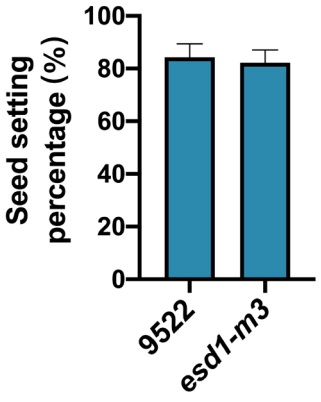
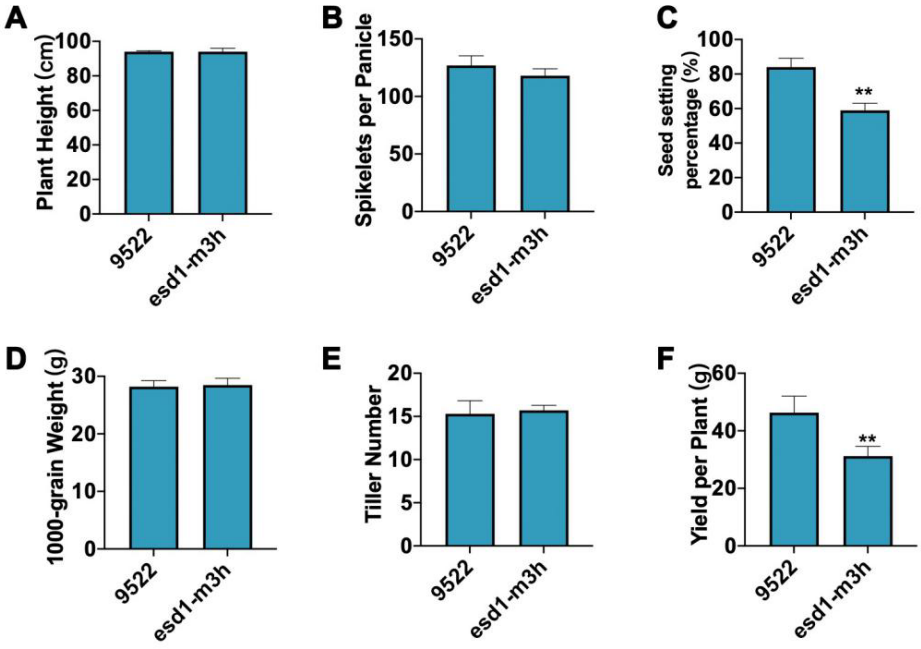
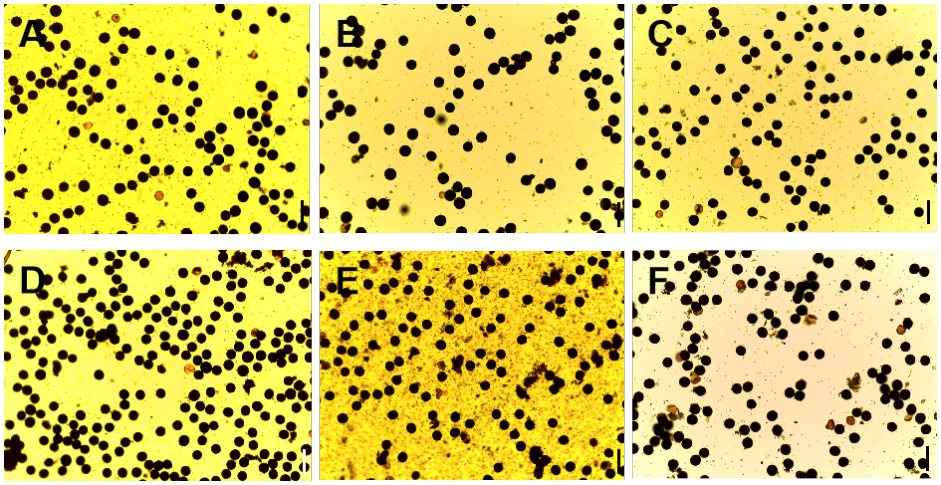


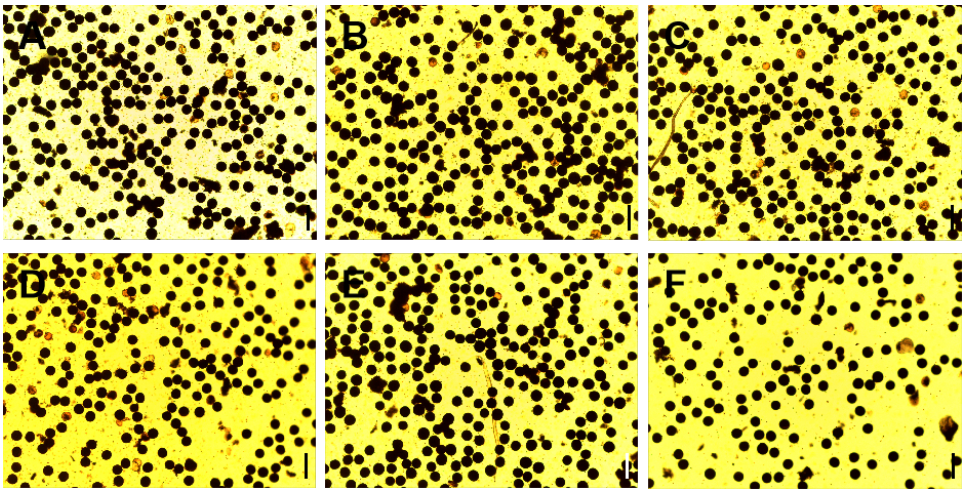
Supplementary Figure S1. The seed setting rate of *esd1-m3.* Error bars indicate SD (n=15).



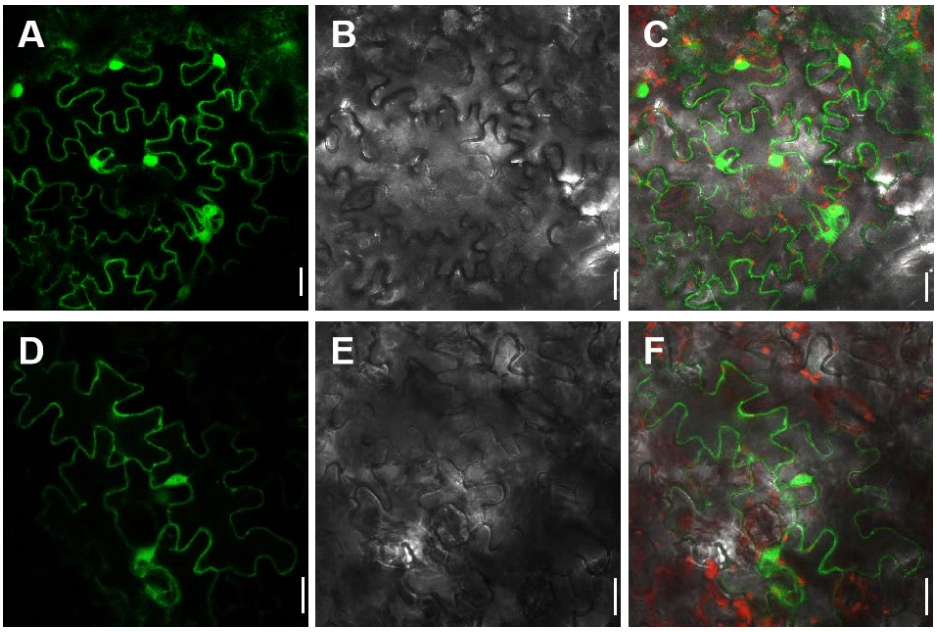
Supplementary Figure S2. Phenotypic data of *esd1-m3h*. A, Plant height of *esd1-m3h*. B, Spikelets per panicle of *esd1-m3h*. C, Seed setting rate of *esd1-m3h*. D, 1000-grain weight of *esd1-m3h*. E, Tiller number of *esd1-m3h*. F, Yield per plant of *esd1-m3h*. Error bars indicate SD (n=15).



Supplementary Figure S3. Effects of photoperiods on the fertility of *esd1* mutants. A, 9522 for long-day treatment. B, *esd1-m1* for long-day treatment. C, *esd1-m2* for long-day treatment. D, 9522 for short-day treatment. E, *esd1-m1* for short-day treatment. F, *esd1-m2* for short day treatment. bars=50 μm.



Supplementary Figure S4. Effects of temperature treatments on the fertility of *esd1* mutants. A, 9522 for high-temperature treatment. B, *esd1-m1* for high-temperature treatment. C, *esd1-m2* for high-temperature treatment. D, 9522 for low-temperature treatment. E, *esd1-m1* for low-temperature treatment. F, *esd1-m2* for low-temperature treatment. bars=50 μm.



Supplementary Figure S5. The subcellular localization of ESD1 in tobacco. A-C, *p1305-GFP*. D~F, *p1305-ESD1-GFP*. A, D were the green fluorescent channel. B, E were the bright field channel. C, F were the green fluorescence + bright field channel. bars=20 μm.

Supplemental Table S1. Subcellular localization prediction for ESD1 protein with TargetP

Protein type	Other	Signal peptide	Mitochondrial transfer peptide	Chloroplast transfer peptide	Thylakoid luminal transfer peptide
Likelihood	0.9431	0.002	0.0019	0.0528	0.0001

Supplemental Table S2. Amplification primers for target sites TS1 and TS2

Primer Names	Sequences (5'-3')
ESD1-U3-F	ggcaGTACCTTGATGATGTCGAGG
ESD1-U3-R	aaacCCTCGACATCATCAAGGTAC
ESD1-U6a-F	gccgGAGTTCCTCAAGTCGATGA
ESD1-U6a-R	aaacTCATCGACTTGAGGAACTC

Supplemental Table S3. Amplification primers for expression cassettes

Primer Names	Sequences (5'-3')
Uctcg-B1'	TTCAGAggtctcTctcgCACTGGAATCGGCAGCAAAGG
gRctga-B2	AGCGTGggtctcGtcagGGTCCATCCACTCCAAGCTC

Uctga-B2’	TTCAGAggtctcTctgaCACTGGAATCGGCAGCAAAGG
gRcggt-BL	AGCGTGggtctcGaccgGGTCCATCCACTCCAAGCTC

Supplemental Table S4. Detection primers for target sites mutation

Primer Names	Sequences (5’-3’)
SP1	CCCGACATAGATGCAATAACTTC
SP2	GCGCGGTGTCATCTATGTTACT
ESD1-JC-F	GGGATGATGCCAAGGAAC
ESD1-JC-R	GAGGAGGAGGTCGGTGAA

Supplemental Table S5. Primers for qRT-PCR reaction

Primer Names	Sequences (5’-3’)
ESD1-qP-F	CCTTGATCCGCCGCTGTA
ESD1-qP-R	GGAATCCACGATGGAGTTCG
Action-F	CCTTCAACACCCCTGCTATG
Action-R	CAATGCCAGGGAACATAGTG

Supplemental Table S6. Primers for *in situ* hybridization

Primer Names	Sequences (5’-3’)
ESD1-ish-F	CGGCCTTTCTTGACGACCTT
ESD1-ish-R	GTCCGGGCGACTCAAAGAA