

Supplementary Information

GDP-D-mannose epimerase regulates male gametophyte development, plant growth and leaf senescence in *Arabidopsis*

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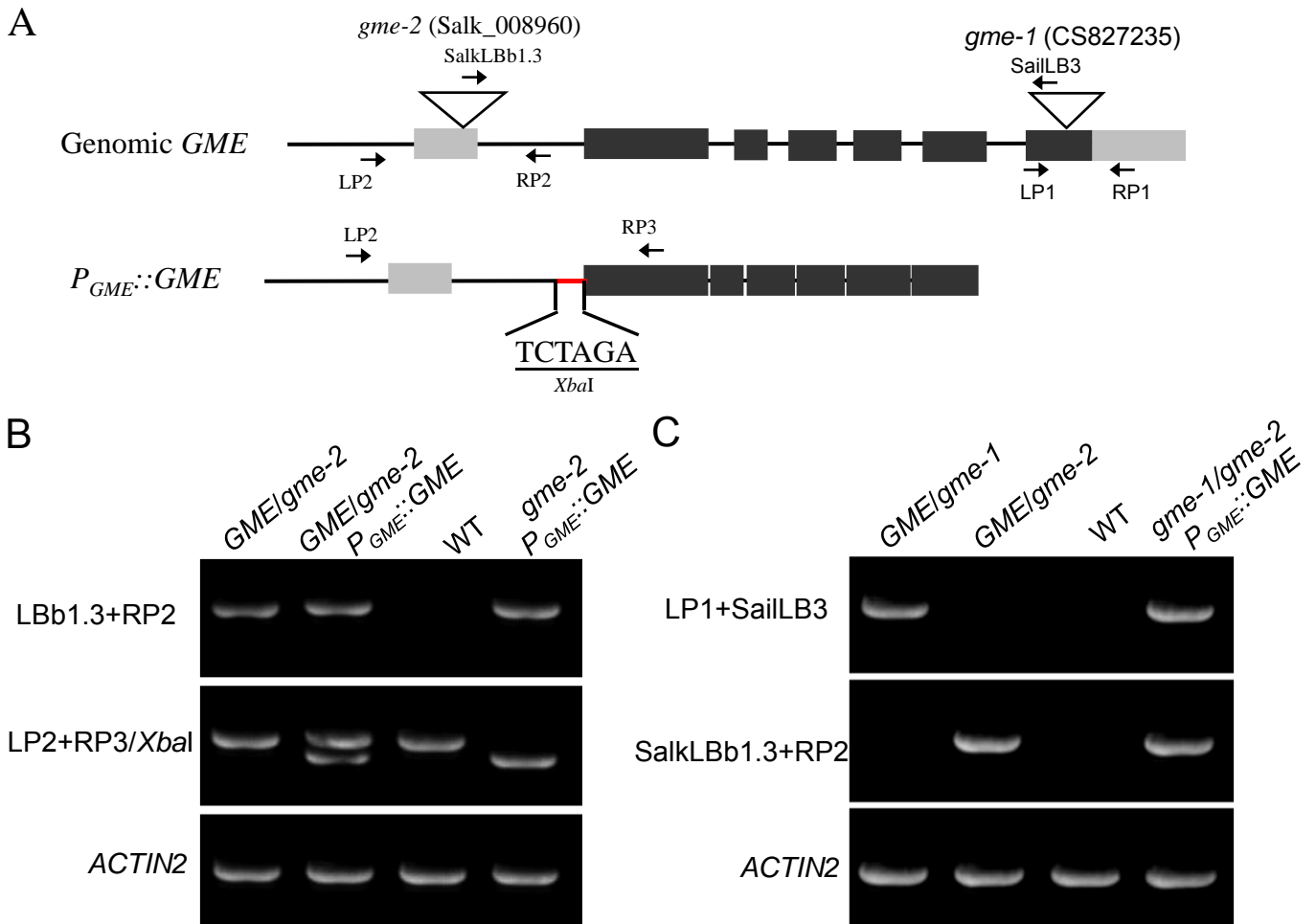
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Supplemental Figure 1. Genotyping of *gme* mutants and complementary transgenic lines.

(A) Schematic diagrams showing the T-DNA insertion site in *gme-2*, and the $P_{GME}::GME$ vector. The grey rectangle, black rectangle and triangle represent the UTR, exon, and T-DNA insertion site, respectively. The red line indicates the inserted artificial sequence in the $P_{GME}::GME$ vector. The primers indicated by arrows were used to identify the mutant background in (B) and (C).

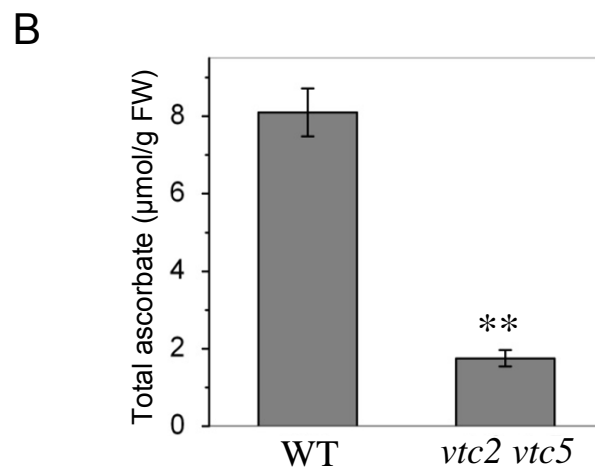
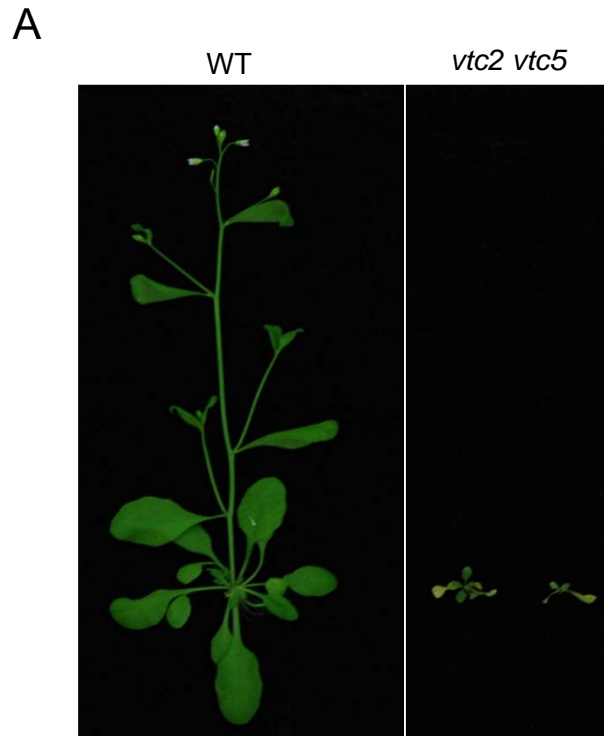
(B) PCR to identify the genetic background of the $GME/gme-2$, $GME/gme-2 P_{GME}::GME$, WT and $gme-2/gme-2 P_{GME}::GME$ plants. The top panel shows the PCR products for the *gme-2* T-DNA insertion using primers RP2 and LBb1.3. The middle panel shows the results of the digestion of the PCR products amplified by primers LP2 and RP3 with *Xba*I. The bottom panel shows the PCR products for *ACTIN2* as a control.

(C) PCR to identify the genetic background of the $GME/gme-1$, $GME/gme-2$, WT and $gme-1/gme-2 P_{GME}::GME$ plants. The top and middle panels, respectively, show the PCR products for the *gme-1* T-DNA insertion (LP1 and LB3) and *gme-2* T-DNA insertion (LP2 and LBb1.3). The bottom panel shows the PCR products for *ACTIN2* as a control.



Supplemental Figure 2. Phenotype of a *GME/gme-1* heterozygous plant.

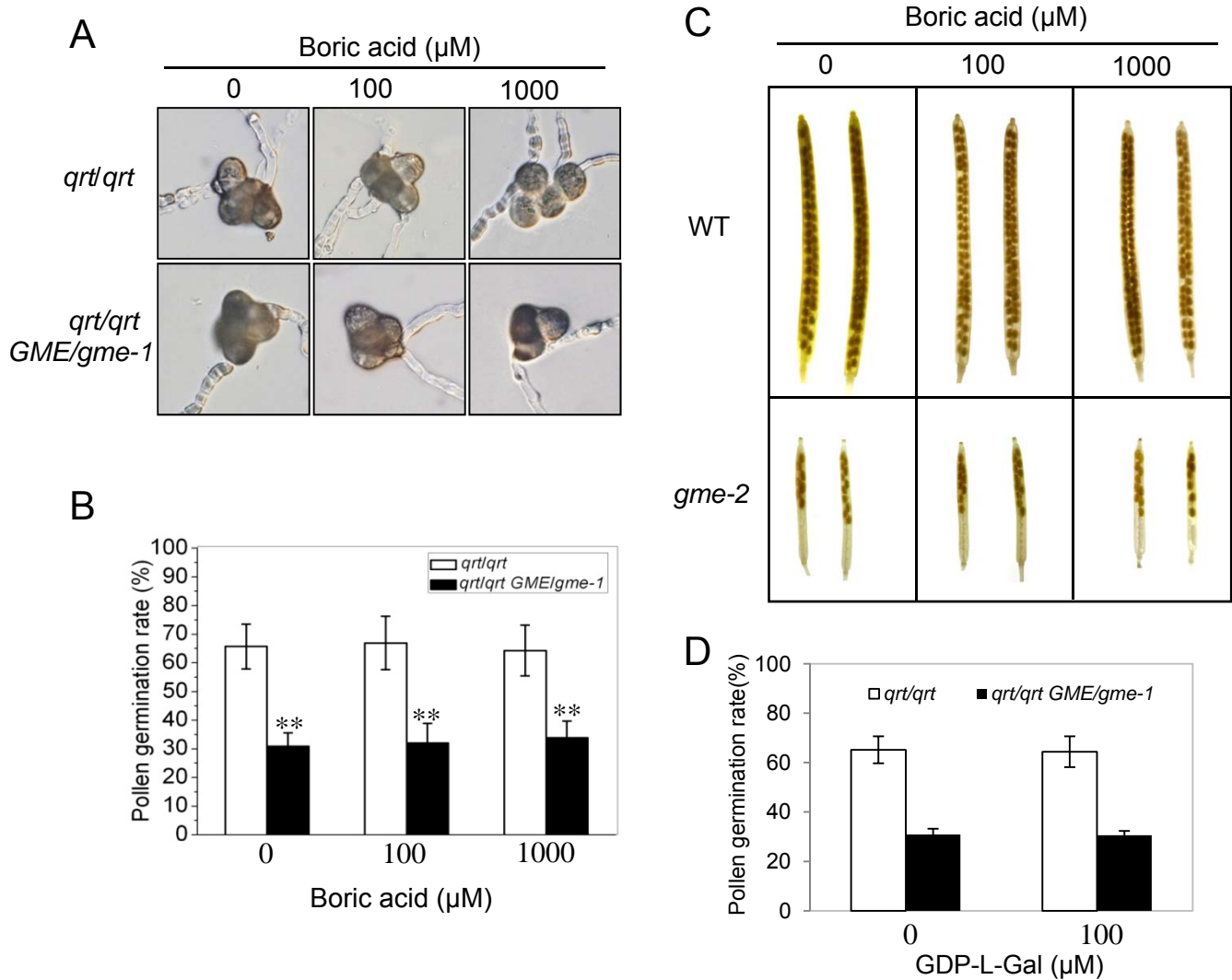
Six-week-old WT and *GME/gme-1* plants (top). The siliques were bleached with ethanol and imaged (bottom). As shown, the *GME/gme-1* plant grew normally and exhibited similar fertility to wild type.



Supplemental Figure 3. Plant phenotype and total ascorbate content in the *vtc2 vtc5* double mutant.

(A) Morphology of 5-week-old WT and *vtc2 vtc5* double mutant plants.

(B) Total ascorbate contents in the leaves of 4-week-old Col-0 WT and *vtc2 vtc5* plants. Error bars represent the SE (n=3). Asterisks represent Student's *t*-test significance compared with wild type (*P<0.05, **P<0.01).

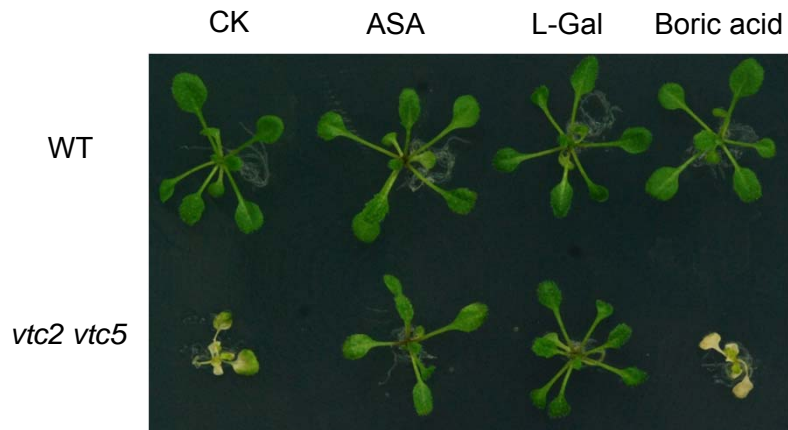


Supplemental Figure 4. Treatment with boric acid or GDP-L-Gal could not rescue the fertility of the *gme* mutant.

(A and B) *In vitro* pollen germination (A) and the pollen germination rates (B) of *qrt/qrt* and *qrt/qrt GME/gme-1* on pollen germination medium containing the indicated concentrations of boric acid. Error bars represent the SE (n=3).

(C) Inflorescences of 5-week-old WT and *gme-2* plants were treated with 0, 100 or 1000 μM boric acid for 10 days. The siliques were derived from flower buds of WT and *gme-2* plants treated with 0, 10 or 100 μM boric acid. Asterisks represent Student's *t*-test significance compared with *qrt/qrt* (**P<0.01).

(E) The pollen germination rates of *qrt/qrt* and *qrt/qrt GME/gme-1* on pollen germination medium containing the indicated concentration of GDP-L-gal sodium salt. Error bars represent the SE (n=3).



Supplemental Figure 5. The growth of *vtc2 vtc5* could be rescued by treatment with ASA or L-Gal.

Three-week-old seedlings of Col-0 WT and *vtc2 vtc5* double mutant plants grown on boric acid-free MS medium treated with mock, 100 μ M ASA, 100 μ M L-Gal or 100 μ M boric acid.

Supplemental Table 1. Genetic transmission analysis of *vtc2* and *vtc5* alleles

Genotype	T-DNA ⁺	T-DNA ⁻	TE(%)
♀ WT × ♂ <i>VTC2/vtc2 vtc5/vtc5</i>	134	141	48.7
♀ <i>VTC2/vtc2 vtc5/vtc5</i> × ♂ WT	107	101	51.4
♀ WT × ♂ <i>vtc2/vtc2 VTC5/vtc5</i>	103	108	48.8
♀ <i>vtc2/vtc2 VTC5/vtc5</i> × ♂ WT	112	108	50.9

Progeny containing a T-DNA insertion (*vtc2* for the top two panels and *vtc5* for the bottom two panels) were identified by PCR. Transmission efficiency (TE)=Number of progeny with a T-DNA insertion/Number of progeny × 100%. WT, Col-0 wild type.

Supplemental Table 2. Primers used for identification of *gme* T-DNA mutants and generation of constructs.

<i>gme-1</i> (CS827235)-LP1	GACAAAATCAGATTTCCGTG
<i>gme-1</i> (CS827235)-RP1	GCTGAACTTAAAAGGCCATATTTG
<i>gme-1</i> (CS827235)-SAILB3	TAGCATCTGAATTTTCATAACCAATCTCGATACAC
<i>gme-2</i> (SALK_008960)-LP2	TTCCTCTTCCTCAAATCTCTG
<i>gme-2</i> (SALK_008960)-RP2	AGAGTTTTGACAGAGGTAAG
<i>gme-2</i> (SALK_008960)-SalkLBb1.3	ATTTTGCCGATTTTCGGAAC
<i>GME</i> -LP2	TTCCTCTTCCTCAAATCTCTG
<i>GME</i> -RP3	AATCACAGAGTGATTACTCTG
<i>GME</i> pro-HindIII-Forward	CCCAAGCTTGATCAAGATGACTAATTTAAC
<i>GME</i> pro-XbaI-Reverse	CTAGTCTAGATCTACATTAACAAAACAAATCTG
<i>GME</i> -XbaI-Forward	TGCTCTAGAATGGGAACTACCAATGGAACAG
<i>GME</i> -SacI-Reverse	ATCGAGCTCTCACTCTTTTCCATCAGCCGC
35S:: <i>GME</i> -GFP-EcoRI-Forward	CCGGAATTCATGGGAACTACCAATGGAACAGACTATG
35S:: <i>GME</i> -GFP-BamHI-Reverse	CGCGGATCCTCACTCTTTTCCATCAGCCGC
35S:: <i>GME</i> -GFP-XhoI-Forward	ACCGCTCGAGATGGGAACTACCAATGGAAC
35S:: <i>GME</i> -GFP-EcoRI-Reverse	GGGGAATTCGCTCTTTTCCATCAGCCGCGC
<i>GME</i> -Realtime-Forward	GGAGCTGGAGGTTTCATTGC
<i>GME</i> -Realtime-Reverse	CGTAATGACCTTCGTGCTTCAA
<i>GME</i> -RT-Forward	ATGCTTCGAGTGCTTGTATC
<i>GME</i> -RT-Reverse	GAAAGCAGCTGGAGCCTTCTC
<i>vtc2-2</i> (CS876707)-LP	CGGAGAGGTAAATCATAGAC
<i>vtc2-2</i> (CS876707)-RP	CCATACTCTATCGGACTAAC
<i>vtc2-2</i> (CS876707)-SAILB3	TAGCATCTGAATTTTCATAACCAATCTCGATACAC
<i>vtc5-2</i> (SALK_135468)-LP	GCTGAAGCCGATAATCCGTA
<i>vtc5-2</i> (SALK_135468)-RP	CCTCCATGCCTTCTCTCTG
<i>vtc5-2</i> (SALK_135468)-SalkLBb1.3	ATTTTGCCGATTTTCGGAAC
<i>CABI</i> -Realtime-Forward	GCAAGGAACCGTGAAGTAGAA

<i>CAB1</i> -Realtime-Reverse	TCCGAACTTGACTCCGTTTC
<i>CAB2</i> -Realtime-Forward	TCAATCTTTTGAATTCGAGTGAGA
<i>CAB2</i> -Realtime-Reverse	TCCACCACAAACACAAACCTAC
<i>RBCS</i> -Realtime-Forward	CGCTCCTTTCAACGGACTTA
<i>RBCS</i> -Realtime-Reverse	AGTAATGTCGTTGTTAGCCTTGC
<i>SAG13</i> -Realtime-Forward	AGGAAAACCTCAACATCCTCGTC
<i>SAG13</i> -Realtime-Reverse	GCTGACTCGAGATTTGTAGCC
<i>SAG21</i> -Realtime-Forward	CATTCCTCGGATATATCTCTCCTT
<i>SAG21</i> -Realtime-Reverse	TTCTTGAAAAGAGAGAAAACAGACTTT
<i>SEN4</i> -Realtime-Forward	AAGGTGACAAAGAGCAACAATTC
<i>SEN4</i> -Realtime-Reverse	CTCTCTAATGGGTGTGTCATCG
<i>ACTIN2</i> -RT-Forward	GCACCCTGTTCTTCTTACCG
<i>ACTIN2</i> -RT-Reverse	AACCCTCGTAGATTGGCACA
<i>ACTIN2</i> -Realtime-Forward	CCGCTCTTTCTTTCCAAGC
<i>ACTIN2</i> -Realtime-Reverse	CCGGTACCATTGTCACACAC