Article:
Villaécija-Aguilar, JA, Körösy, C, Maisch, L et al. (6 more authors) (2022) KAI2 promotes Arabidopsis root hair elongation at low external phosphate by controlling local accumulation of AUX1 and PIN2. Current Biology, 32 (1). pp. 228-236. ISSN 0960-9822

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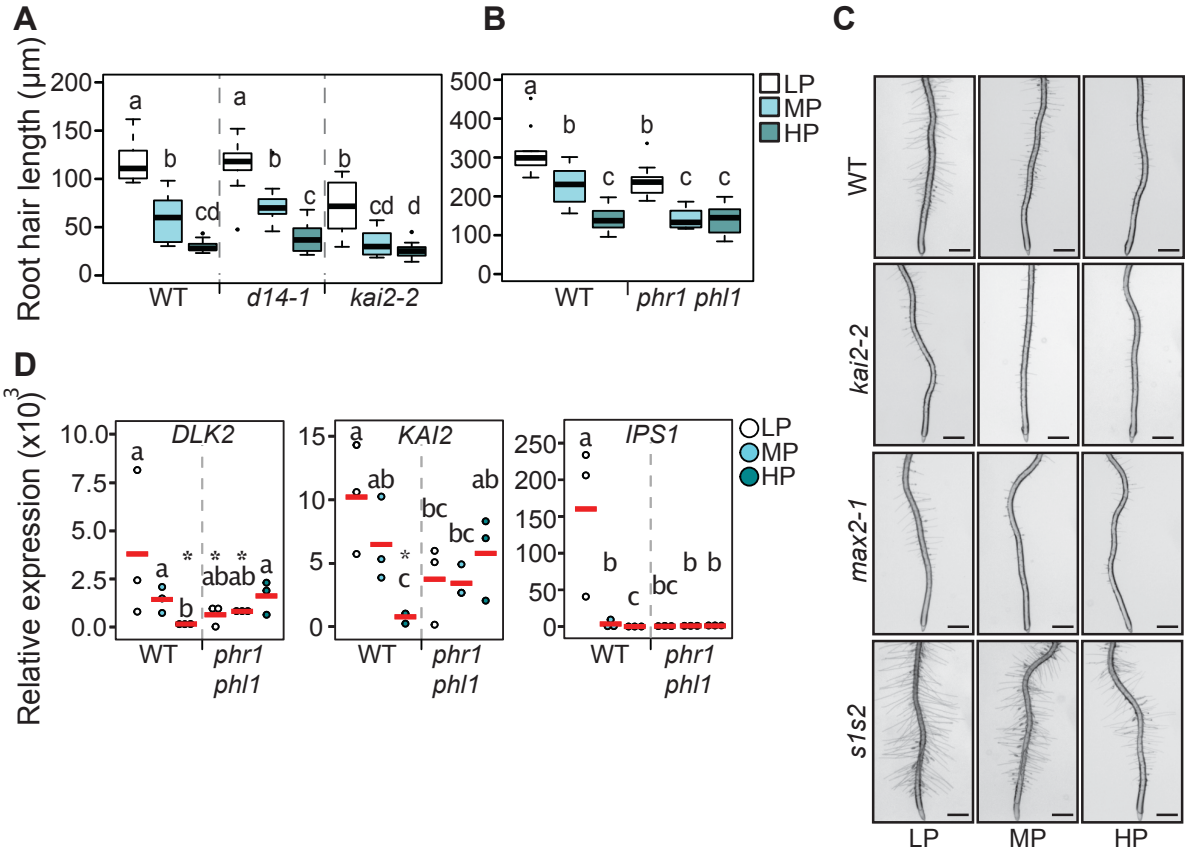

Figure S1. Root hair elongation in response to low external P_i requires KAI2 but not D14.

(A) Root hair length of Col-0 wild type, *d14-1* and *kai2-2* mutants at low (LP, 2 µM), medium (MP, 625 µM) or high (HP, 2000 µM) external P_i. Result obtained in 2 independent experiments. n = 10. (B) Representative images of roots from Figure 1A. (C) Root hair length of Col-0 wild type and *phr1phl1* mutants at low (LP, 2 µM), medium (MP, 625 µM) or high (HP, 2000 µM) external P_i. n = 10. (B) Transcript accumulation of the indicated genes in Col-0 wild type and *phr1phl1* mutant roots at LP, MP and HP. Expression levels were normalized with those of *UBIQUITIN10*, n = 3 biological replicates. Red lines indicate the means. (A, C-D) Different letters indicate different statistical groups (Kruskal-Wallis test with post-hoc Student's t-test, $p \leq 0.05$). Asterisks indicate statistical difference compared to wild type LP (Kruskal-Wallis pairwise comparison, $p \leq 0.05$).

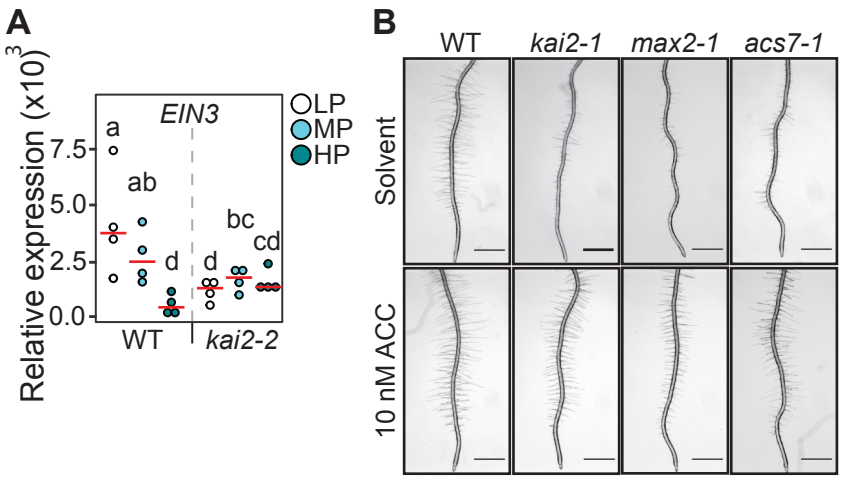

Figure S2. KAI2 promotes root hair elongation at low external P_i through ethylene biosynthesis and signalling.
(A) Transcript accumulation of *EIN3* in Col-0 wild type and *kai2-2* mutant roots at low (LP, 2 µM), medium (MP, 625 µM) or high (HP, 2000 µM) external P_i. Expression levels were normalized with those of *UBIQUITIN10*, n = 4 biological replicates. Red lines indicate the means. Different letters indicate different statistical groups (Kruskal-Wallis test with post-hoc Student's t-test, $p \leq 0.05$). (B) Representative images of roots in Figure 2D. Scale bar 1 mm.

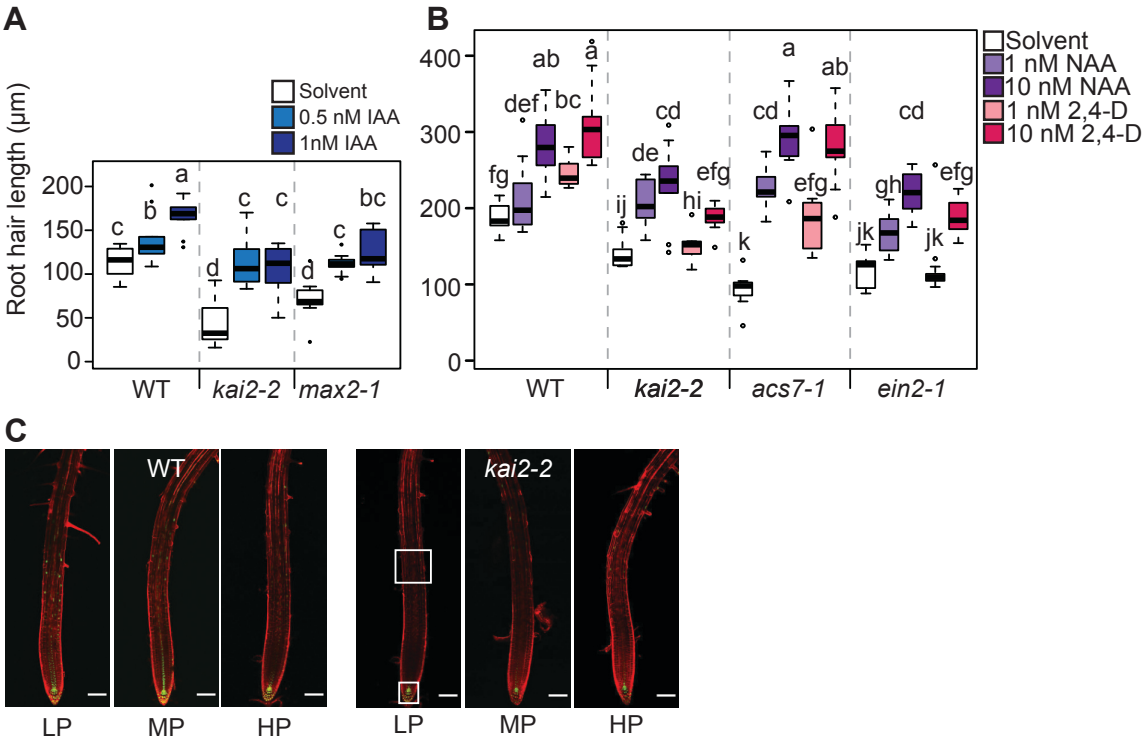

Figure S3. IAA and NAA but to a lesser extent 2,4-D restore root hair length in KL and ethylene signalling mutants.
(A) Root hair length in Col-0 wild type, *kai2-2* and *max2-2* at MP treated with solvent, 0.5 nM IAA or 1 nM IAA. (B) Root hair length in Col-0 wild type, *kai2-2, acs7-1* and *ein2-1* mutants at MP and treated with solvent, 1 nM NAA, 10 nM NAA, 1 nM 2,4-D or 10 nM 2,4-D. (A-B) Results were obtained in 2 independent experiments. n = 10. Different letters indicate different statistical groups (Kruskal-Wallis test with post-hoc Student's t-test, p≤0.05). (C) Representative images of roots used for measurements shown in Figure 3A. White boxes represent the region used for fluorescence quantification. Apical root meristem from 0 µm to 100 µm and E-D zone from 500 to 650 µm above the root tip. Scale bar, 100 µm.

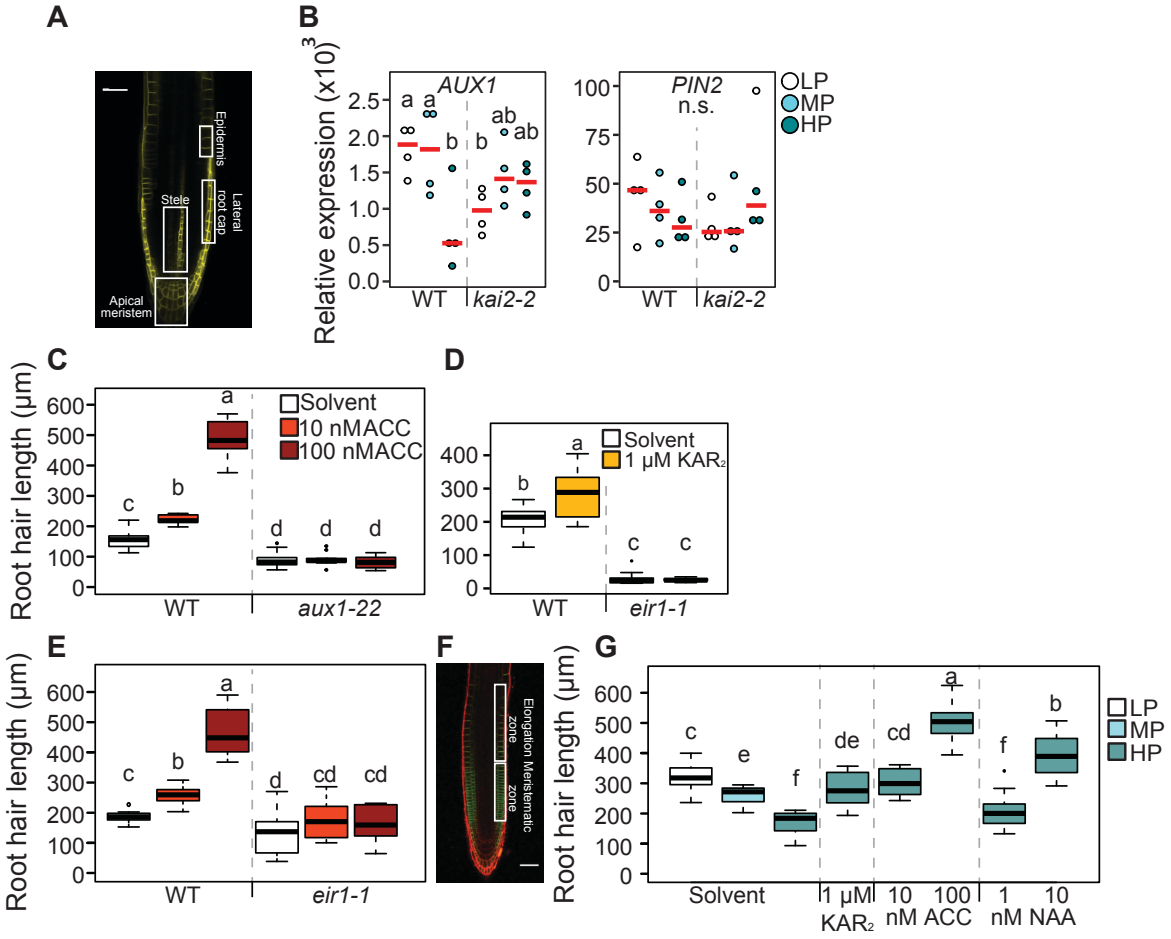

Figure S4. AUX1 and PIN2 act downstream of ethylene signalling and PIN2 accumulation increases at high P_i.

(A) Root tip regions used for measurement in Figure 4A and B. Epidermis from 210 µm to 250 µm from the root tip. Lateral root cap from 100 µm to 180 µm from the root tip. Stele from 70 µm to 150 µm from the root tip. Apical root meristem from 0 µm to 60 µm. Scale bar 40 µm. (B) Transcript accumulation of *AUX1* and *PIN2* in Col-0 wild type and *kai2-2* mutant roots at low (LP, 2 µM), medium (MP, 625 µM) or high (HP, 2000 µM) external P_i. Expression levels were normalized with those of *UBIQUITIN10*, n = 4 biological replicates. Red lines indicate the means. (C) Root hair length of Col-0 wild type and *aux1-22* mutants at MP and treated with solvent, 10 nM ACC or 100 nM ACC. n = 10. (D) Root hair length of Col-0 wild type and *eir1-1* mutants at MP and treated with solvent or 1 µM KAR_2. n = 10. (E) Root hair length of Col-0 wild type and *eir1-1* mutants at MP and treated with solvent, 10 nM ACC or 100 nM ACC. n = 10. (F) Root tip regions used for measurement in Figure 4G and H. Elongation zone from 300 µm to 500 µm from the root tip and meristematic zone from 150 µm to 300 µm. (G) Root hair length in Col-0 wild type at HP and treated with solvent, 1 µM KAR_2, 10 nM ACC, 100 nM ACC, 1 nM NAA or 10 nM NAA. n = 10. (B-D) Different letters indicate different statistical groups (Kruskal-Wallis test with post-hoc Student's t-test, $p \leq 0.05$).

Use	Gene ID	Name	Sequence	Refs.
qPCR *UBQ10/ UBI10*	AT4G05320	Ubi10 F	GGCCTTGTATAATCCCTGATGAATAAG	S1
		Ubi10 R	AAAGAGATAACAGGAACGGAAACATAGT	
qPCR *IPS1*	AT3G09922	IPS1F	AGACTGCAGAAGGCTGATTCAGA	S2
		IPS1R	TTGCCCAATTTCTAGAGGGAGA	
qPCR *PHT1;4*	AT2G38940	PHT1;4 F	CCACGATTCCTCAAGCTGAT	S3
		PHT1;4 R	CAACCAAAGCCGTGTACCTT	
qPCR *KAI2*	AT4G37470	KAI2 F	TGATCTCTGCTTCTCCGAGATACG	S4
		KAI2 R	CCACGCTTTGTAGTTGCTTCGG	
qPCR *DLK2*	AT3G24420	DLK2 F	GCTGCTTCTCCAAGGTATATAA	
		DLK2 R	GAAATCAACCGCCCAAGCT	
qPCR *MAX2*	AT2G42620	MAX2 F	CCGGAGAACGATATGAGCAC	S5
		MAX2 R	CAAGTTTCAGTCAATGATGTTGC	
qPCR *ACS7*	AT4G26200	ACS7 F	CCGTATTATCCAGGATTCGAT	S6
		ACS7 R	CTTTTGGACCGTCGCCCCTA	
qPCR *EIN2*	AT5G03280	EIN2 F	CGCAAGCATCGTCCCTCACAA TTT	S7
		EIN2 R	ACAAGTGACAGTCCGCTGAAGACA	
qPCR *EIN3*	AT3G20770	EIN3 F	TCCTGCACCTTACAAGAAGCCTCA	
		EIN3 R	TTGCCTCACGAGCTTACGGATCTT	
qPCR *AUX1*	AT2G38120	AUX1 F	CTTCTCCGCCGCATTCTGA	This study
		AUX1 R	CTTCTCCGCCGCATTCTGA	
qPCR PIN2	AT5G57090	PIN2 F	TCACGACAACCTCGCTACTAAAGC	This study
		PIN2 R	GTCTTGGTCCATTTCCACATGCC	
Genotyping *smax1-2*	AT5G57710	*smax1-2* F	CATATGAGAGCTGGTTTAAGT	S8
		smax1-2 R	CATATGTCATCGGGAAAACGC	
		SALK LBb1.3	ATTTTGCCGATTTCGGAAC	
Genotyping *smxl2-1*	AT4G30350	*smxl2-1* F	TGACATACACCGATCACCAC	S9
		smxl2-1 R	GTATCATCATCCCACTTTGCATAC	
		SAIL LB1	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	

Table S1. Primers used in this study. Related to STAR methods

Supplemental References

S1. Haydon, M.J., Kawachi, M., Wirtz, M., Hillmer, S., Hell, R., and Krämer, U. (2012). Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in *Arabidopsis*. Plant Cell *24*, 724-737.

S2. Murakawa, M., Shimojima, M., Shimomura, Y., Kobayashi, K., Awai, K., and Ohta, H. (2014). Monogalactosyldiacylglycerol synthesis in the outer envelope membrane of chloroplasts is required for enhanced growth under sucrose supplementation. Front. Plant Sci. *5*, 280-280.

S3. Mayzlish-Gati, E., De-Cuyper, C., Goormachtig, S., Beeckman, T., Vuylsteke, M., Brewer, P.B., Beveridge, C.A., Yermiyahu, U., Kaplan, Y., Enzer, Y., et al. (2012). Strigolactones are involved in root response to low phosphate conditions in Arabidopsis. Plant Physiol. *160*, 1329-1341.

S4. Waters, M.T., and Smith, S.M. (2013). KAI2- and MAX2-mediated responses to karrikins and strigolactones are largely independent of HY5 in Arabidopsis seedlings. Mol. Plant *6*, 63-75.

S5. Mashiguchi, K., Sasaki, E., Shimada, Y., Nagae, M., Ueno, K., Nakano, T., Yoneyama, K., Suzuki, Y., and Asami, T. (2009). Feedback-regulation of strigolactone biosynthetic genes and strigolactone-regulated genes in Arabidopsis. Biosci. Biotechnol. Biochem. *73*, 2460-2465.

S6. Carbonnel, S., Das, D., Varshney, K., Kolodziej, M.C., Villaécija-Aguilar, J.A., and Gutjahr, C. (2020). The karrikin signaling regulator SMAX1 controls *Lotus japonicus* root and root hair development by suppressing ethylene biosynthesis. Proc. Natl. Acad. Sci. USA. *117*, 21757-21765.

S7. Huang, L., Jiang, Q., Wu, J., An, L., Zhou, Z., Wong, C., Wu, M., Yu, H., and Gan, Y. (2020). Zinc finger protein 5 (ZFP5) associates with ethylene signaling to regulate the phosphate and potassium deficiency-induced root hair development in Arabidopsis. Plant Mol. Biol. *102*, 143-158.

S8. Stanga, J.P., Smith, S.M., Briggs, W.R., and Nelson, D.C. (2013). *SUPPRESSOR OF MAX2 1* controls seed germination and seedling development in *Arabidopsis thaliana*. Plant Physiol. *163*, 318-330.

S9. Stanga, J.P., Morffy, N., and Nelson, D.C. (2016). Functional redundancy in the control of seedling growth by the karrikin signaling pathway. Planta *243*, 1397-1406.