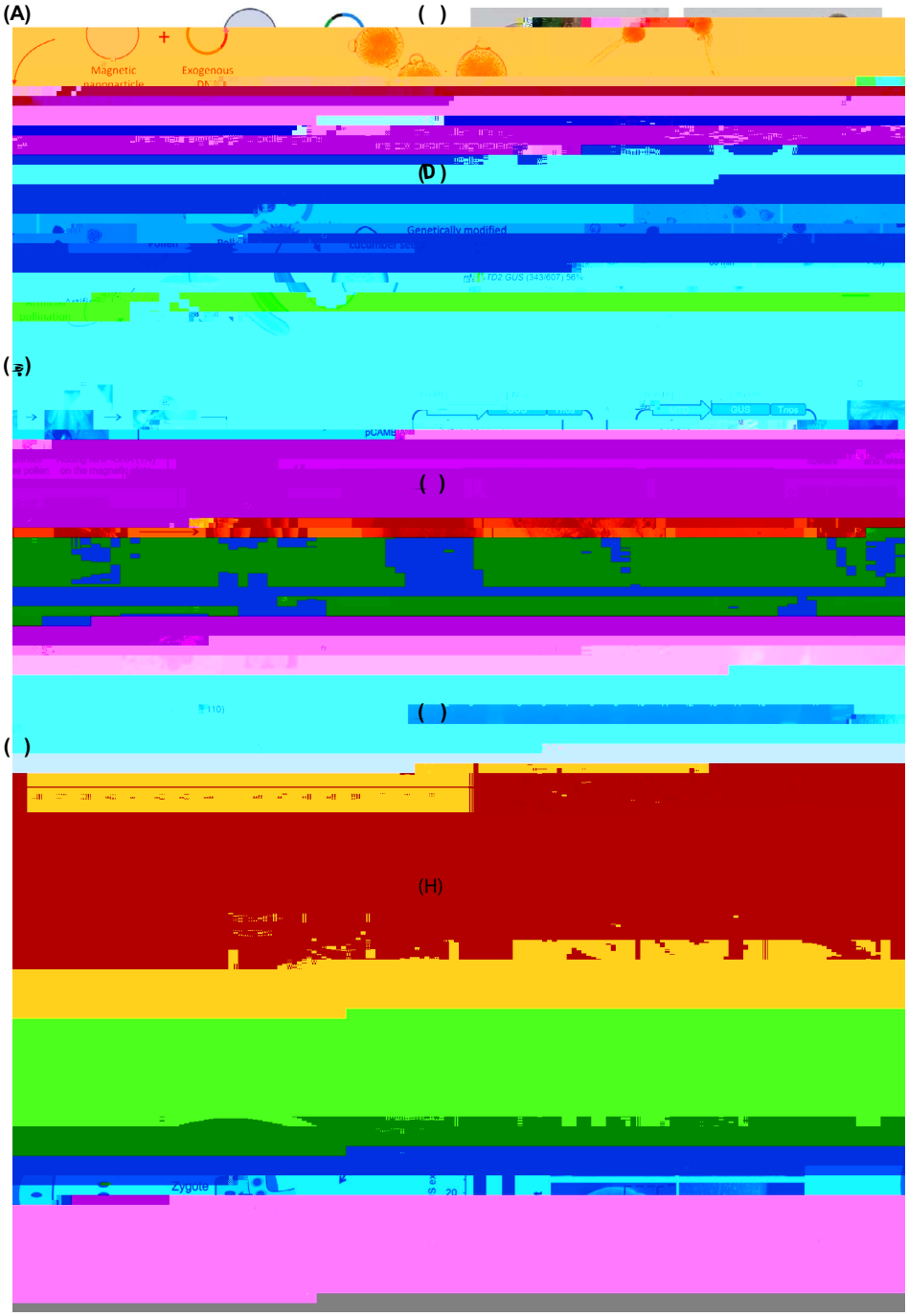

Figure 1. Magnetofected pollen gene delivery system applied to *C. sativus*. (A) Schematic illustration of pollen magnetofection. (B) Steps of production of magnetofected pollen gene delivered cucumber. 0.5 μg of MNPs (PolyMag200, Chemicell, Germany)) and 2 μg of plasmid DNA (200–250 ng/μg) were combined at a 1:4 ratio and allowed to stand for 15 minutes at room temperature for one magnetofection reaction. The MNP–DNA complexes were then added to a magnetofection buffer (15% sucrose, 0.01% boric acid, 1 mM Ca(NO3)2) containing pollen grains (Approximately 22 000 pollen grains, collected from 20 male flowers, were magnetofected with the MNP–DNA complexes. This corresponds to around 1100 pollen grains per male flower. [6]), and placed in a magnetic field (MagnetoFACTOR-24, Chemicell, Germany) for 30 minutes. Subsequently, the magnetofected pollen grains were carefully spread onto filter paper to remove the buffer and allowed to dry at room temperature overnight, followed by storage at 4°C. The next day, the magnetofected pollen grains were manually pollinated onto the stigma of female flowers. (C) Viability test of processed pollen. Dried magnetofected pollen grains were observed after the drying process and germination of magnetofected pollen grains which had dried 1 day before (Scale bar = 100 μm). (D) Increase in exogenous gene expression activity over time in the magnetofected pollen. Pollen-specific promoter (*OsMTD2* promoter) showed stronger GUS activity than the for constitutive promoter (Scale bar = 100 μm). (E) Statistical analysis of GUS expression was conducted with T1 seedlings ($n > 130$ seedlings for each group). Error bars represent the standard error of three repeats. No significant difference w-c7007712T.4(d9)130enc d(f)279.6(a)-h

1. Primers used in this study.

	(5′ t 3′)	
Cas1_F	TTCATCCAGCTCGTGCA	DNA certification
Cas1_R	GGCTTGATGAACTTGTAGAACT	DNA certification
Cas2_F	TTCATCCAGCTCGTGCA	DNA certification
Cas2_R	GGCTTGATGAACTTGTAGAACT	DNA certification
Hyg_F	GTGCTTGACATTGGGGAGTT	DNA certification
Hyg_R	GATGTTGGCGACCTCGTATT	DNA certification
CseIF4E_F	GAAGCCCAAGGGATAAAAGG	Genotyping
CseIF4E_R	TCTCTCCAGCCCTCACATTC	Genotyping
CsPDS_F	TCTCGGTTTCATTTCATCCA	Genotyping
CsPDS_R	CTGCCCCAGCAATCACTACT	Genotyping
eIF4E_sgRNA7_F		

Data availability

All data supporting this study are available in the article.

Conflict of interest statement

The authors declare no conflicts of interest.

References

1. Hasegawa T, Wakatsuki H, Ju H. *et al.* A global dataset for the