Landes Bioscience www.landesbioscience.com

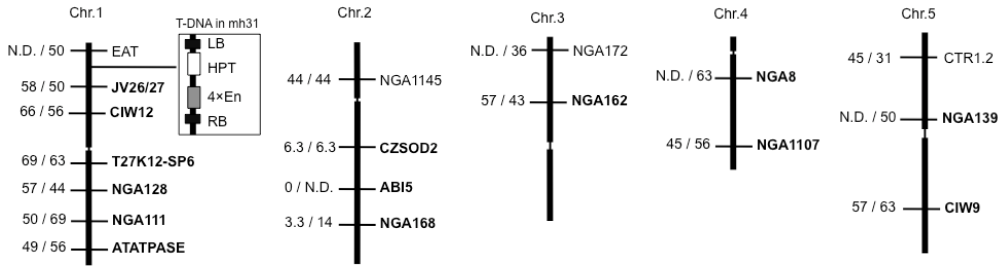
Supplemental Material to:

Kenji Tezuka, Teruaki Taji, Takahisa Hayashi and Yoichi Sakata

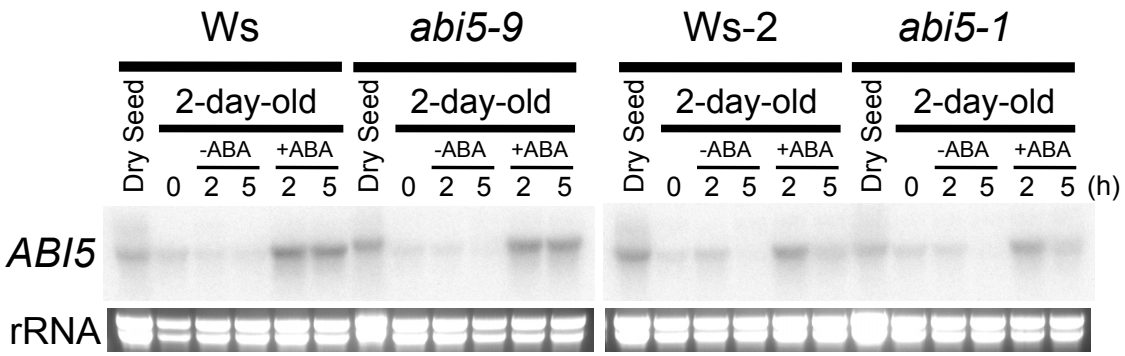
A novel abi5 allele reveals the importance of the conserved Ala in the C3 domain for regulation of downstream genes and salt tolerance during germination in Arabidopsis

Plant Signaling & Behavior 2013; 8(3)
http://dx.doi.org/10.4161/psb.23455

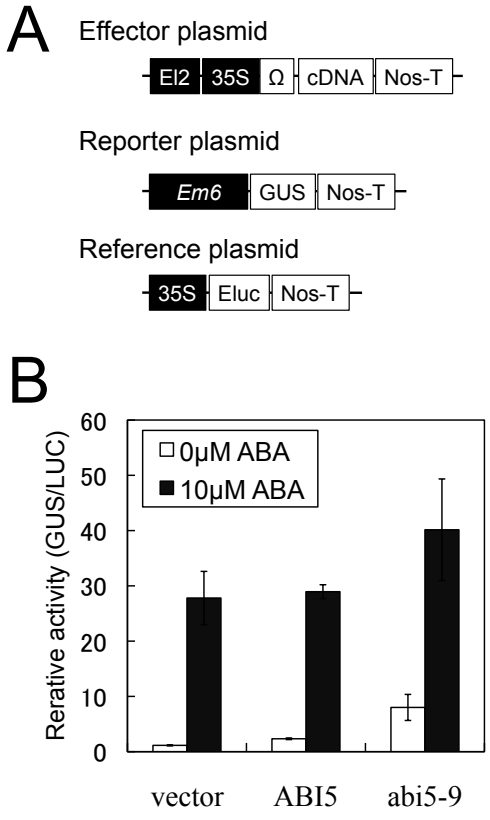
http://www.landesbioscience.com/journals/psb/article/23455



Supplementary Figure S1 (Sakata)



Supplementary Figure S2 (Sakata)



Supplementary Figure S3 (Sakata)

Supplementary Figure S1. Genetic linkage analysis of *mh31* gene. F_1 was obtained by crossing the *mh31* mutant plant (Ws wild-type background) with the Col-0 plant, and F_2 progeny was produced by self-fertilization of F_1. In the F_2 progeny, genetic linkage analysis of *mh31* was performed using SSLP markers. In the *mh31* mutant, a point mutation, a nucleotide substitution of C to G, was identified in the *ABI5* gene. This mutated nucleotide of *ABI5* derived from the *mh31* mutant was also used for genetic linkage analysis. Recombination values for the results of ABA response and mannitol response are indicated to the left and the right of the slash, respectively.

Supplementary Figure S2. RNA expression of *ABI5* in the *abi5-9* mutant. RNA expression levels were determined by northern blot. Two-day-old seedlings were transferred onto plates with or without 5 μM ABA, and incubated at 23°C for the indicated duration under continuous light (2000 lux). Total RNA was extracted, and 15 μg total RNA was used per lane. The ribosomal RNA bands stained with ethidium bromide in the gel were used to verify equal loading.

Supplementary Figure S3. Transactivation activity of abi5-9. (A) Schematic diagram of the effector and reporter constructs used in co-transfection experiments. El2: enhancer-like element of the cauliflower mosaic virus (CaMV) 35S promoter; 35S: CaMV35S; Ω: tobacco mosaic virus (TMV) omega sequence; Nos-T: nopaline synthase terminator. The cDNA indicated in the box is *ABI3*, *ABI5,* or *abi5-9* cDNA. The reporter construct, *Em6* promoter-*GUS*, contains 1.2 kb of the *Em6* promoter. (B)

Transactivation activity of the *Em6* promoter based on *abi5-9* expression. Transactivation experiments were performed using protoplasts prepared from Arabidopsis Col-0 leaves. The "vector" is pGHX as a control treatment. Transfected cells were cultured for 20 hours with or without 10 μM ABA, and were assayed for GUS and luciferase activities. The values shown are the average of GUS activities normalized by LUC activity. The bars indicate the standard errors. The empty vector pGHX was also included in other transfections to adjust the total level of transfected DNA content. In each experiment, *CaMV35S* promoter-*Emerald luciferase* (*Eluc*) was co-transfected.

Supplementary Table S1. Genetic inheritance of *mh31* phenotype

Genotype	Medium	Sensitive	Tolerant
Ws	ABA	140	0
	Mannitol	37	39
Col-0	ABA	148	0
	Mannitol	50	30
mh31	ABA	32	115
	Mannitol	0	79
F_2 Col-0×*mh31*	ABA	196	74
	Mannitol	14	61

Stratified seeds were sown on plates with 2 μM ABA or 375 mM mannitol. After being sown, plates were incubated at 23℃ under continuous light (2000 lux). Growth-arrested seedling and greening seedling are indicated as “Sensitive” and “Tolerant”, respectively.

Supplementary Table S2. Genetic inheritance of salt tolerance

Genotype	NaCl sensitive	NaCl tolerant
Ws	17	11
mh31	0	24
F_1 Ws×*mh31*	0	27

Stratified seeds were sown on plates supplemented with 150 mM NaCl. After being sown, plates were incubated at 23℃ under continuous light (2000 lux). Growth-arrested seedling and greening seedling are indicated as “NaCl sensitive and “NaCl tolerant”, respectively. F_1 seeds were obtained by crossing *mh31* (male) with Ws (female).