

Supplementary Information:

**CRISPR/Cas9 mediated mutagenesis of *MORE AXILLARY GROWTH 1* in tomato confers resistance to root parasitic weed *Phelipanche aegyptiaca***

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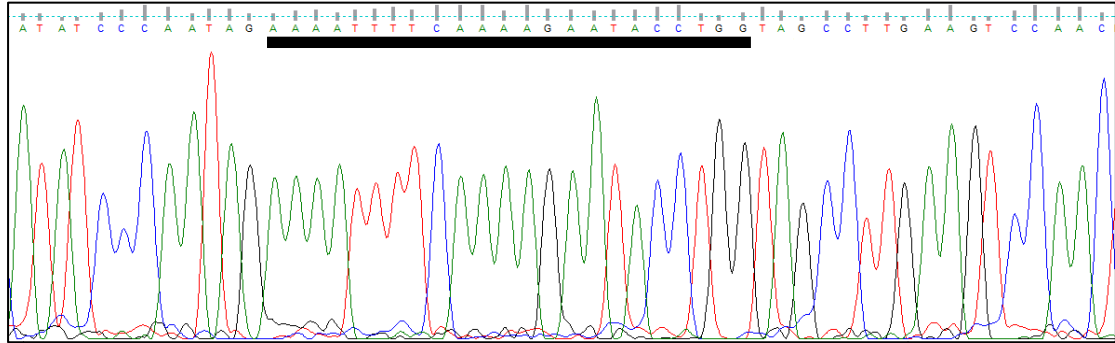
Radi Aly: [radi@volcani.agri.gov.il](mailto:radi@volcani.agri.gov.il)

**MAX1-sgRNA target**

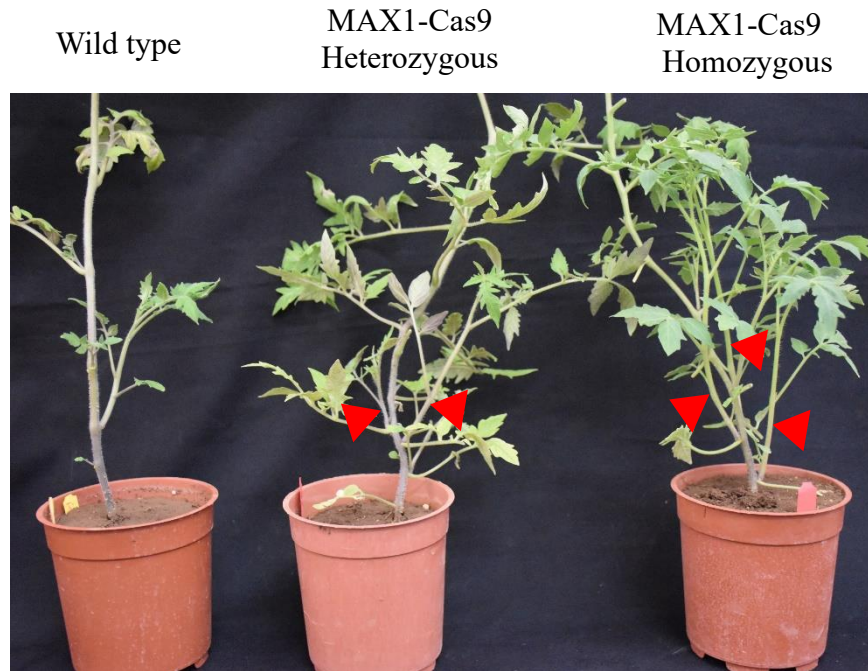
CAAGTTCTCAAAGAATCCCGGG

**Off target** (4 mismatch)

AAAATTTTTCAAAGAATACCTGG



**Fig. S1.** PCR product DNA sequencing chromatogram of potential off-target of the MAX1-sgRNA. No mutation at off-target site detected in the genome of MAX1-Cas9 edited homozygous lines. Region highlighted in black bar correspond to off target sites.



**Fig. S2.** Characteristic axillary bud outgrowth phenotype of 1-month-old MAX1-Cas9 heterozygous and homozygous T<sub>1</sub> plants as compare to wild type tomato plants grown in green house.

**Table S1.** Segregation pattern of MAX1-Cas9 mutation from T<sub>0</sub> to the T<sub>1</sub> generation. The zygosity of heterozygote in T<sub>0</sub> plant lines was putative. +, Foreign DNA (nptII) was detected; -, Foreign DNA (nptII) was not detected.

MAX1-Cas9 Line	T <sub>0</sub> generation		T <sub>1</sub> generation		
	Mutation type	Zygosity	Mutation type/ total test	Mutation exists no./ total test (%)	Foreign DNA (nptII) segregation (%)
Line 1	-9nt	Heterozygous	homozygous (5/24), heterozygous (12/24), wt (7/24)	17/24 (70.8%)	9+; 5- (35%)

**Table S2.** Mutational analysis of off-target sites predicted to associated with MAX1-sgRNA edited lines. To analyse the off target using CRISPR-P, high score value was considered as top hit. The PAM motif (NGG) is marked by blue; mismatching bases are shown in red colour.

<b>Name of putative off -target sites &amp; score</b>	<b>Putative off-target locus</b>	<b>Sequence of the Putative off-target site</b>	<b>Number of mismatching bases</b>	<b>Presence of mutation detected</b>
OFF1 (0.394)	SL2.50ch09:+61329926 Solyc09g064200.2 (intron)	AAAATTTTCAAAAAGAATACCTGG	4	0
OFF2 (0.331)	SL2.50ch01:+47873454 Intergenic	AAAGTTCTCTAAAAAATCCATGG	4	0
OFF3 (0.321)	SL2.50ch08:-36699521 Intergenic	AAAGTTATCAAAAAGAATCACAGG	3	0
OFF4 (0.318)	SL2.50ch04:-54647171 Intergenic	AAAATTGTCAAAA AATCCCTGG	4	0

**Table S3.** List of primers used in this study

Oligo's name	Sequence (5' → 3')	Use
U6R-HindIII	TAAGCTAAGCTTCGATCTAAAAAAGCACCGACT	MAX1 Exon-3 targeting sgRNA cloning in Cas9 expression vector
MAX1sgRNA-F	AGAGTCGACATAGCGATTCAAGTTCTCAAAAGAA TCCCGTTTTAGAGCTAGAAATAGCAAG	
RCSMAX1-DG-F	CAAGTTCTCAAAAGAATCCC	Diagnostic PCR and sequencing
sgRNA-SEQ-F	GGACACTGACGGCTTTATG	
sgRNA-SEQ-R	GACGAACGGATAAACCTTTTC	
Npt-II-F	TCTTGTCGATCAGGATGATC	Transgene validation
Npt-II-R	AGAAGAACTCGTCAAGAAG	
pcoCas9-F	GATTGGCTTGATGAACTTGTAG	
pcoCas9-R	ATGATGATCTTGATAACCTTCTTG	
TmMAX1-Int-F	GAGGGAGATCTCACATTCTCTG	Mutation detection and sequencing
TmMAX1-Int-R	CCAGCAAGTGCTCATAAGTTAC	
TmMAX1-Nest-F	GGTGTGGATTTTGGACTTTC	
SICCD8-qF	CCAATTGCCTGTAATAGTTCC	Real time PCR
SICCD8-qR	GCCTTCAACGACGAGTTCTC	
ABCG45-qF	TGATTATCCAAGACCAGAGCTG	
ABCG45-qR	GCACCAGCAATAACAATCTCC	
SIPDS1-qF	TGATTATCCAAGACCAGAGCTG	
SIPDS1-qR	GCACCAGCAATAACAATCTCC	
SIEF $\alpha$ 1-qF	GATTGGTGGTATTGGAAGTGC	
SIEF $\alpha$ 1-qR	AGCTTCGTGGTGCATCTC	
SIMAX1-qF	GCAAGGGAGTCAGAGAAGTTAG	
SIMAX1-qR	GATGGCAAGCAACCAGATAGA	
OFF9-F	CTCCTGAAGTTTTAGATGGAAG	Off target PCR and sequencing analysis
OFF9-R	AGGCTATGTTTCAGAAAGCTAAC	
OFF1-F	TATTTAAGGTAAGGTAAG	
OFF1-R	ATTCACCATAACTGAG	