGATGTATGCTATGGTCTGTAC
7B-7	CTTGCTCAACATCAGAAGCTGTTAA
7B-8	AGCAGCAGTCTCTTCATCCGAGAC
M13R	CAGGAAACAGCTATGAC
csp-LTR-F	GCTGTTGGGTGACAAGTGACTG
csp-LTR-R	TCTAACCGTAAAGGGCATGTTGA
csp-LTR-R1	GGTCCGATTCAAGGCATTCAA

Table S4 The primers used in Real-Time PCR

Primer name	Forward primer (5'→3')	Reverse primer (5'→3')
TM6-Q	CTCCATTCGCAAATGCCACAGC	GAAGGCGAGGAACTTAGAAGAGCAA
TAP3-Q	TATAAGTCCCTCAATCACGACCA	GATCATTTAGGCTTTCTCCCATC
TPI-Q	TCTGGGAGGAGACTATGGGATG	TCAGACTGCTTGGCACTGATACTA
SlGLO1-Q	GCTTACTGGAAGAAGATTGTGGG	CTCATTCTGTTTTTCACGGATACC
MC-Q	AAGTAGCAGAAGCAAGGAGGA	CAAGCGATTAGCAAAGAGTGA
TAG1-Q	ATGAACTTGATGCCAGGGAGT	GGGGTTGGTCTTGTCTAGGGTA
TM5-Q	CTTTGTGATGCTGAGGTTGCTC	TTTCCAGTGCTTCTCGTGTTG
TAGL2-Q	CAGCAGCAACATCCTCAATCTC	CACAGCATCCAACCAGGTATCA
Asparatic protease-Q	GTGATATTAATTGGCTTCAATGTGAACC	ATACTCGCCGGAACCTGTAACATC
Sister chromatid cohesion-Q	AGTGAGATCATGAGAATTACAGCTCC	GATGAAGTTTGACAGCACTTTCTTG
SlTTS	AAGCCACCATCACCTTAT	TCAGCCTGTTCAACTAATG
TAGL11	TCTACTGAGGAGGAAGGAA	AAGTTGAGATGTCCAGAGTAT
SlACTIN	GGGATGGAGAAGTTTGGTGGTGG	CTTCGACCAAGGGATGGTGTAGC

Table S5 The primers for CRISPR/Cas9 gene editing

Experiment	Primer name	Primer sequence (5'→3')
Detection of target sequences of genetically modified materials	CX-F	GTGACAAGTGACTGATAACTGAG
	CX-R	CCAATAGTCTTCTGGTACAGATCG
gRNA amplify	CSP-F	TTCATCTCGGGTCTCGTTTGAGAAATGGGC TATTCAAGAGTTTTAGAGCTAGAAATAG
	CSP-R	TTCATCTCGGGTCTCTAAACGTACTAGAAAT CATAACAATGACCGTTGATAGTGGATAG
Positive clone detection and sequencing	MGET-F1	GTCGTCACACAACTGGGCTTC
	MGET-R1	AACTTGAGACAGTCATTTCGGA
	MGET-S1	ATCTTAATCTCTTCTGGTGGCT
T-DNA detection	Cas9-909F	GCAGCTCTCCAAGGACACAT
	Cas9-2450R	CGTGAGTTCTTCTGGCCCTT
Target mutation detection	M13F	GTTGTAAAACGACGGCCAG
	M13R	CAGGAAACAGCTATGAC

Table S6 PCR amplification system

Component	Volume (μL)
Template DNA: pCBC-S1	2
2×Phanta Max Master Mix (Dye Plus)	25
CSP-F (10 μmol/L)	1
CSP-R (10 μmol/L)	1
ddH_2O	21

Table S7 Golden Gate reactions system

Component	Volume (μL)	Reaction conditions
CRISPR Target DNA (0.5-50 umol/L)	2	
pMGET-GFP (~ 100 ng/μL)	2	5 hours at 37℃;
10 ×T4 DNA Ligase Buffer (NEB)	1.5	5 min at 50℃;
10 ×BSA	1.5	10 min at 80℃;
BsaI (NEB)	1	Note: It is essential to use a High
Hi-T4™ DNA Ligase (HC, NEB)	5	Concentration (HC) Ligase
ddH_2O	2	(2 million units/ml, NEB).
Total volume	15	