Pyrimidine degradation influences germination and production of Arabidopsis seeds

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Supplementary information

Table S1 PCR primers used in this study

Quantitative RT-PCR

Pyd1-RT_fwd	ACCGGTGTGATGATGCACG
Pyd1-RT_rev	TCAAGCCTCTCTTCTCAGC
ABI1_fwd	CGTTGGGTTTGGAGCGGG
ABI1_rev	GAGTGGACAACTCGGGTTCC
ABF4_fwd	GCTCAGGTTGCTGCAAGAGC
ABF4_rev	GCACCAACGCCTAAAGCTCC
EF1α q-fwd	GAGACCAAGTACTACTGCAC
EF1α q-rev	GTTGGTCCCTTGTACCAGTCAAG

T-DNA mutant screening

SAIL_LB	TAGCATCTGAATTTCATAACCAATCTCGATACAC
GK_LB	CCCATTTGGACGTGAATGTAGACAC
Pyd1-KO_fwd	GCGCAGAATGGGTGCCGCTGTTGG
Pyd1-KO_rev	CCAATACCCGAAAGCGAGCGATCC
Pyd1_fwd	TTTCATATGGCTTCCATGATGTTCGCC
Pyd1_rev	TTTCTCGAGGTTAGAAACCATACTCTCAGTCTCC

Generation of overexpressor mutants

Pyd1_fwd TTTCATATGGCTTCCATGATGTTCGCC

Pyd1_rev TTTCTCGAGGTTAGAAACCATACTCTCAGTCTCC

Table S2: Contents of storage compounds in Wildtype and *Pyd1* mutant seeds

Mature seeds were analyzed in an element analyzer (A) or lipid and protein were extracted and quantified subsequently (B). DW, dry weight, FW, fresch weight. Data represent the mean ± standard errors of five biological replicates.

(A)

	total nitrogen (N) [% DW]	total carbon (C) [% DW]	C/N
WT	4.2 ± 0.16	50.58 ± 0.49	11.80 ± 0.59
Pyd1-1	4.46 ± 0.14	51.35 ± 0.53	11.79 ± 0.49
Pyd1-2	4.69 ± 0.13	49.87 ± 0.34	10.80 ± 0.33
35S: Pyd1 #11	4.69 ± 0.05	49.97 ± 0.11	10.67 ± 0.13
35S: Pyd1 #19	4.74 ±0.08	50.32 ± 0.29	10.65 ± 0.25

(B)

	lipid content [mg g^{-1} FW]	protein content [mg g^{-1} FW]
WT	211.71 ± 15.71	269.21 ± 19.93
Pyd1-1	209.29 ± 12.85	269.21 ± 11.69
Pyd1-2	216.57 ± 8.94	287.29 ± 14.49
35S: Pyd1 #11	219.00 ± 2.49	289.12 ± 11.75
35S: Pyd1 #19	224.33 ± 16.04	306.65 ± 11.09

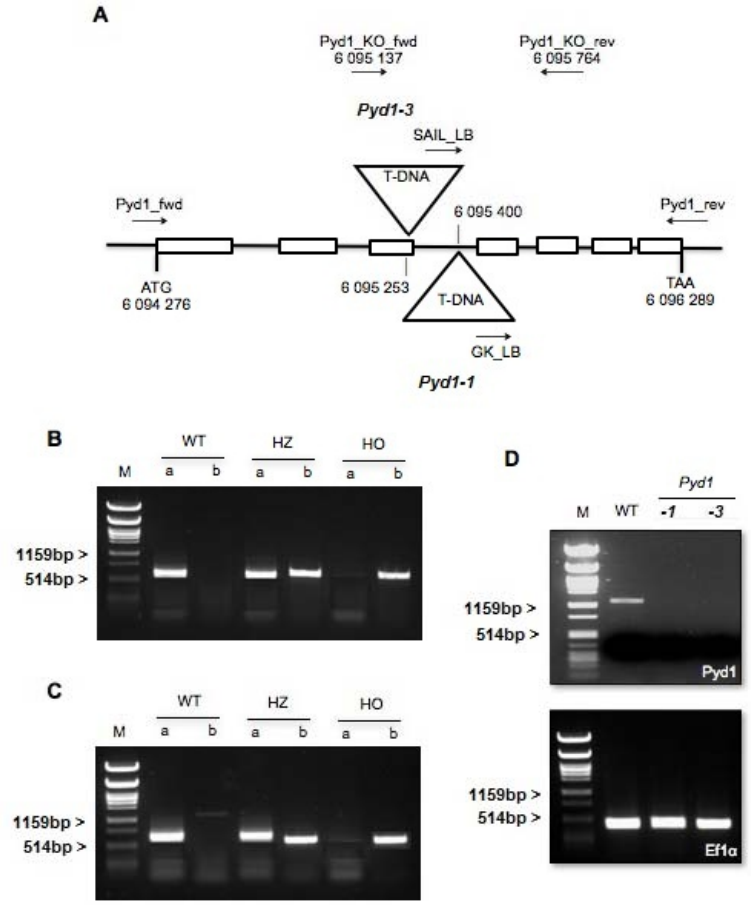

Figure S1

Genetic characterization of *Pyd1* T-DNA insertion mutants. A, Localization of T-DNA insertions in *Pyd1*. B, PCR analysis of genomic DNA from SAIL_363_E04, (*pyd1-3*), plants with gene specific (a) and T-DNA specific (b) primer combinations. C, PCR analysis of genomic DNA from GK-2511F09, (*pyd1-1*), plants with gene specific (a) and T-DNA specific (b) primer combinations. D, PCR analysis with gene specific primer combinations (Pyd1_fw, PYD1_rev, Table S1) on cDNA from WT, *pyd1-1* and *pyd1-3* mutants. Elongations factor EF1α served as control for the presence of cDNA. Lambda-DNA cut with PSTI was used as a marker; HZ, heterozygous plants; HO, homozygous plants.

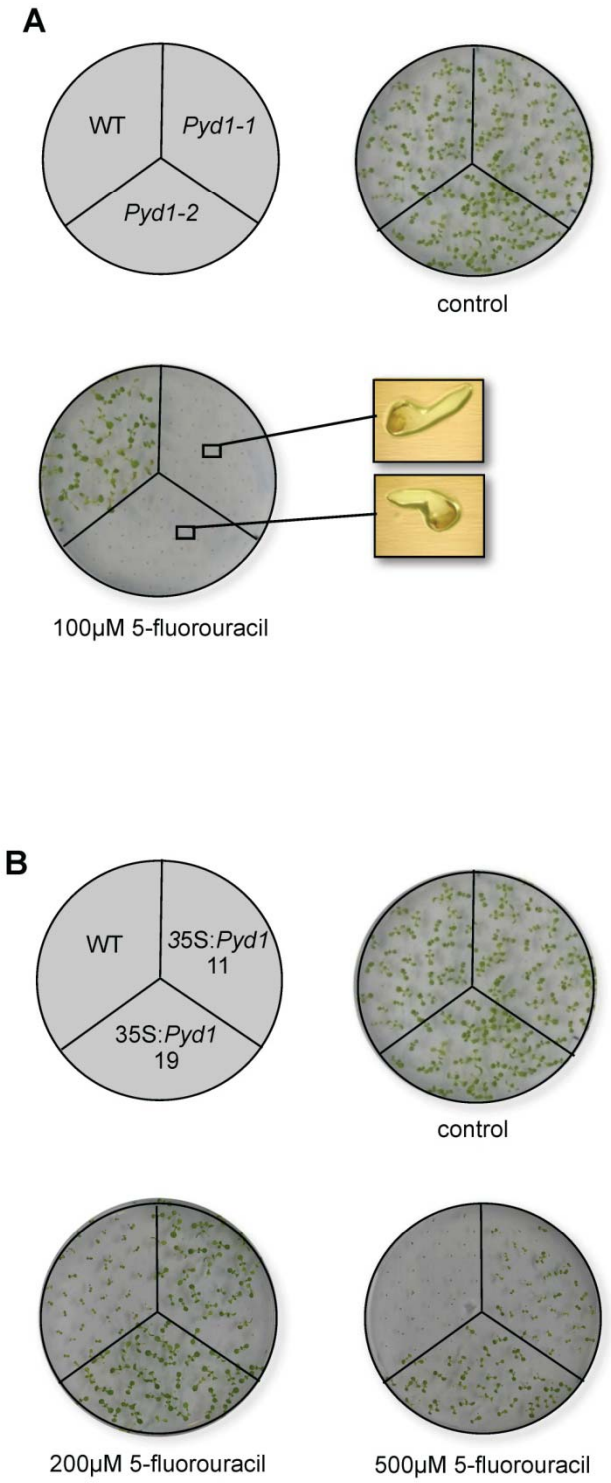

Figure S2
Growth of WT and *Pyd1* mutants in the presence and absence of toxic 5-fluorouracil. A, WT, *pyd1-1* and *pyd 1-3* T-DNA insertion lines. B ,WT and *35S:Pyd1* lines were compared.

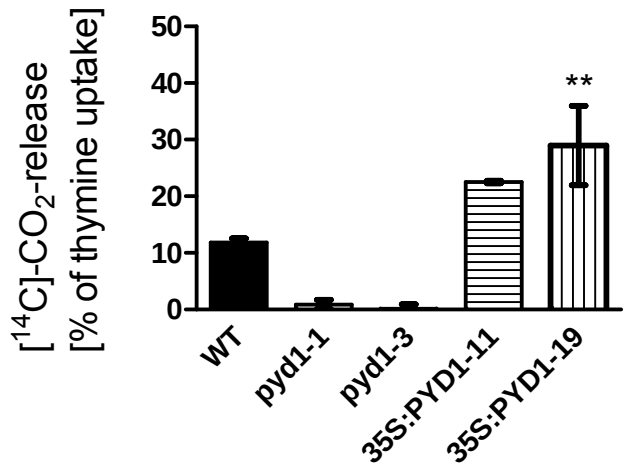

Figure S3
Catabolism of [^{14}C]-labelled thymine in *Pyd1* mutants. Degradation was measured as released [^{14}C]-CO_2 based on import of the corresponding substrate. Values represent means ± standard error of at least five biological replicates. The asterisks indicate significant differences between WT and mutants, based on a one way ANOVA test.

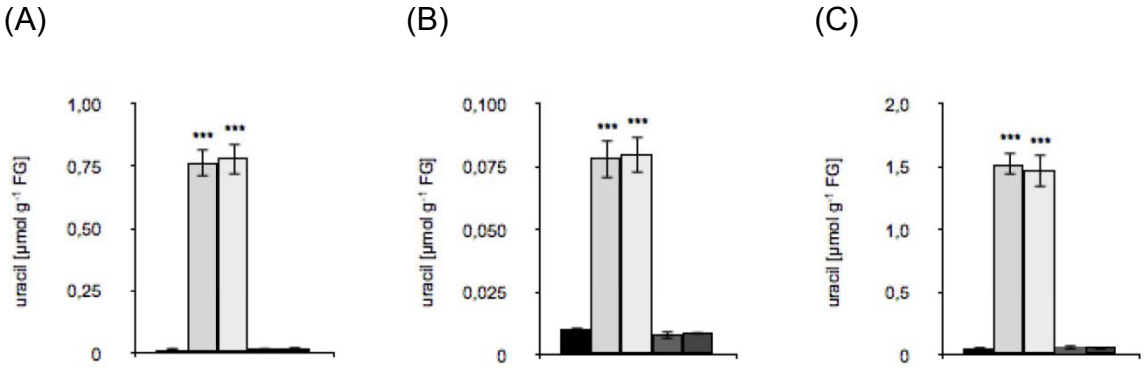

Figure S4
Uracil contents of WT and *Pyd1* mutants as indicated. The following tissues were analyzed: (A) senescent leaves, (B) stems (C) siliques of 10 weeks old plants. Values represent means ± standard error of at least five biological replicates. The asterisks indicate significant differences between WT and mutants, based on a one way ANOVA test (**P*<0.05, ***P*<0.01, ****P*<0.005).