Article

TSA Promotes CRISPR/Cas9 Editing Efficiency and Expression of Cell Division-Related Genes from Plant Protoplasts

Seung Hee Choi[1], Myoung Hui Lee[1], Da Mon Jin[1,2], Su Ji Ju[1,3], Woo Seok Ahn[1,2], Eun Yee Jie[1], Ji Min Lee[1], Jiyoung Lee[1], Cha Young Kim[1] and Suk Weon Kim[1,*]

[1] Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsingil, Jeongeup-si, Jeollabuk-do, 580-185, Korea; csh@kribb.re.kr (S.H.C.); mhlee17@kribb.re.kr (M.H.L.); damon@kribb.re.kr (D.M.J.); dntjr0412@kribb.re.kr (W.S.A.); jeannie@kribb.re.kr (E.Y.J.); jimin1215@kribb.re.kr (J.M.L); jiyoung1@kribb.re.kr (J.L); kimcy@kribb.re.kr (C.Y.K)

[2] Department of Bioenergy Science and Technology, Chonnam National University, Gwangju 500-757, Korea

[3] Department of Applied Plant Science, Chonnam National University, Gwangju 500-757, Korea; suji@kribb.re.kr (S.J.J.)

* Correspondence: kimsw@kribb.re.kr; Tel.: +82-63-570-5650; Fax: +82-63-570-5609

Figure S1. The changes in gene editing efficiency according to TSA treatment in tobacco. (**A**) The schematic illustration of *NbPDS* CRISPR/Cas9 target site. The target region is shown in green letters followed by PAM (NGG; red). (**B**) Relative efficiency of indel frequency (%) of at target site in protoplasts examined at 48hr after transfection of Cas9 and gRNAs as RNP complexes with DMSO or TSA treatment. The indel frequency of the DMSO treatment group was set to 100%, and those of the TSA treatment groups were shown relatively. Bars represent means ± SE (n = 3) of independent experiments. Different letters on the bars indicate significant differences between each treatment (ANOVA with the Duncan's test, $p < 0.05$).

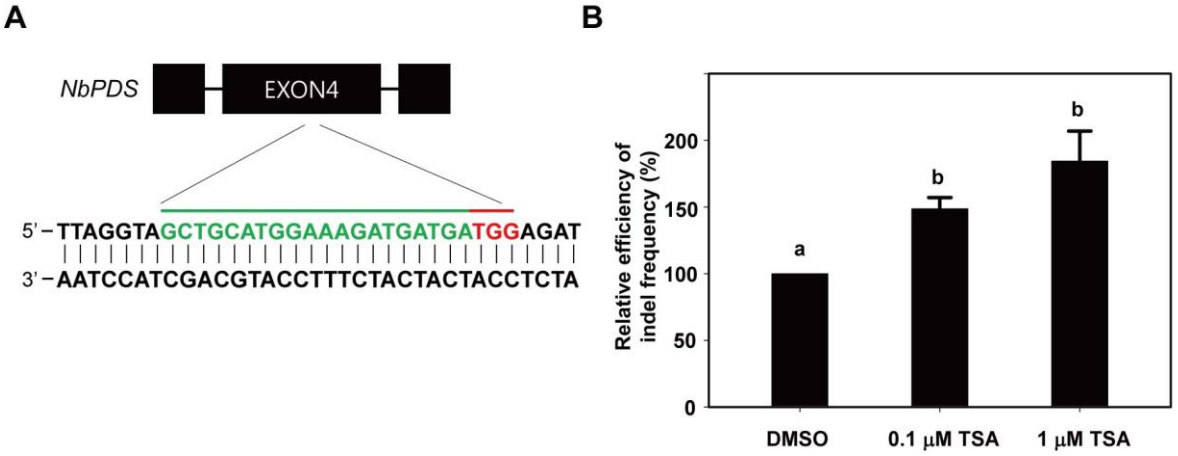

Table S1. Mutation rates based on deep sequencing of *NbPDS* target region.

Plant species	#	Concentration of TSA (uM)	Total reads	WT	Insertions	Deletions	In-del frequency (%)
N. benthamiana	1ST	0 (DMSO)	63,162	62,562	259	341	600 (0.9%)
		0.1	49,324	48,678	247	399	646 (1.3%)
		1	43,538	42,676	459	403	862 (2.0%)
	2nd	0 (DMSO)	61,563	59,689	515	1,359	1,874 (3%)
		0.1	37,193	35,667	565	961	1,526 (4.1%)
		1	50,570	47,754	792	2,024	2,816 (5.6%)
	3rd	0 (DMSO)	23,498	22,239	573	686	1,259 (5.4%)
		0.1	23,710	21,604	879	1,227	2,106 (8.9%)
		1	38,027	35,067	1,454	1,506	2,960 (7.8%)

Table S2. The primers that were used in this study

Primer name	Target	Sequence (5'→3')	No. bases
LsSOC1 1ST PCR F	*LsSOC1*	TAGTCCACACACTCCATCGC	20
LsSOC1 1ST PCR R	*LsSOC1*	AGGAAAAGAACCCACAAACCAG	22
LsSOC1 adapter PCR F	*LsSOC1*	AAGAAGACTTGACATTTGATTGGT	24
LsSOC1 adapter PCR R	*LsSOC1*	TGGAGAAGGTCACTTGTCTACTTG	24
LsSOC1 gRNA F	*LsSOC1*	taatacgactcactatagGAGGGAAGACTCAAAT	34
LsSOC1 gRNA R	*LsSOC1*	ttctagctctaaaacCCGCATTTGAGTCTTCCCT	34
LsSOC1 genomic PCR F	*LsSOC1*	TGTAACTTGAACATGCGACCATAC	24
LsSOC1 genomic PCR R	*LsSOC1*	CATTATAAGGAAAAGAACCCACAAAC	26
LsCyclinD1-1 qF	*LsCyclinD1-1*	TCTCGAACCAACGAACTCCA	20
LsCyclinD1-1 qR	*LsCyclinD1-1*	CTCTCCGGTACATCCACTCC	20
LsCyclinD3-2 qF	*LsCyclinD3-2*	ACCCATTACGGATTCGTTGC	20
LsCyclinD3-2 qR	*LsCyclinD3-2*	TTGCAGGTCGAGGAGAAGAG	20
LsCyclinD6-1 qF	*LsCyclinD6-1*	CGGGAAGCGTTGACAGTTAT	20
LsCyclinD6-1 qR	*LsCyclinD6-1*	GCGAGAGAAAGGCACGAAAT	20
LsCyclinU4-1 qF	*LsCyclinU4-1*	TCAAGTACGCCAATTGTAGCC	21
LsCyclinU4-1 qR	*LsCyclinU4-1*	CATCCATGAACTTGGCAGCA	20
LsKPR3 qF	*LsKPR3*	CCGTCATGACCAGGAACAAC	20
LsKPR3 qR	*LsKPR3*	AGCGGAATTACACCAATGCC	20
NbPDS 1ST PCR F	*NbPDS*	CCTGTTGGTTGCATTTCTCA	20
NbPDS 1ST PCR R	*NbPDS*	GAAAATCAAAGCGGCTGAAC	20
NbPDS adapter PCR F	*NbPDS*	CTGAAGCAGTCACCAAGAATC	21
NbPDS adapter PCR R	*NbPDS*	GTGCAACCCAGTCTCGTACC	20
NbPDS gRNA F	*NbPDS*	taatacgactcactatagGCTGCATGGAAAGATG	34
NbPDS gRNA R	*NbPDS*	ttctagctctaaaacTCATCATCTTTCCATGCAG	34
NbPDS genomic PCR F	*NbPDS*	GAGTCAATTTTACCCGTCCTGTTG	24
NbPDS genomic PCR R	*NbPDS*	GAATGTTCCTTCCACTGCAACC	22

Figure S2. The effect of TSA on callus proliferation from the transfected or non-transfected tobacco protoplasts. Representative images of callus development after DMSO or TSA treatment during tobacco protoplast cultures. Scale bars: 1 cm.

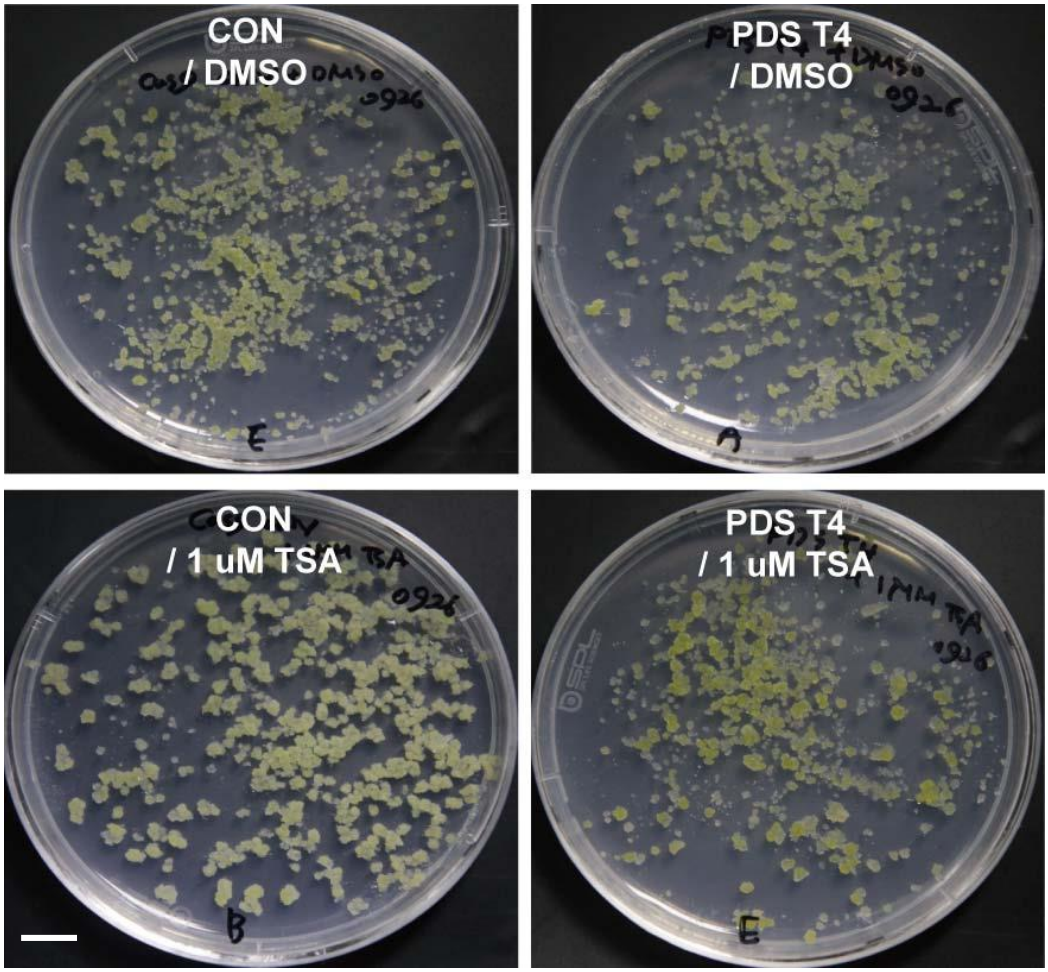

Figure S3. Representative images of *PDS* gene-edited tobacco plants from the transfected green calluses. Scale bars: 1 cm.