

RNA guided genome editing for target gene mutations in wheat

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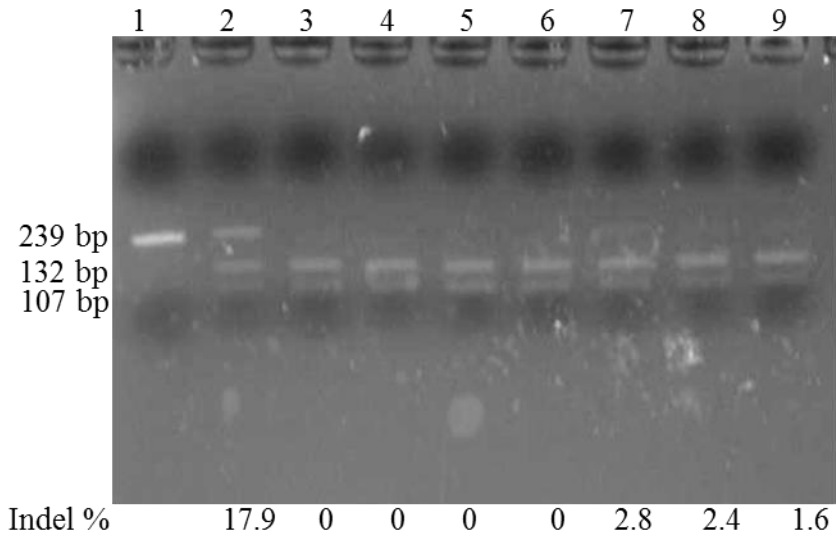


Figure S1 Sequence specificity analysis of CRISPR-Cas system by using different mutated cgRNAs. List of mutant cgRNA is given in Supplementary table 2. Normal and mutant cgRNAs (targeting protospacer 1 of *inox* gene) were co-expressed with Cas9 in suspension cells of *T. aestivum*. Targeted region was amplified, and digested with BsgI restriction enzyme (BsgI restriction site is present at cleavage position in *inox* protospacer1). BsgI could not digest the mutated DNA amplicon. Lane 1 undigested amplicon and lane 2-9 digested with BsgI. Lane 2, mutated with normal cgRNA; 3, wild type amplicon; 4-9, mutated with mutant cgRNA 1, 6, 9, 11, 12 and 13, respectively. Figure shows that mutations at 3' end of target binding region of cgRNA upto 12th bases completely abolished the cleavage activity. However mutations were detected in case of 5' end mutant cgRNA.

File S1

Partial sequence of (A) *inositol oxygenase (inox)* and (B) *phytoene desaturase (pds)* genes of wheat, and (C) *pds* gene of *Nicotiana benthamiana* used to decide the target for modification by CRISPR-Cas System. Targeted regions are shown in blue font and PAM in red. Primer used for amplification of gene after editing is shown in green.

(A)

Forward primer →

GCTCGACGGCGGCTTCACCGTGCCGACTCCAACGCCTTCGGCCACACCTT**CAGGGACTACGACGCGGAG**TCGGA BASE PAIRS
CGAGCTGCCGCCGAAGTGGCACGGCCTGAGGTTGCGGAAGCCGGTGTGGAAGTCCCTGATGCTGCGCCTCAGCCT 1 TO 75

Protospacer 1

GCGGAAGAAGACGGTGGAGGAGTTCTACAGCGTGAACCACATCAACC**AGACGTACGAGTTTGTGCAG**CGGATGCG BASE PAIRS
CGCCTTCTTCTGCCACCTCCTCAAGATGTCGCACTTGGTGTAGTTGGTCTGCATGCTCAAACACGTTCGCCTACGC 76 TO 150

Protospacer 2

GGACGCATACGGGCGGCTGGA**CAAGACGGAGATGAGCATCTGGG**AGTGCATCGAGCTGCTCAACGAGTTCATCGA BASE PAIRS
CCTGCGTATGCCC GCCGACCTGTTCTGCCTCTACTCGTAGACCCTCACGTAGCTCGACGAGTTGCTCAAGTAGCT 151 TO 225

CGACAGTGACCCCGACCTGGACATGCCGCAGATCGAGCACCTCCTCCAAACCGCCGAGGCCATCCGCAAGGACTA BASE PAIRS
GCTGTCACTGGGGCTGGACCTGTACGGCGTCTAGCTCGTGGAGGAG**GTTTGGCGGCTCCGGTAGG**CGTTCCTGAT 226 TO 300

← Reverse primer

Yellow highlighted region is the site for BsgI used for digestion of amplified DNA.

(B)

Forward primer

Protospacer 1

GCTGAGCTTGGTATTAGTGATCGCTTGCAATGGAAGGAACACTCCATGATATTTGCCATGCCAAACAAACCAGGA BASE PAIRS
CGACTCGAACCATAATCACTAGCGAACGTTACCTTCCTTGTGAGGTACTATAAACGGTACGGTTTGTTTGGTCCT 1 TO 75

Protospacer 2

GAATACAGCCGTTTTGATTTTCCAGAGACTTTGCCGGCGCCCTTAAATGGAGTGTGGCCATACTGAAAACAAT BASE PAIRS
CTTATGTCGGCAAACTAAAAGGTCTCTGAAACGGCCGCGGAATTTACCTCACACCCGGTATGACTTTTTGTTA 76 TO 150

GAAATGCTTACTTGGCCGGAAGGTGAAG BASE PAIRS
CTTTACGAATGAACCGGCCTCTTCCACTTC 151 TO 180

Reverse primer

(C)

Forward primer

GAATTGGTTTTTGCACCTGCAGAAGAGTGGATAAATCGCAGTGACTCTGAAATTATTGATGCTACAATGAAGGAA BASE PAIRS
CTTAACCAAAAACGTGGACGTCTTCTCACCTATTTAGCGTCACTGAGACTTTAATAACTACGATGTTACTTCCTT 1 TO 75

Protospacer 1

Protospacer 2

CTAGCAAAGCTTTTCCCTGATGAAATTTGGCAGATCAGAGCAAAGCAAATAATTGAAGTATCATGTTGTCAA BASE PAIRS
GATCGTTTCGAAAAGGGACTACTTTAAAGCCGTCTAGTCTCGTTTCGTTTTTATAACTTCATAGTACAACAGTTT 76 TO 150

ACTCCAAGTCTGTTTATAAACTGTGCCAGGTTGTGAACCTGCCGGCCCTTGCAAAGATCCCCTATAGAGGGG BASE PAIRS
TGAGGTTCCAGACAAATATTTTGACACGGTCCAACACTTGGGACGGCCGGGAACGTTTCTAGGGGATATCTCCC 151 TO 225

Reverse primer

File S2

Chimeric guide RNA (cgRNA) for targeting (A) *inositol oxygenase (inox)* and (B) *phytoene desaturase (pds)* genes of wheat, and (C) *pds* gene of *Nicotiana benthamiana*. (i) cgRNA for protospacer 1, (ii) cgRNA for protospacer 2 and (iii) cgRNA for targeting both protospacer 1 and 2. Target region is given in blue font and guide RNA scaffold in red font. Restriction site SpeI (highlighted in yellow) was used for ligation of the two cgRNAs.

(A)

(i)

NNNNNAGACGTACGAGTTTGTGCAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTTNNNNNNNN

(ii)

NNNNNCAAGACGGAGATGAGCATCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTTNNNNNNNN

(iii)

NNNNNAGACGTACGAGTTTGTGCAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTACTAGTNNNNCAAGACGGAG
ATGAGCATCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GAAAAAGTGGCACCAGTCGGTGCTTTTNNNNNNN

(B)

(i)

NNNNN**TTTGCCATGCCAAACAAACC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNNN

(ii)

NNNNN**GGCGCCCTTAAATGGAGTGT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNN

(C)

(i)

NNNNNGCTTTTCCCTGATGAAATTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNNN

(ii)

NNNNNATCATGTTGTCAAACCTCCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNNN

Table S1 Plant expression vectors used in the study.

Vector	Detail	Target gene
pCas9	Expression of FLAG tag and NLS containing Cas9 protein.	NA
pTpds1	Expression of cgRNA targeting protospacer 1 of <i>Nicotiana benthamiana phytoene desaturase (pds)</i> gene	<i>N. benthamiana pds</i> gene
pTpds2	Expression of cgRNA targeting protospacer 2 of <i>N. benthamiana pds</i> gene	<i>N. benthamiana pds</i> gene
pCtpds1	Co-expression of Cas9 and cgRNA targeting protospacer 1 of <i>N. benthamiana pds</i> gene	<i>N. benthamiana pds</i> gene
pCtpds2	Co-expression of Cas9 and cgRNA targeting protospacer 2 of <i>N. benthamiana pds</i> gene	<i>N. benthamiana pds</i> gene
pCinox1	Co-expression of Cas9 and cgRNA targeting protospacer 1 of <i>T. aestivum inositol oxygenase (inox)</i> gene	<i>T. aestivum inox</i> gene
pCinox2	Co-expression of Cas9 and cgRNA targeting protospacer 2 of <i>T. aestivum inox</i> gene	<i>T. aestivum inox</i> gene
pCwpds1	Co-expression of Cas9 and cgRNA targeting protospacer 1 of <i>T. aestivum pds</i> gene	<i>T. aestivum pds</i> gene
pCwpds2	Co-expression of Cas9 and cgRNA targeting protospacer 2 of <i>T. aestivum pds</i> gene	<i>T. aestivum pds</i> gene
pCinox12	Co-expression of Cas9 and duplex cgRNAs targeting protospacer 1 and 2 of <i>T. aestivum pds</i> gene	<i>T. aestivum pds</i> gene
pCpin1	Co-expression of Cas9 and duplex cgRNAs targeting protospacer 1 of both <i>inox</i> and <i>pds</i> gene of <i>T. aestivum</i> .	<i>T. aestivum inox</i> and <i>pds</i> gene

Table S2 Specificity analysis of cgRNA. Table shows average mutation percentage at targeted locus with different mutant cgRNA.

	Sequence	Average mutation (%)
Normal cgRNA	AGACGTACGAGTTTGTGCAG	17.9
Mutant cgRNA	1 AGACGTACGAGTTTGTCAA	0
	2 AGACGTACGAGTTTGTGCGA	0
	3 AGACGTACGAGTTTGTGGCA	0
	4 AGACGTACGAGTTTGGTGCA	0
	5 AGACGTACGGAGTGCAGTTT	0
	6 AGACGTACTAGTTTGTGCAG	0
	7 AGACGTACGAGTTTGTGAAG	0
	8 AGACGTACGAGTTTGTCTAG	0
	9 AGACGTACAAGTTTATGCAG	0
	10 AGACGTACGAGTGGGTGCAG	0
	11 TGACGTACGAGTTTGTGCAG	2.8
	12 CAACGTACGAGTTTGTGCAG	2.4
	13 CAGCGTACGAGTTTGTGCAG	1.6
	14 AGACAGTCGAGTTTGTGCAG	0
	15 AGACTGCAGAGTTTGTGCAG	0