

Flavonoids Redirect PIN-mediated Polar Auxin Fluxes during Root Gravitropic Responses

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Supplemental data

<http://www.jbc.org/>

Supplemental Figures S1, S2, S3

Supplemental Table S1, S2

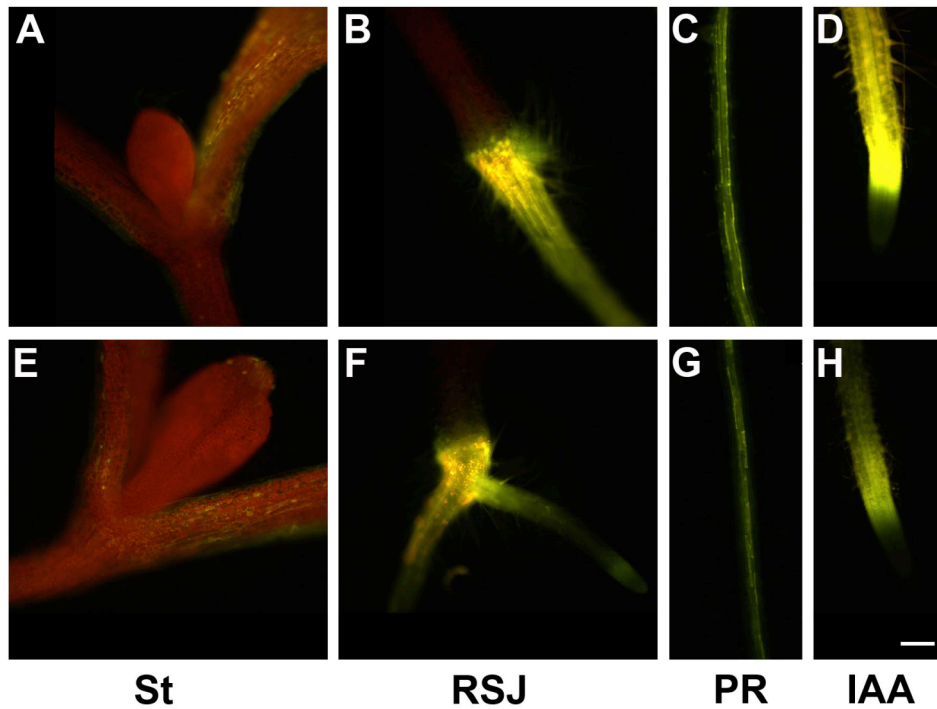


Figure S1. Flavonoid distribution in cotyledons, root-shoot junction and primary root of *pin2* mutant is not affected, but exogenous supply of IAA resulted in increased flavonoid accumulation in the EZ.

In situ flavonoid visualization of *Arabidopsis* seedlings using diphenylboric acid 2-aminoethyl ester (DPBA, a fluorescent dye that interacts with flavonoids) by epifluorescence microscopy (yellow fluorescence). These patterns were observed in all stained seedlings. **A-D**, wild type (Col Wt); **E-H**, *pin2*. St, stem; RSJ, root-shoot junction; PR, primary root; IAA, application of 100 nM IAA. Bar, 100 μ m.

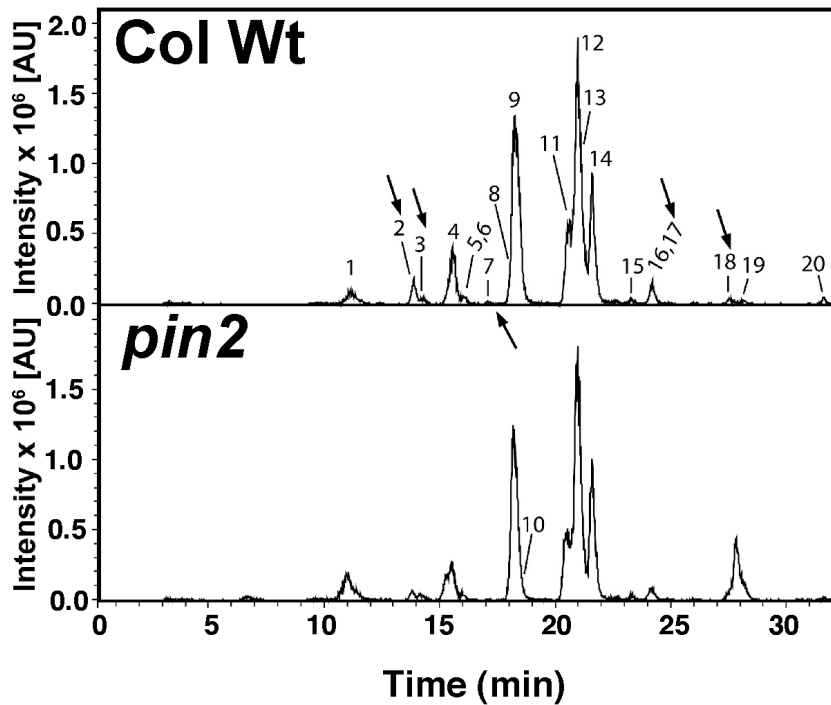


Figure S2. Accumulation of specific flavonoid glycosides in the entire *pin2* root is altered qualitatively but not quantitatively.

Representative sum of EIC of flavonoid derivatives found in wild type (*Col Wt*) and *pin2* (*eir1-4*) entire roots analysed by LC-ESI-MS. Selected ions are m/z 431, 447, 463, 477, 577, 593, 609, 623, 739, and 755, 771. Altered compounds are indicated by arrows. Peak numbers correspond to flavonoid derivatives listed in Table 1. Note 10-fold higher intensities at the whole root level for *pin2* compared to the root tip-elongation zone (Fig. 1G).

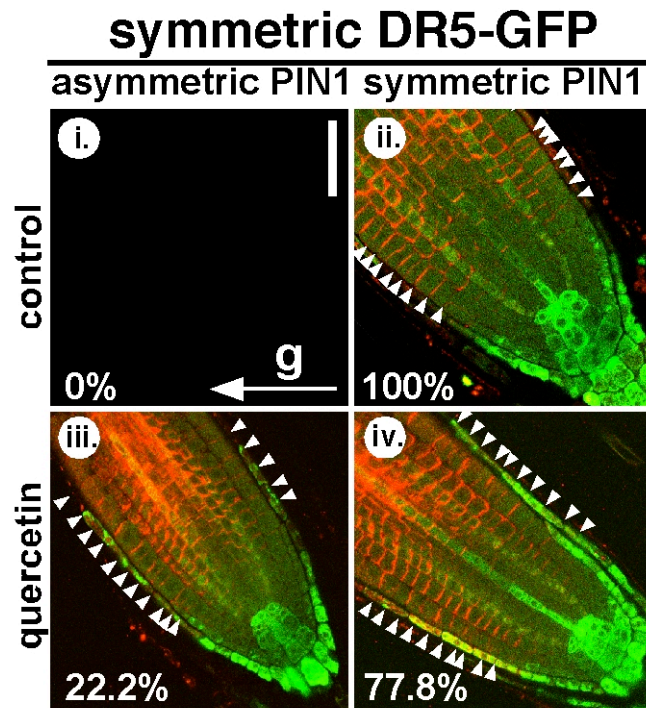


Figure S3. Flavonoid-dependent rescue of basipetal auxin reflux in *pin2* is correlated with PIN1 asymmetric distribution across gravity-stimulated tissues.

Whole-mount *in situ* immunolocalization of PIN1 protein in *pin2* after 2h of gravity stimulation. 4 dag *pin2* seedlings were transferred on media supplemented with 100nM quercetin or the solvent (control) for 24h. Red, PIN1; green, DR5_{rev}-GFP expression. White arrows indicate more pronounced PIN1 proteins levels at the lower or upper side of gravity stimulated root tip. Bar, 30 mm. Gravity vectors relative to the root are indicated by an arrow. Percentages indicate relative occurrence of asymmetric or symmetric PIN1 distributions with symmetric DR5-GFP signals; the total number of analysed roots showing both clear DR5-GFP and PIN1 signals was 47.


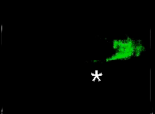
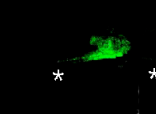
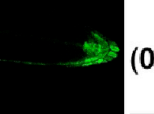






DR5-GFP		0: symm. 1: dLRC 2: pLRC 3: DEZ				total (0+1+2+3)	% (2+3) of total (0+1+2+3)
root tip orientation							
Solvent control							
A 	3 17	10 3	17 1	2 0	32 21	59.4 4.8	
B 	4 14	15 7	9 5	0 8	28 34	32.1 38.2	
C 	0 4	0 2	0 4	0 0	0 10	0 40	
Total	7 35	25 12	26 10	2 8	60 65	46.7 27.7	
% of total	11.7 53.8	41.7 18.5	43.3 15.4	3.3 12.3			
Quercetin							
A 	12 11	27 9	32 12	6 4	77 36	49.6 44.4	
B 	2 5	9 5	11 13	5 20	27 43	59.3 76.7	
C 	1 5	0 3	0 7	0 6	1 21	0 61.9	
total	15 21	36 17	43 32	11 30	105 100	51.4 62	
% of total	14.3 21	34.3 17	41 32	10.5 30			

Table S1: The majority of quercetin-treated *pin2* roots form IAA gradients upon gravity stimulation.

Absolute and relative numbers of wild type (black) and *pin2* (red) seedling showing IAA gradients. Definition of IAA gradients is based on the strength of IAA movement from the LRC to the EZ, as assessed by confocal imaging of DR5-GFP fluorescent signals upon 2h gravity stimulation. Gradient symmetry is quantified in arbitrary units (0= symmetric signal; 1= weak signal asymmetry, up to distal lateral root cap (dLRC); 2= intermediate signal asymmetry, up to proximal lateral root cap (pLRC); 3= strong signal asymmetry, up to elongation zone (DEZ)). Classification of root tip orientation is relative to the gravity stimulation vector, as depicted (A= neutral; B= towards the gravity stimulation vector; C= opposite to the gravity stimulation vector). Number of analysed wild type (*pin2*) seedlings was 60 (65) on solvent control and 105 (100) on 100 nM quercetin plates, respectively. Gravity vector (g) relative to the root is indicated by an arrow; symmetry of DR5-GFP signals by asterisks.

DR5-GFP 0: symm. 1: dLRC 2: pLRC 3: DEZ

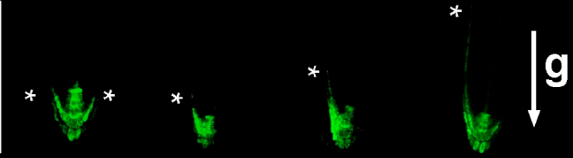



root tip orientation	* 				total (0+1+2+3)	% (2+3) of total (0+1+2+3)
A 	51 12	5 3	0 4	0 0	56 19	0 21.1
B 	2 11	0 3	0 6	0 9	2 29	0 51.7
C 	1 10	0 4	1 0	0 1	2 15	50 6.7
total	54 33	5 10	1 10	0 10	60 63	1.7 31.7
% of total	90 52.4	8.3 15.9	1.7 15.9	0 15.9		

Table S2. Weak asymmetric IAA gradients were occasionally observed in vertically oriented *pin2* roots.

Absolute and relative numbers of wild type (black) and *pin2* (red) seedling showing IAA gradients. Definition of IAA gradients is based on the strength of IAA movement from the LRC to the EZ, as assessed by confocal imaging of DR5-GFP fluorescent signals upon 2h gravity stimulation. Gradient symmetry is quantified in arbitrary units (0= symmetric signal; 1= weak signal asymmetry, up to distal lateral root cap (dLRC); 2= intermediate signal asymmetry, up to proximal lateral root cap (pLRC); 3= strong signal asymmetry, up to elongation zone (DEZ)). Classification of root tip orientation is relative to the gravity stimulation vector, as depicted (A = neutral; B = to the left (lower than 90°); C = to the right (higher than 90°)). Number of analysed wild type (*pin2*) seedlings was 60 (63). Gravity vector (g) relative to the root is indicated by an arrow.