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Silencing of CsDMC1 Suppresses Meiotic Recombination and Reduces Pollen Viability in Cucumber (Cucumis sativus L.)

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Abstract

Cucumber (*Cucumis sativus* L.) is a globally significant vegetable crop, and targeted manipulation of its recombination frequency offers opportunities to broaden genetic diversity and enable novel breeding applications. In the first stage of this study, we characterized the spatial activity of the cucumber *DMC1* (*CsDMC1*) promoter in cucumber. Subsequently, we investigated the effects of *CsDMC1* silencing on meiotic recombination and pollen viability of cucumber. Transgenic plants expressing the *GUS* reporter gene under the control of the *CsDMC1* promoter exhibited strong GUS activity in the microspores, but no detectable expression in vegetative tissues or seeds, confirming the meiosis-specific activity. RNAi-mediated silencing of *CsDMC1* in cucumber resulted in variable downregulation (67–94%) across transgenic lines, correlating strongly with pollen abortion rates (16–90%, r = 0.87). Meiotic crossover frequency was evaluated using two polymorphic SSR markers in a non-transgenic cucumber plant and a transgenic line exhibiting approximately 80% reduction in *CsDMC1* expression and 55% pollen abortion, revealing a 46% decrease in crossover frequency in the transgenic line compared with the control. Importantly, the results indicate that although *CsDMC1* silencing effectively suppresses recombination, the cellular abundance of *CsDMC1* likely exceeds the minimum requirement for normal crossover formation, and its overexpression alone may not increase crossover frequency beyond a natural threshold. Our findings highlight *CsDMC1* silencing as a practical tool for reverse breeding in cucumber and underscore the utility of the *CsDMC1* promoter as a meiosis-specific driver for future studies.

1. Introduction

Cucumber (*Cucumis sativus* L.) is a globally important vegetable crop, with 2023 production reaching nearly 98 million metric tons (FAOSTAT 2024). Its considerable economic value arises from its roles in fresh markets, pickling, and processing sectors, underpinning its significance in agricultural economies. However, cucumber cultivation faces challenges such as susceptibility to biotic stresses (e.g., powdery mildew, downy mildew, nematodes) and abiotic stresses (e.g., temperature extremes, water deficits), necessitating advanced breeding strategies to enhance yield, quality, and resilience in the context of global food security and climate change.

Management of crossovers is a target for breeders to reduce the time and cost of the selection process (Taagen et al. 2020). Crossovers play a crucial role by creating favorable gene combinations and disrupting undesirable linkages, thereby facilitating the development of improved crop varieties. In most plant species, at least one crossover per homologous chromosome pair or obligate crossover is required for accurate chromosome segregation during meiosis, and more than three crossovers on a single chromosome are uncommon due to crossover interference and chromosomal constraints (Fernandes et al. 2018; Mercier et al. 2015). While this restriction ensures proper chromosome segregation, it also constrains the genetic variation accessible to breeders (Lambing and Heckmann 2018).

Escalation in crossover rate improve the genetic variation, disrupt undesirable linkages, facilitate the formation of desirable linkages and provide a better material for genetic mapping (Mieulet et al. 2018).

On the other hand, a decrease or absence of crossover rate is favorable for keeping a beneficial linkage and the haplotype organization on chromosomes. Reducing crossover frequency through genetic engineering has practical applications in breeding systems requiring the maintenance of parental haplotypes. The most notable example is reverse breeding, which enables the seed-based reproduction of desirable heterozygous plants by generating complementary homozygous parents. Reverse breeding is achieved by three steps, (i) suppression of meiotic crossovers to produce non-recombinant gametes; (ii) generation of doubled haploids from these haploid gametes; and (iii) selection of complementary parents using molecular markers (Dirks et al. 2009; Wijnker et al. 2012).

Silencing or mutating one of several genes involved in meiotic crossover is the best choice to prevent chromosome recombination. The *DMC1*(disrupted meiosis cDNA1) gene, first identified in *Saccharomyces cerevisiae* in 1992, encodes a meiosis-specific protein and is indispensable for proper meiotic progression, homologous recombination, and synaptonemal complex formation in yeast (Bishop et al. 1992). In many eukaryotes, the initial stages of homologous recombination are mediated by Rad51 and *DMC1*, which are orthologs of the bacterial *RecA* protein. *Rad51* promotes strand invasion between sister chromatids, but during meiosis, in cooperation with *DMC1*, it facilitates recombination between homologous chromosomes (Neale and Keeney 2006). The application of *DMC1* gene silencing in plants are primarily centered on manipulating meiotic recombination to advance crop breeding strategies. In Arabidopsis, RNAi-mediated silencing of *DMC1* has been used to generate non-recombinant gametes, enabling reverse breeding (Wijnker et al. 2012). This approach has been extended to tetraploid crops like potato (*Solanum tuberosum*), where RNAi disruption of the *StDMC1* gene produces asynaptic mutants with reduced pollen viability, potentially aiding the development of true potato seeds for stable, virus-free propagation and overcoming challenges in heterozygous breeding (Kumar et al. 2023).

Elucidating the regulatory mechanisms and functional dynamics of the *DMC1* gene in cucumber (*CsDMC1)* not only enhances our understanding of meiotic control in this economically important crop but also unlocks potential applications in recombination engineering for accelerated breeding. In this study, we characterized the tissue-specific activity of the *CsDMC1* promoter and evaluated the impact of *CsDMC1* silencing on meiotic crossover frequency and pollen viability.

2. Material and Methods

2.1. Plasmid construction *CsDMC1* Promoter isolation and evaluation

To assess the activity of *CsDMC1* promoter, a 554-bp fragment from the 5' UTR was amplified and subsequently cloned upstream of the *GUS* reporter gene in the pCAMBIA2301 plasmid (Fig. 1a). Based on bioinformatic analysis, the region spanning 201–374 bp upstream of the start codon was predicted to contain the core promoter. Nevertheless, the primers selected for amplification and cloning extended beyond this range, resulting in the isolation of a 554 bp fragment upstream of the start codon.

The silencing plasmid for the *CsDMC1* gene was constructed using a 325-bp fragment of the *CsDMC1* ORF arranged in a hairpin structure with a 192-bp intron from the catalase gene (*cat*). The *CsDMC1* promoter was employed to drive expression of the hairpin construct, whereas the CaMV 35S promoter was used to regulate *GUS* reporter gene expression in the transformed plants (Fig. 1b).

2.2. Transformation and plant regeneration

Cucumber transformation was performed according to the method described by Nanasato et al. (2013), with minor modifications, using seeds of the local cultivar 'Dastgerdi'. After surface sterilization of peeled seeds and germination on MS medium for 3–4 days, cotyledons were cut into four parts and used for transformation. *Agrobacterium tumefaciens* strain EHA105 harboring either pCAMBIA2301-CsDmPr-GUS or pCAMBIA2301-CsDmPr-RNAi plasmids was cultured until OD600 0.6. Then centrifuged and dissolved in liquid MS medium containing 100 µM acetosyringone, and the culture used for inoculation after 1 hr. Explants were vacuum-infiltrated with the bacterial suspension and co-cultivated on shoot induction (SI) medium including MS medium supplemented with 2 mg/L BAP (benzylaminopurine), 1 mg/L ABA (abscisic acid) and 2.5 gr/l gelrite for 2–3 days at 28° C in the dark. Subsequently, explants were transferred to selection medium including SI medium containing kanamycin (50 mg/l), meropenem (50 mg/l) and $AgNO_3$ (3 mg/L). Transformation of the tomato cultivar 'Micro-Tom' with the pCAMBIA2301-CsDmPr-GUS construct was conducted using the same procedure, except that the SI medium consisted of MS salts supplemented with 2 mg/L BAP and 0.2 mg/L thidiazuron (TDZ). Regenerated shoots were transferred to root induction media, MS medium containing 0.5 mg/L IBA (indole-3-butyric acid), and well-developed transgenic plantlets were acclimatized and transferred to greenhouse.

2.2. Histochemical GUS assay, and *csDMC1* gene expression

Fresh plant samples (male flower buds, anthers, leaf, cotyledons and roots) from transgenic lines and control plants were subjected to histochemical GUS staining. The tissues were immersed in GUS assay buffer containing 1 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc), 10 mM EDTA, 0.1% Triton X-100, and 50 mM sodium phosphate (pH 7.0), and incubated for 16 hrs at 37°C. Following incubation, samples were washed and fixed in 70% ethanol for 4–16 hrs to remove chlorophyll and enhance visualization of GUS activity.

2.3. Pollen viability evaluation

To evaluate the pollen viability or abortion in transgenic and control plants, a modified method from Peterson et al. (2010) was utilized (Peterson et al. 2010). Initially, anthers were detached from floral samples and placed in Carnoy's fixative for ~ 15 min and in 70% ethanol for ~ 10 min. Subsequently, the anthers were placed in 0.5 ml microcentrifuge tubes containing 200 µl of the staining buffer and incubated at 80°C for 15 min. After staining, the anthers were mounted on a slide, and minced in a drop of staining solution. Coverslips were applied to the samples, and images were captured using an Eclipse 90i microscope (Nikon).

2.4. Assessment of *csDMC1* gene expression

To analyze *CsDMC1* expression, 10–15 flower buds at the meiotic stage (~ 3 mm in length) were collected, and total RNA was extracted using TRIzol™ reagent. The *UBC1* gene was used as a housekeeping gene for normalization. Primers for relative expression analysis of the *CsDMC1* gene, CsDmRT-F (ACCAACCAAGTCGTTGCAGA) and CsDmRT-R (GGGGCATCGAACACCTTACA), were designed to amplify a region of the CsDMC1 transcript located outside the sequence targeted for gene silencing. Real-time PCR was performed using SYBR Green qPCR Master Mix on a StepOne Real-Time PCR System (Applied Biosystems). Relative differences in *CsDMC1* expression between transgenic and non-transgenic plants were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.5. Crossover rate evaluation

Simple sequence repeat (SSR) markers were employed to evaluate crossover frequency in transgenic and non-transgenic cucumber plants. Of the 56 SSR loci tested from the cucumber genetic map (Ren et al. 2009), two polymorphic markers—1B24 and 1B56—located on chromosome 1 were identified and selected for analysis. The immature seeds were isolated ~ 3 weeks after pollination and cultured on the MS medium containing 0.1 mg/l IAA (indole- 3-acetic acid) for 1–2 weeks. The leaves of germinated embryos were used for DNA extraction using the CTAB method. Amplification was performed using a Veriti Thermal Cycler (Applied Biosystems) under the following protocol: an initial denaturation at 95°C for 3 min, followed by a touchdown phase consisting of 12 cycles (95°C for 15 s; annealing at 66°C for 30 s, decreasing by 0.5°C per cycle; and extension at 72°C for 30 s), then 22 additional cycles of standard amplification (95°C for 15 s, 60°C for 30 s, 72°C for 30 s), and a final extension at 72°C for 6 min. The resulting amplicons were resolved on 7% polyacrylamide gels, visualized by silver nitrate staining, and scanned using an Image Scanner III (GE Healthcare). Banding patterns were scored manually from the acquired images.

3. Results

3.1. Verification of transformed plants and *CsDMC1* promoter activity

Plants transformed with the pCAMBIA2301-CsDmPr-RNAi plasmid were first verified by histochemical GUS staining of root tips (Fig. 2a, b). To assess *CsDMC1* promoter activity, non-transgenic and transgenic cucumber plants carrying the T-DNA of pCAMBIA2301-CsDmPr-GUS plasmid were subjected to GUS staining. Strong GUS activity was detected in the anthers of transgenic cucumber plants (Fig. 2c–f). In contrast, no staining was observed in seeds, roots, leaves, or cotyledons of the same transgenic lines, indicating that the *CsDMC1* promoter drives meiosis-specific expression.

3.2. Pollen viability and *CsDMC1* expression

Disruption of crossover formation, which compromises the balanced segregation of homologous chromosomes, directly affects pollen viability. Following staining, viable pollen grains appeared red and non-shrunken, whereas aborted pollen grains were green and shrunken, observed at varying frequencies among transgenic plants (Fig. 3).

Among 11 transgenic lines harboring the T-DNA of the pCAMBIA2301-CsDmPr-RNAi construct, verified by GUS staining of root tissues, six lines showed no discernible difference in pollen abortion compared with control plants. Likewise, cucumber and tomato plants transformed with the pCAMBIA2301-CsDmPr-GUS construct, confirmed by GUS staining of flower buds and anthers, exhibited no signs of pollen abortion. In contrast, five transgenic lines carrying the pCAMBIA2301-CsDmPr-RNAi construct displayed evident pollen abortion, with the proportion of aborted grains ranging from 16% to 90%. Quantitative analysis revealed variable levels of *CsDMC1* downregulation among the examined lines (T1–T5), with the T2 line showing the most pronounced reduction (93%) decrease in *CsDMC1* transcript abundance (Fig. 4).

Figure 4. Relative *CsDMC1* gene expression and pollen viability in control plant (WT) and transgenic cucumber plants (T1-T5) in which the *CsDMC1* is downregulated by RNAi method.

3.3. Evaluation of Crossover Frequency

The T3 transgenic line, exhibiting 80% reduction in *CsDMC1* expression along with 55% pollen abortion, was selected to evaluate crossover frequency through test crossing. For comparison, the normal crossover rate in wild type plant was assessed in the plantlets obtained from self-pollination. The results indicated the 46% reduction of crossover in T3 line compared with control plant (Table 1).

Table 1
Method and results of crossover frequency evaluation in a transgenic line (T3) and non-transgenic plant.

Plant type	Crossing method	No. of plants analyzed	No. of scorable gametes	No. of recombinant gametes	Distance (cM)
Non-transgenic	Self-pollination	50	72	22	30.5
Transgenic (T3)	Testcross	72	72	12	16.6

4. Discussion

The successful verification of *CsDMC1* promoter activity through GUS staining in the anthers of transgenic plants confirms its meiosis-specific expression, in agreement with previous reports showing that *DMC1* orthologs are predominantly active during meiotic prophase I across diverse plant species (Da Ines et al. 2013; Neale and Keeney 2006). In contrast to Brassica rapa, where *BrDMC1* expression has been reported during seed germination under salt stress (Wang et al. 2023a); no promoter activity was detected in the seeds, leaves and roots of transgenic cucumber lines.

Pollen viability assessments across transgenic lines revealed a broad spectrum of abortion rates (16–90%), correlating strongly with *CsDMC1* downregulation (67–94%; $r = 0.87$). These results confirm that *CsDMC1* plays a central role in crossover formation and pollen fertility, consistent with findings in *Arabidopsis*, potato, and barley, where *DMC1* disruption led to defective homologous recombination, pollen abortion, and chromosomal abnormalities (Kumar et al. 2023; Szurman-Zubrzycka et al. 2019; Wijnker et al. 2012). Mechanistically, *DMC1* acts in partnership with RAD51 to mediate homologous strand invasion, chiasma formation, and proper chromosome segregation during meiosis (Da Ines et al. 2022). Suppression of *DMC1* expression is therefore expected to reduce crossover frequency and consequently pollen viability. If crossover formation is completely suppressed through *CsDMC1* silencing, random segregation of chromosomes during meiosis theoretically reduces the frequency of viable pollens to $1/2^n$, where n is the haploid chromosome number; for cucumber (n = 7), this equates to a probability of 1 in 128.

The frequency of meiotic crossovers in non-transgenic cucumber revealed a genetic distance of 30.5 cM between two SSR markers, closely aligning with the previously reported value of 32 cM (Ren et al. 2009). In the T3 transgenic line—characterized by 80% CsDMC1 silencing and 55% pollen abortion—testcross evaluation demonstrated a 46% reduction in crossover frequency (from 30.5 cM to 16.6 cM), highlighting a direct, dose-dependent relationship between DMC1 function, recombination rate, and gamete viability (Draeger et al. 2023). In cucumber, a recent multi-omic map revealed how genomic and epigenetic features influence crossover localization, implying that chromatin structure - such as DNA methylation or chromatin state - could modulate recombination (Wang et al. 2023b). Consequently, the observed variation in pollen abortion rates across transgenic lines likely reflects differential degrees of crossover suppression, attributable to *CsDMC1* silencing as well as underlying genomic and epigenetic influences. To facilitate predictive modeling of recombination rates based on pollen viability data, we recommend establishing correlations between viability and crossover frequencies across multiple loci. Such correlations would enhance reverse breeding efficiency by enabling the selection of transgenic lines that balance adequate microspore viability with targeted recombination suppression.

The absence of pollen abortion in several RNAi transgenic lines, as well as in lines expressing the GUS reporter under the control of the *CsDMC1* promoter, together with the observed *CsDMC1* expression patterns and pollen viability data, indicates that increasing *CsDMC1* expression alone is unlikely to enhance meiotic crossover frequency. In *Arabidopsis thaliana*, three major pathways limit meiotic recombination, including (i) the *FANCM* helicase and its cofactors; (ii) the *BLM/Sgs1* homologs *RECQ4A* and *RECQ4B* together with their partners *TOP3α* and *RMI1*; and (iii) the *FIGL1* AAA-ATPase complex (Crismani et al. 2012; Fernandes et al. 2018; Girard et al. 2015; Rafiei and Ronceret 2023; Séguéla-Arnaud et al. 2017). Together, these anti-crossover pathways establish an upper limit on meiotic recombination, ensuring genome stability while maintaining sufficient crossovers for accurate chromosome segregation.

Reverse breeding relies on suppressing crossover while maintaining meiotic integrity so that non-recombinant gametes can produce doubled haploids that recapitulate parental genotypes. In the

absence of crossovers, the proportion of viable gametes decreases exponentially with increasing chromosome number. For instance, in Arabidopsis (n = 5) and cucumber (n = 7), the expected ratios of viable gametes are 1/32 and 1/128, respectively, thereby restricting the practical application of reverse breeding to species with relatively low chromosome numbers (Dirks et al. 2009). Furthermore, although several protocols for doubled haploid production in cucumber have been developed (Claveria et al. 2005; Salehian et al. 2023), their efficiency in generating adequate numbers of plants from lines with low gamete viability remains unclear.

5. Conclusion

This study demonstrates that targeted silencing of *CsDMC1* effectively suppresses meiotic recombination in cucumber, leading to a significant reduction in crossover frequency accompanied by variable pollen abortion. Functional analysis confirmed that the *CsDMC1* promoter drives highly specific expression during meiosis, making it a reliable regulatory element for microspore-targeted gene manipulation. The strong correlation between *CsDMC1* downregulation, pollen viability, and crossover suppression underscores the central role of *CsDMC1* in homologous recombination and suggests that its endogenous abundance exceeds the minimum requirement for normal crossover formation. These findings establish *CsDMC1* silencing as a promising molecular approach for reverse breeding and haplotype preservation in cucumber. Further optimization of silencing strategies and validation across additional genomic regions will enhance its practical application in advanced breeding and meiosis-specific genome engineering.

Declarations

Conflict of interest

The authors declare no competing interests.

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Author Contribution

R. A. performed experiments and wrote the manuscript; P. A. supervised the research and edited the manuscript; Z. H. supervised the research; I. A. assisted in practical experiments. All authors read the manuscript and verified its contents.

References

1. Bishop DK, Park D, Xu L, Kleckner N (1992) : a meiosis-specific yeast homolog of. coli recA required recombination synaptonemal complex formation cell cycle progression Cell 69:439–456. https://doi.org/10.1016/0092-8674(92)90446-J. DMC1
2. Claveria E, Garcia-Mas J, Dolcet-Sanjuan R (2005) Optimization of cucumber doubled haploid line production using in vitro rescue of in vivo induced parthenogenic embryos. J Am Soc Hortic Sci 130:555–560. https://doi.org/10.21273/JASHS.130.4.555
3. Crismani W, Girard C, Froger N, Pradillo M, Santos JL, Chelysheva L, Copenhaver GP, Horlow C, Mercier R (2012) FANCM limits meiotic crossovers. Science 336:1588–1590. https://doi.org/10.1126/science.1220381
4. Da Ines O, Bazile J, Gallego ME, White CI (2022) attenuates. PLoS Genet 18:e1010322. https://doi.org/10.1371/journal.pgen.1010322. DMC1
5. Da Ines O, Degroote F, Goubely C, Amiard S, Gallego ME, White CI (2013) Meiotic recombination in. is catalysed DMC1 RAD51 Play supporting role PLoS Genet 9:e1003787. https://doi.org/10.1371/journal.pgen.1003787
6. Dirks R, Van Dun K, De Snoo CB, Van Den Berg M, Lelivelt CL, Voermans W, Woudenberg L, De Wit JP, Reinink K, Schut JW et al (2009) Reverse breeding: a novel breeding approach based on engineered meiosis. Plant Biotechnol J 7:837–845. https://doi.org/10.1111/j.1467-7652.2009.00450.x
7. Draeger TN, Rey M-D, Hayta S, Smedley M, Martin AC, Moore G (2023) stabilizes crossovers at high and low temperatures during wheat meiosis. Front Plant Sci 14:1208285. https://doi.org/10.3389/fpls.2023.1208285. DMC1
8. FAOSTAT (2024) FAOSTAT Statistical Database. Food and Agriculture Organization of the United Nations (FAO). https://www.fao.org/faostat/en/#home
9. Fernandes JB, Séguéla-Arnaud M, Larchevêque C, Lloyd AH, Mercier R (2018) Unleashing meiotic crossovers in hybrid plants. Proc Natl Acad Sci USA 115:2431–2436. https://doi.org/10.1073/pnas.1713078114
10. Girard C, Chelysheva L, Choinard S, Froger N, Macaisne N, Lehmemdi A, Mazel J, Crismani W, Mercier R (2015) AAA-ATPase FIDGETIN-LIKE 1 and helicase FANCM antagonize meiotic crossovers by distinct mechanisms. PLoS Genet 11:e1005369. https://doi.org/10.1371/journal.pgen.1005369
11. Kumar A, Siddappa S, Bhardwaj V, Dalamu, Singh B, Sharma N, Dipta B, Kumar V, Goutam U, Sood S (2023) Generation of asynaptic mutants in potato by disrupting. gene using rna interference approach Life 13:174. https://doi.org/10.3390/life13010174
12. Lambing C, Heckmann S (2018) Tackling plant meiosis: from model research to crop improvement. Front Plant Sci 9:829. https://doi.org/10.3389/fpls.2018.00829
13. Mercier R, Mézard C, Jenczewski E, Macaisne N, Grelon M (2015) The molecular biology of meiosis in plants. Annu Rev Plant Biol 66:297–327. https://doi.org/10.1146/annurev-arplant-050213-035923
14. Mieulet D, Aubert G, Bres C, Klein A, Droc G, Vieille E, Rond-Coissieux C, Sanchez M, Dalmais M, Mauxion J-P et al (2018) Unleashing meiotic crossovers in crops. Nat Plants 4:1010–1016. https://doi.org/10.1038/s41477-018-0311-x

15. Nanasato Y, Konagaya K-i, Okuzaki A, Tsuda M, Tabei Y (2013) Improvement of Agrobacterium-mediated transformation of cucumber (. L) combination vacuum infiltration co-cultivation filter paper wicks Plant Biotechnol Rep 7:267–276. https://doi.org/10.1007/s11816-012-0260-1
16. Neale MJ, Keeney S (2006) Clarifying the mechanics of DNA strand exchange in meiotic recombination. Nature 442:153–158. https://doi.org/10.1038/nature04885
17. Peterson R, Slovin JP, Chen C (2010) A simplified method for differential staining of aborted and non-aborted pollen grains. Int J Plant Biol 1:e13–e13. https://doi.org/10.4081/pb.2010.e13
18. Rafiei N, Ronceret A (2023) Crossover interference mechanism: New lessons from plants. Front Cell Dev Biol 11:1156766. https://doi.org/10.3389/fcell.2023.1156766
19. Ren Y, Zhang Z, Liu J, Staub JE, Han Y, Cheng Z, Li X, Lu J, Miao H, Kang H et al (2009) An integrated genetic and cytogenetic map of the cucumber genome. PLoS ONE 4:e5795. https://doi.org/10.1371/journal.pone.0005795
20. Salehian H, Shahnazi S, Nazari M (2023) Production of doubled haploid plants in cucumber (. L) via parthenogenesis Vitro Cell Dev Biol Plant 59:467–474. https://doi.org/10.1007/s11627-023-10345-1
21. Séguéla-Arnaud M, Choinard S, Larchevêque C, Girard C, Froger N, Crismani W, Mercier R (2017) and. limit meiotic CO formation through their C-terminal domains Nucleic Acids Res 45:1860–1871. https://doi.org/10.1093/nar/gkw1210. RMI1
22. Szurman-Zubrzycka M, Baran B, Stolarek-Januszkiewicz M, Kwaśniewska J, Szarejko I, Gruszka D (2019) The. mutant allows insight into DNA double-strand break repair Dur meiosis barley (Hordeum vulgare L) Front Plant Sci 10:761. https://doi.org/10.3389/fpls.2019.00761
23. Taagen E, Bogdanove AJ, Sorrells ME (2020) Counting on crossovers: controlled recombination for plant breeding. Trends Plant Sci 25:455–465. https://doi.org/10.1016/j.tplants.2019.12.017
24. Wang X, Xie Z, Tian Z, Wang S, Shi G, Chen W, Cao G, Tian B, Wei X, Zhang L (2023a), a recombinase gene, is involved in seed germination in. under salt stress Agron 13:595. https://doi.org/10.3390/agronomy13030595. BrDMC1
25. Wang Y, Dong Z, Ma Y, Zheng Y, Huang S, Yang X (2023b) Comprehensive dissection of meiotic DNA double-strand breaks and crossovers in cucumber. Plant Physiol 193:1913–1932. https://doi.org/10.1093/plphys/kiad415
26. Wijnker E, van Dun K, de Snoo CB, Lelivelt CL, Keurentjes JJ, Naharudin NS, Ravi M, Chan SW, de Jong H, Dirks R (2012) Reverse breeding in. generates homozygous Parent lines heterozygous plant Nat Genet 44:467–470. https://doi.org/10.1038/ng.2203

Figures

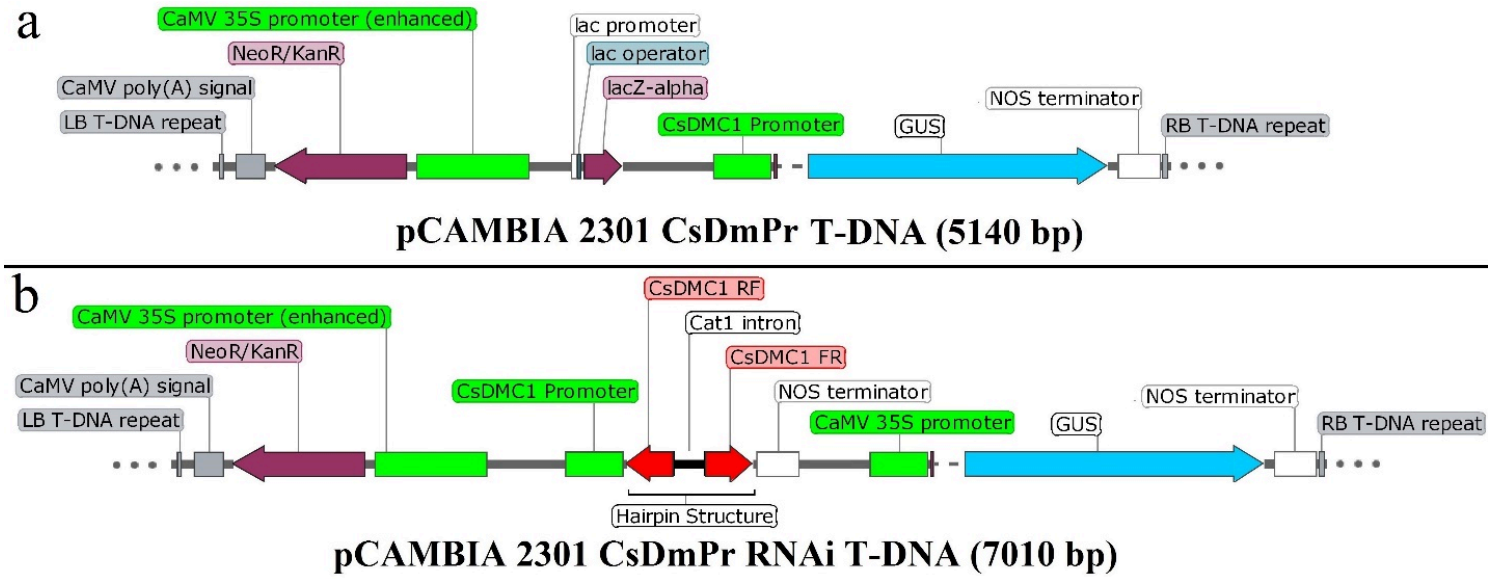


Figure 1

The T-DNA region of binary plasmids. (a) pCAMBIA2301-CsDmPr-GUS plasmid which was used to assess the activity of *CsDMC1* promoter, (b) pCAMBIA 2301-CsDmPr-RNAi plasmid, containing a 325-bp *CsDMC1* ORF hairpin structure for silencing the *CsDMC1*gene, with *GUS* reporter expression driven by the CaMV 35S promoter.

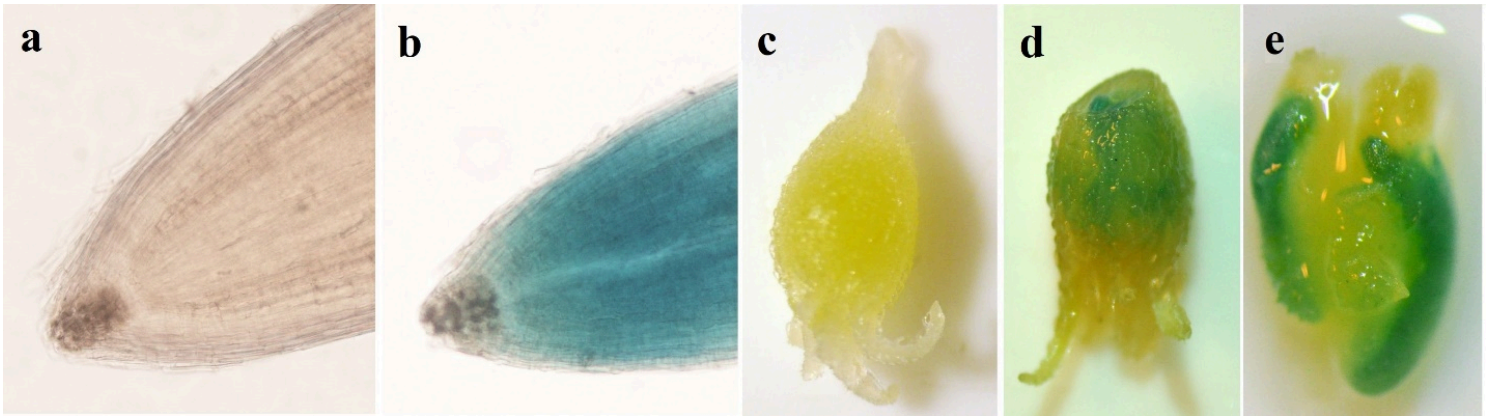


Figure 2

GUS staining in transgenic and non-transgenic plants, (a) Roots of non-transgenic cucumber. (b) Root of plants transformed with the pCAMBIA2301-CsDmPr-RNAi plasmid displaying clear GUS activity, (c) Male flower of non-transgenic cucumber. (d) and (e) strong GUS expression in male flowers and anthers of cucumber plants carrying the T-DNA of pCAMBIA2301-CsDmPr-GUS plasmid.

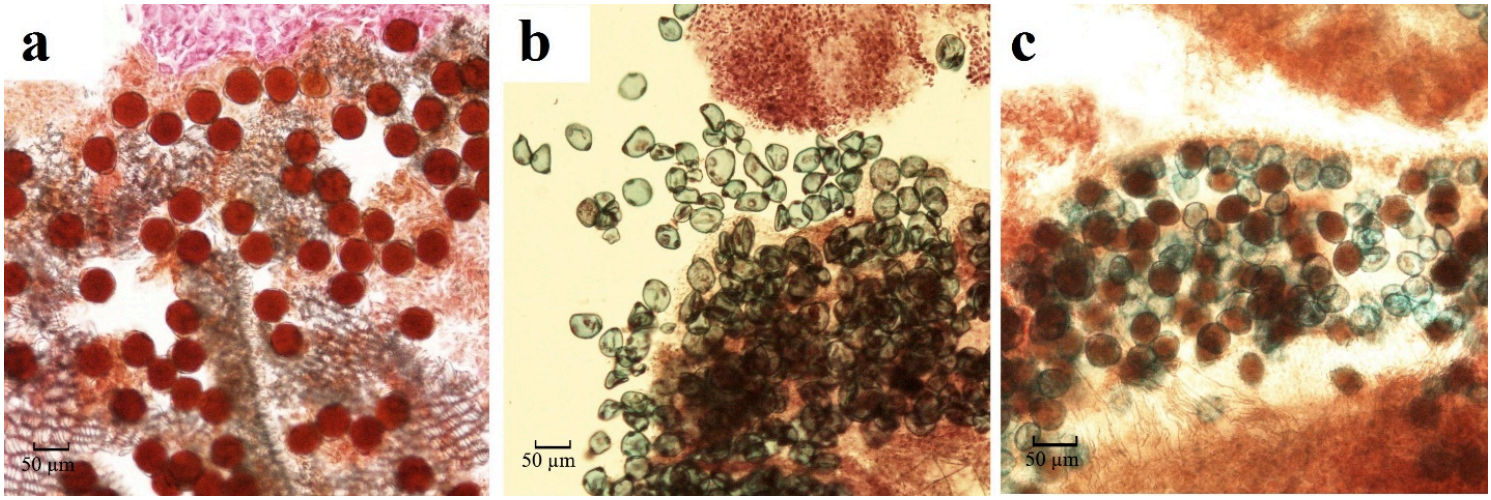


Figure 3

Morphology and staining patterns of pollen grains in transgenic and non-transgenic cucumber plants. (a) Viable pollen grains from a non-transgenic (control) plant, appearing red and non-shrunken. (b) Aborted pollen grains from the T2 transgenic line, appearing green and shrunken due to *CsDMC1* gene silencing and resultant crossover inhibition. (c) Anther from the T3 transgenic line containing a mixture of viable and aborted pollen grains.

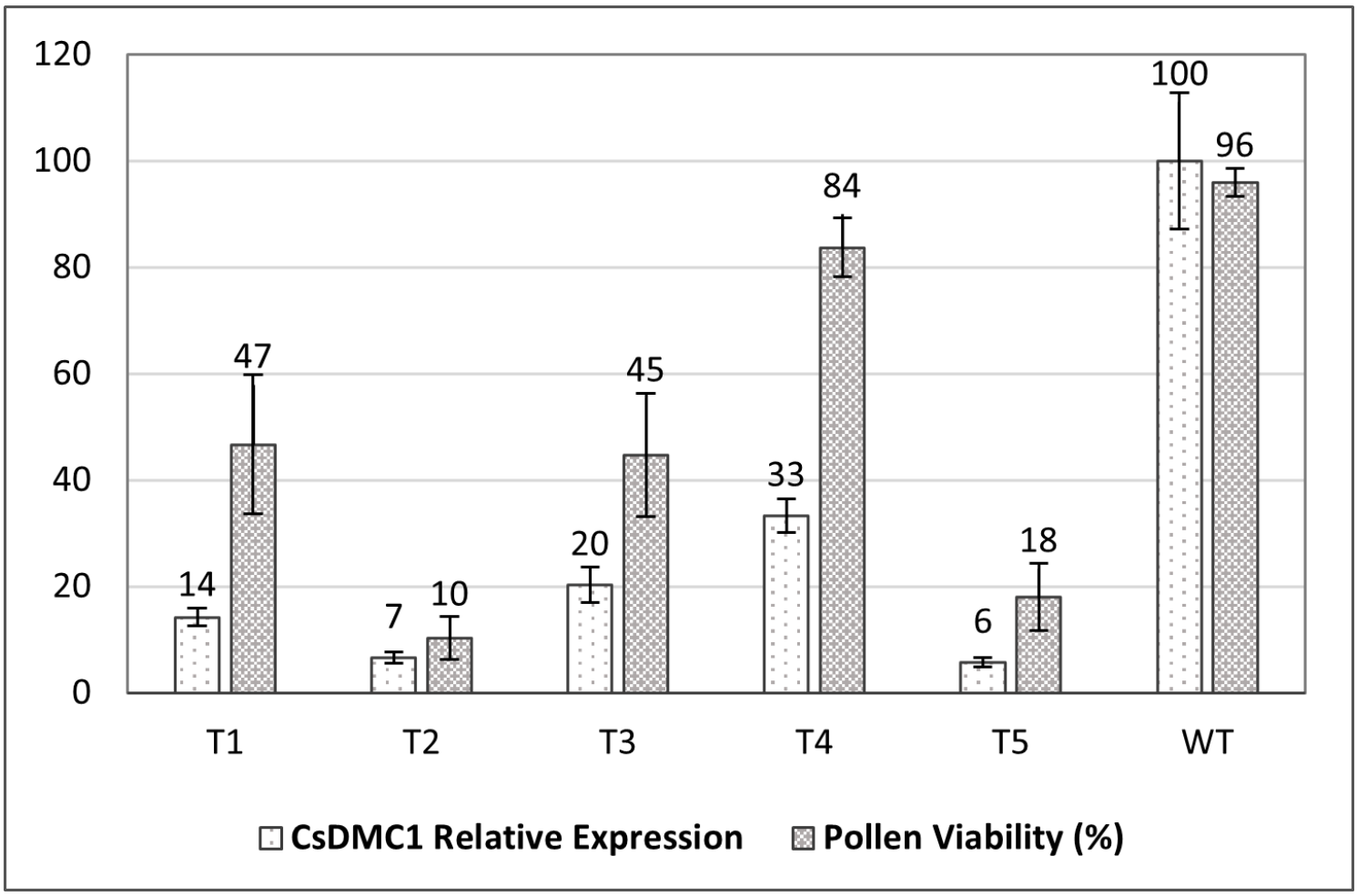


Figure 4

Relative *CsDMC1* gene expression and pollen viability in control plant (WT) and transgenic cucumber plants (T1-T5) in which the *CsDMC1* is downregulated by RNAi method.