Robust CRISPR/Mb2Cas12a genome editing tools in cotton plants

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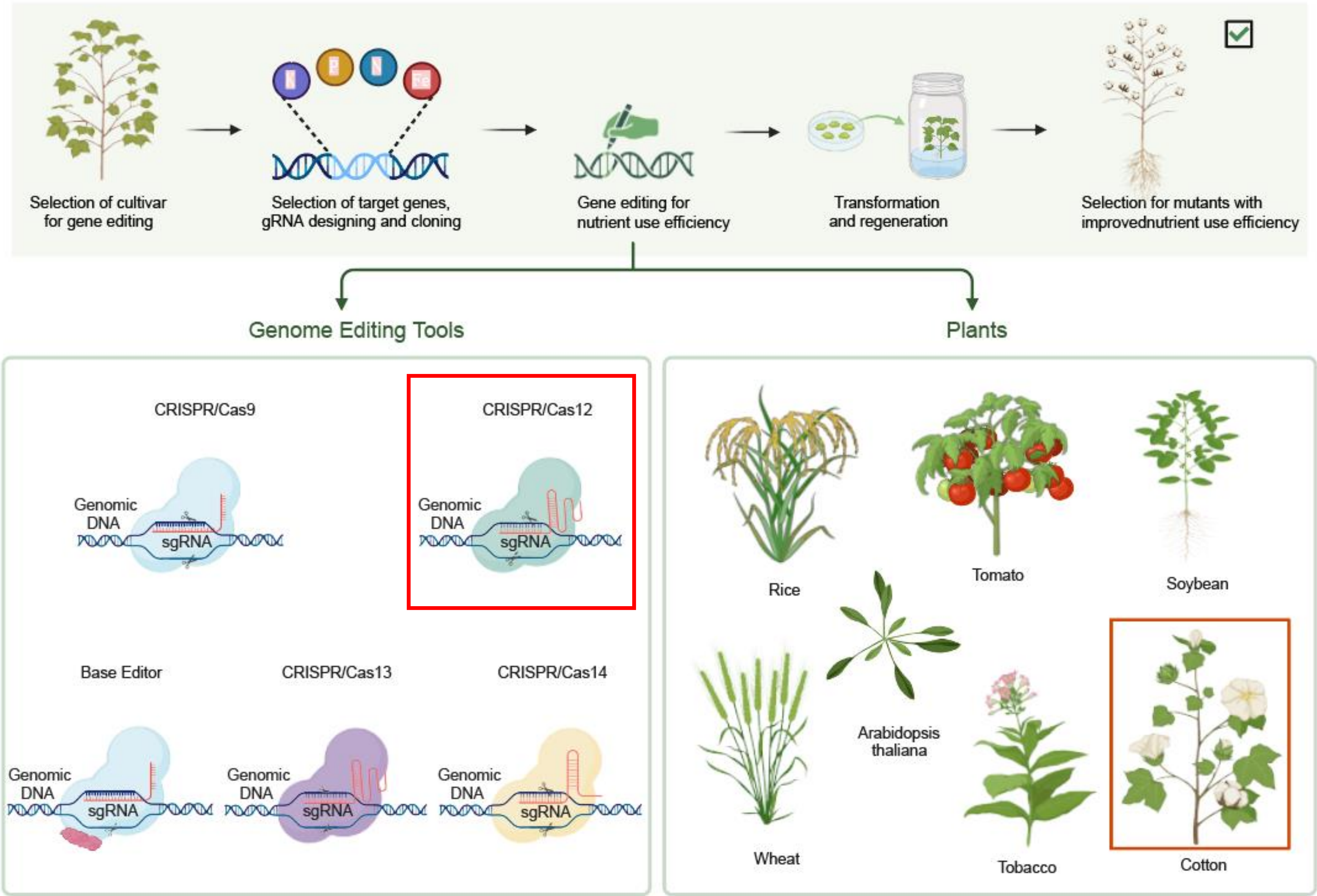
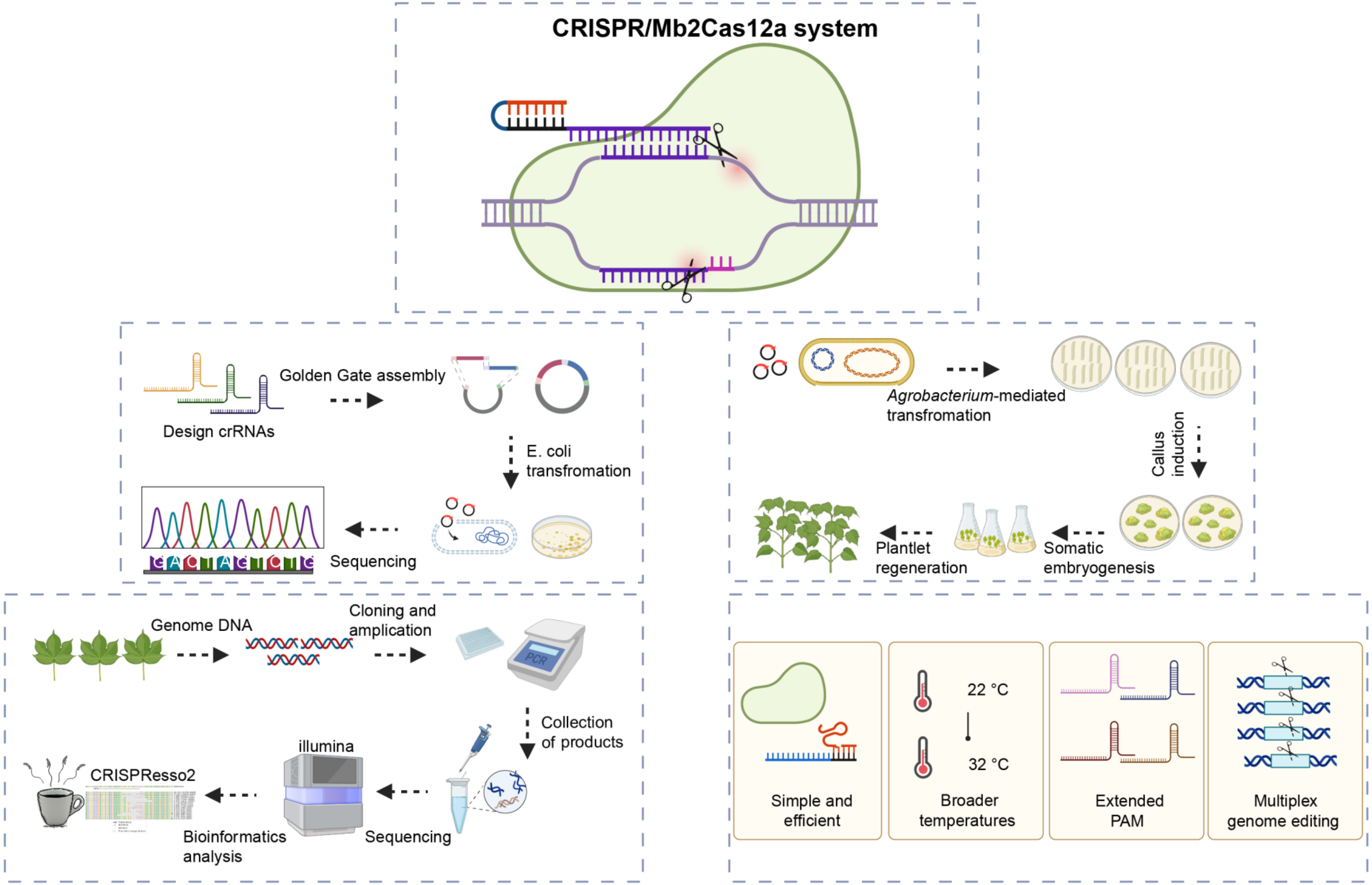
Introduction



Graphical Abstract



Results: Enable highly effective targeted mutations

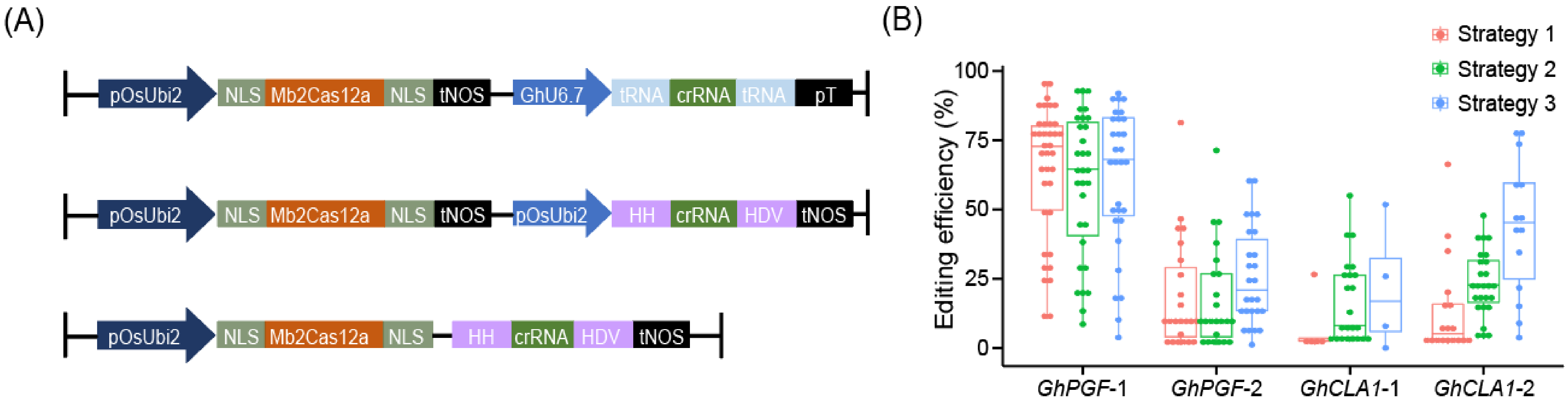
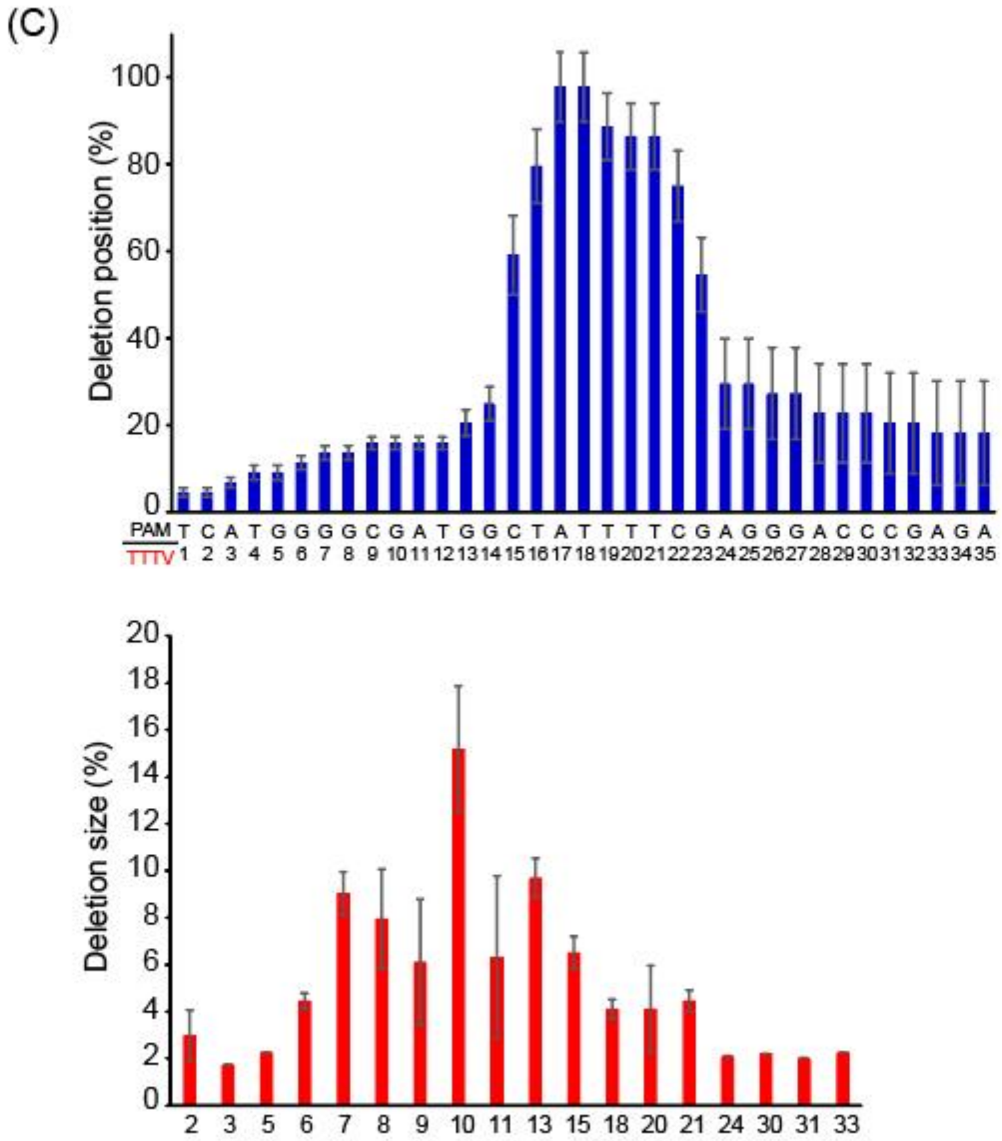
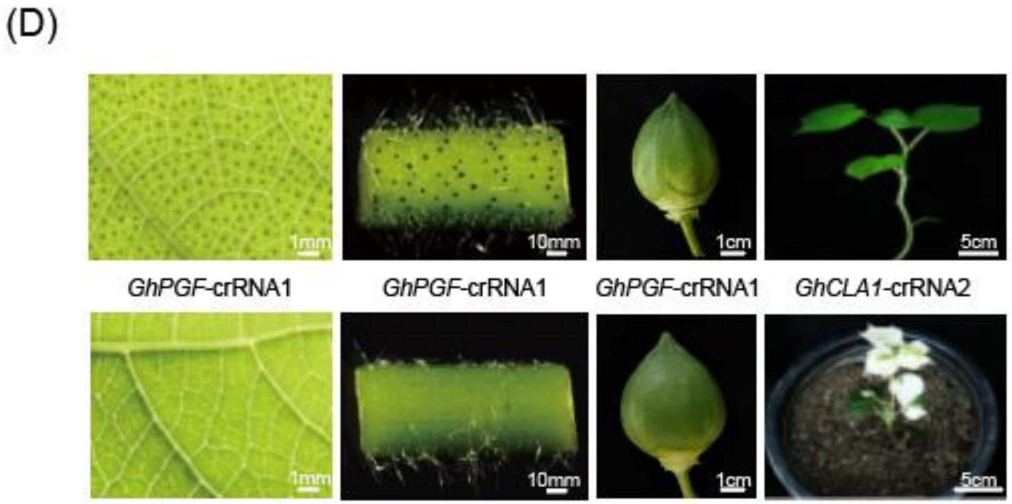


Figure 1. (A) A schematic illustration of the T-DNA region of the CRISPR/Mb2Cas12a binary vector for single site targeted editing. (B) Editing efficiency (%) at target sites of *GhPGF* and *GhCLA1* with different vectors.

Results



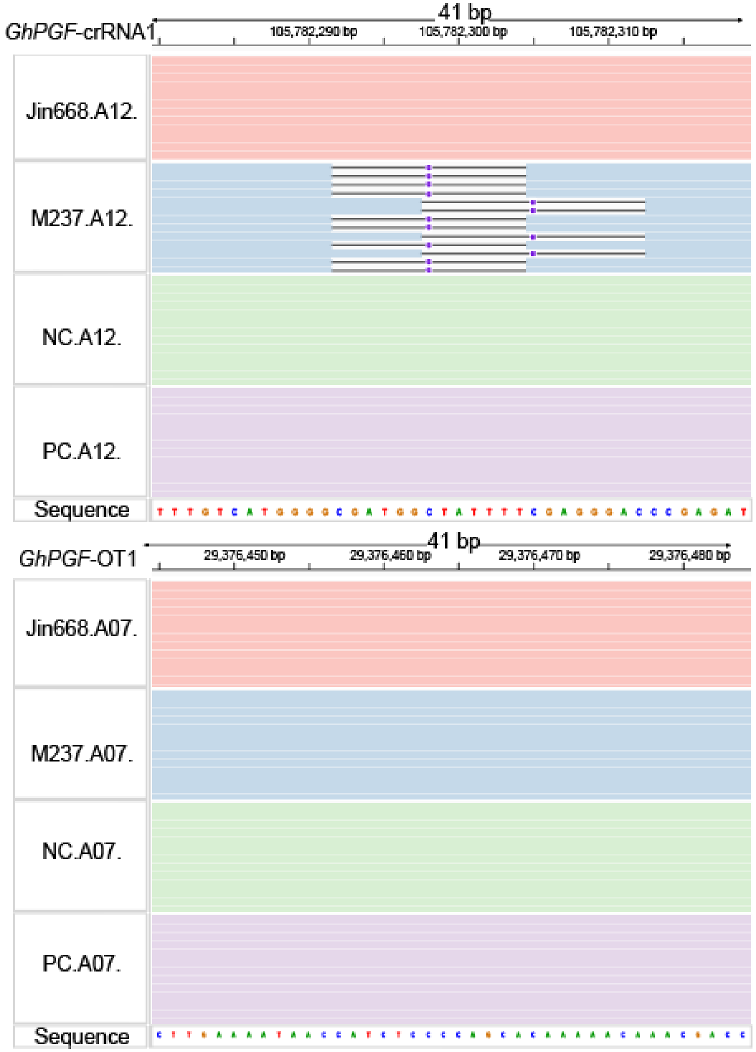


- ☐ The main editing window of Mb2Cas12a ranged from 13 to 26 nt downstream of the PAM sites;
- ☐ The deletion sizes mainly ranging from 7 to 18 bp;
- ☐ The density of gossypol glands in both the leaf and stem tissues was significant decreased;
- ☐ Plants carrying the *GhCLA1*-crRNA2 component exhibited a partially bleached phenotype on their leaves.

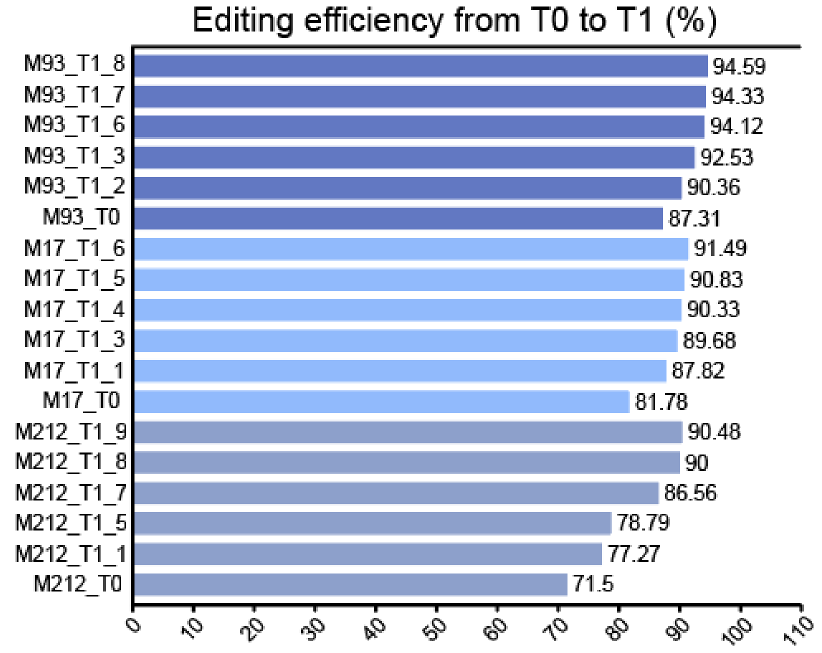
Figure 1. (C) Frequency of DNA deletion position and size at the target site of *GhPGF*-crRNA1 induced by Mb2Cas12a. (D) Phenotype of the T0 cotton plants with the target mutations in *GhPGF* and *GhCLA1*.

Results: Target specificity and genetic stability

(E)



(H)



- ☐ No off-target mutation was detected at any predicted off-target sites;
- ☐ The genotypes at the edited loci in cotton plants were faithfully inherited from the T0 to the T1 progeny

Figure 1. (E) Evaluation of potential off-target editing through whole genome sequencing technology. (H) Determination of the target mutation efficiency at the *GhPGF*-crRNA1 target site in T1 lines using NGS.

Results

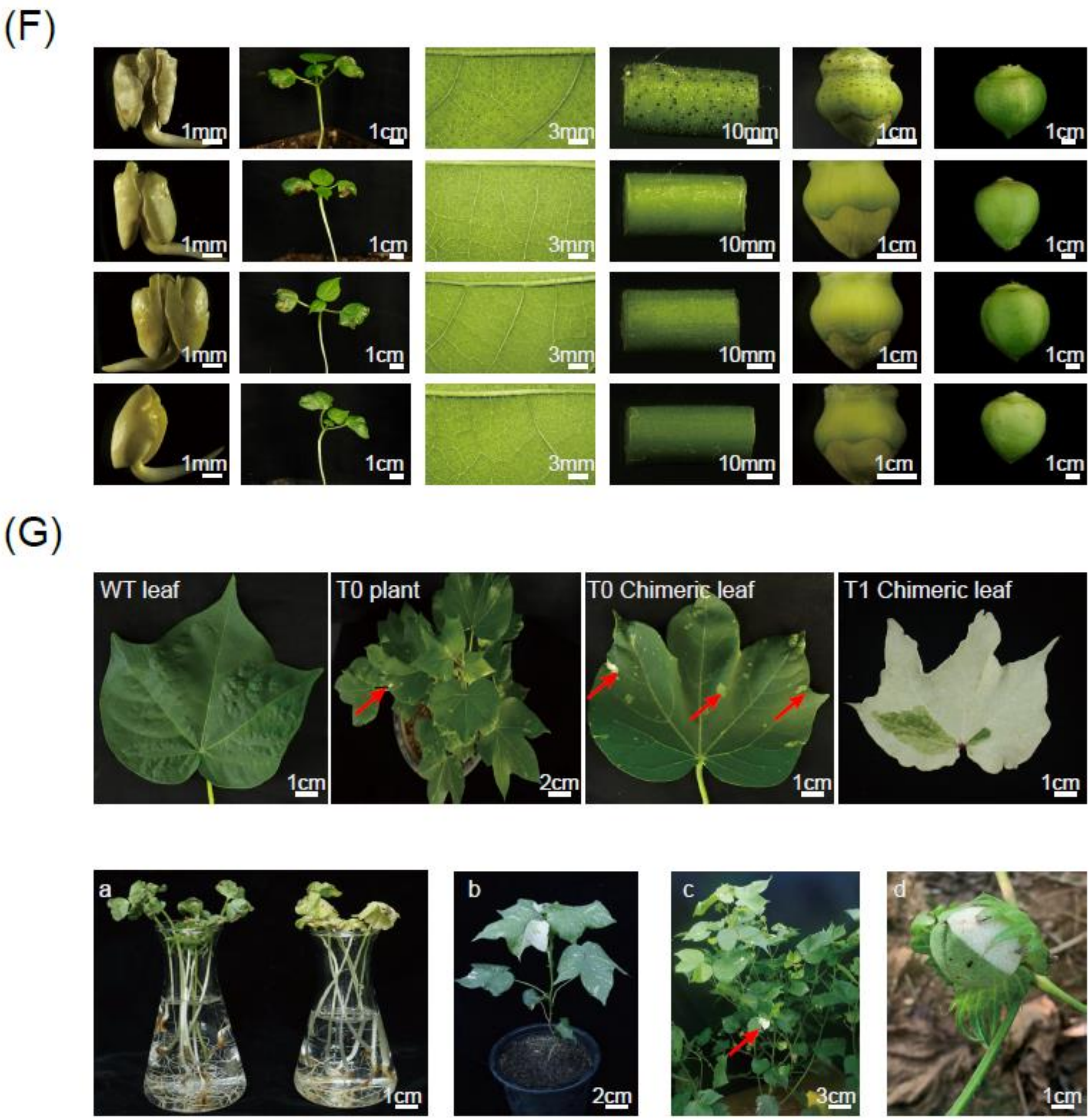


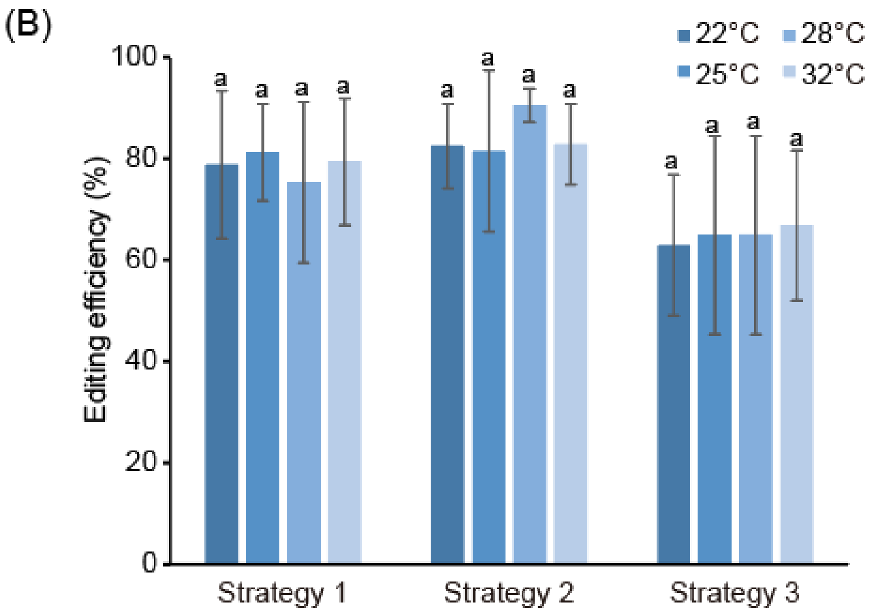
Figure 1. (F) Phenotypic characterization of T1 lines at the *GhPGF*-crRNA1 site. (G) T1 cotton plants with mutations in *GhCLA1*-crRNA2 site showed leaf bleaching in different environments.

Results: Exhibit wide temperature adaptability

(A)

GhPGF-crRNA1	Temperature (°C)	Line 66 (%)	Line 67 (%)	Line 71 (%)	Line 200 (%)
Strategy 1	22	54.66	79.98	90.26	90.21
	25	69.65	75.44	94.62	85.12
	28	50.42	72.9	91.18	86.53
	32	59.26	79.27	92.29	86.53
GhPGF-crRNA1		Line 149	Line 70	Line 77	Line 95
Strategy 2	22	92.53	81.73	69.54	86.21
	25	84.67	94.33	54.66	92.33
	28	86.57	94.92	92.26	88.18
	32	92.44	86.37	70.51	82
GhPGF-crRNA1		Line 156	Line 208	Line 212	Line 76
Strategy 3	22	47.5	77.4	50.63	76.15
	25	39.21	67.81	59.16	93.4
	28	35.59	60.17	76.04	88.03
	32	46.67	61.66	71.5	87.47

(B)



(C)

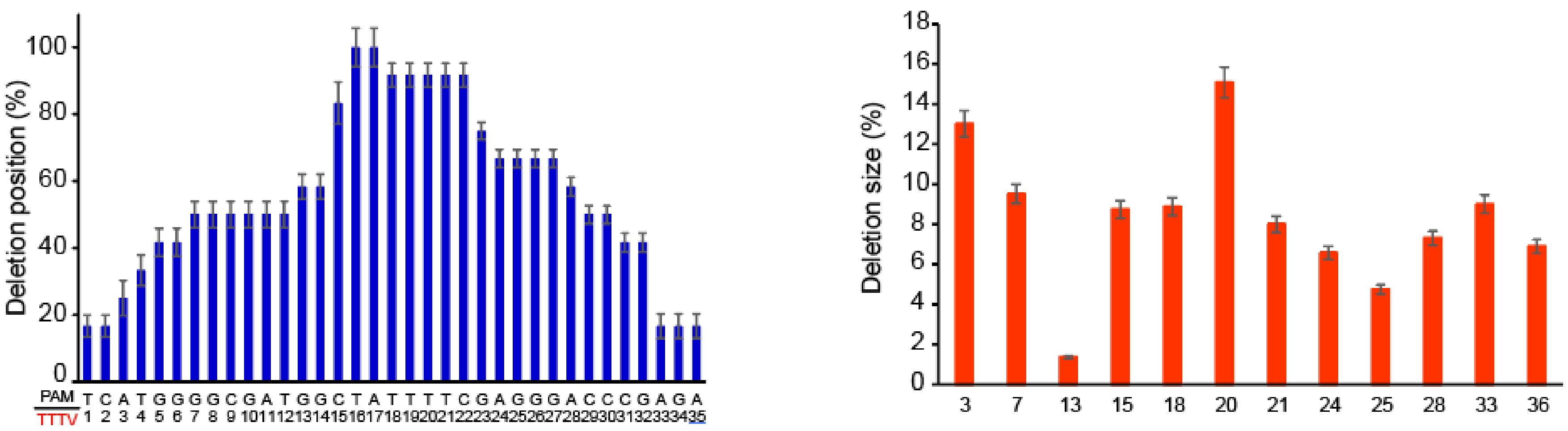
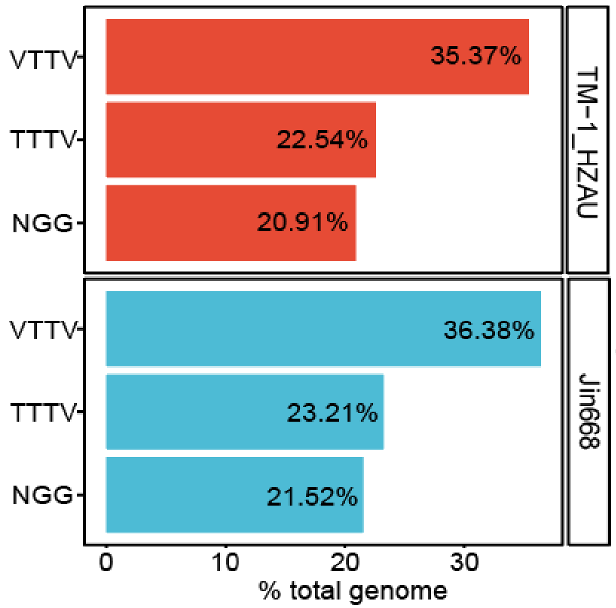


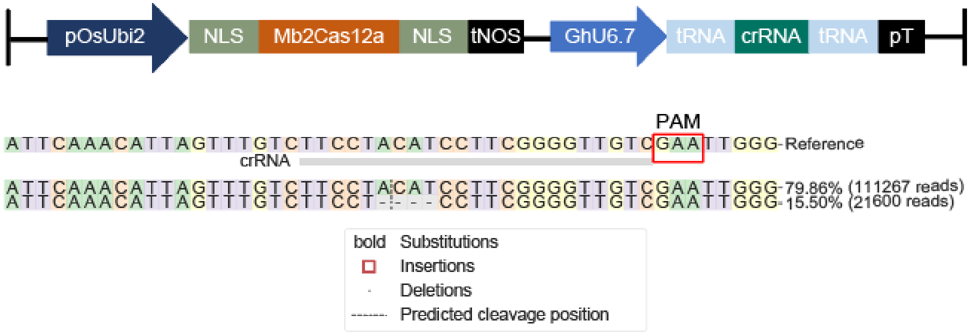
Figure 2. Wide temperature tolerance and multiplex genome editing by Mb2Cas12a in cotton.

Results: Expands the scope of cotton genome editing

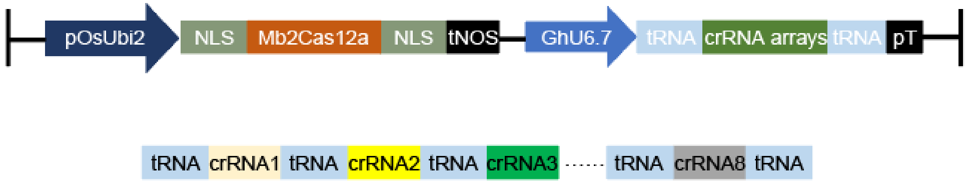
(D)



(E)



(F)



(G)

Strategy	Target	Gene	crRNA	No. of transgenic events identified	Editing efficiency	No. of events with edited
Strategy 1 with TTTV	crRNA1	*GhPGF*	TCATGGGGCGATGGCTATTTTCG	79	1.8-39.7%	29 (42.03%)
	crRNA2	*GhPGF*	TCTTCCTACATCCTTCGGGGTTG	79	4.93-5.47%	2 (2.53%)
	crRNA3	*GhCLA1*	TCTGCCAACTGCCAATCTCGACG	79	7.46%	1 (1.45%)
	crRNA4	*GhTAC1*	CTGGAACAAGTGGCACTGGTTGA	79	1.82-17.93%	6 (8.70%)
	crRNA5	*GhTAC1*	ATGGGACGTGAATCCTCGCCAAC	79	4.08-26.43%	33 (47.83%)
	crRNA6	*GhMYB25*	GCAAATGAGCCGCAATAGCCGAC	79	1.06-19.92%	28 (40.58%)
	crRNA7	*GhMYB25*	GTAGCATGAGCCAAACCAACATC	79	10.02-71.47%	59 (85.51%)
	crRNA8	*GhFAD*	ACTTTCTCCCACAACCCTTTTCC	79	17.12-58.62%	60 (86.96%)

Summary

- CRISPR/Mb2Cas12 significantly enhances the capability and accuracy in cotton genome editing;
- Mb2Cas12a exhibits wide temperature adaptability;
- Mb2Cas12a greatly expands the scope of cotton genome editing;
- We have established an efficient multiplex genome editing system based on Mb2Cas12a.

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