

# Supporting Information

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## SI Materials and Methods

**Plant Material and Growth Conditions.** The 88 *Arabidopsis thaliana* (L.) Heynh. accessions (N22660) (Fig. 1 and Table S1) and 86 recombinant inbred lines (RILs) from a cross between Nok-3 and Ga-0 (N717142) were obtained from the Nottingham *Arabidopsis* Stock Centre. The *receptor-like protein kinase1-1* (*rpkl-1*) and *rpkl-5* mutants and the *pRPK1::RPK1-GFP* line, all in Columbia-0 (Col-0) background (1), were provided by Frans Tax (University of Arizona, Tucson, AZ). The *rpkl-1* mutant has an exonic transfer DNA insertion 502 bp downstream of the translation start and downstream of the leucine-rich repeat; no full-length transcripts are detected in this line (1). The *rpkl-5* mutant has been identified in a population generated by targeting induced local lesions in genomes and has a nonsense mutation that is predicted to result in an early premature stop codon in the leucine-rich repeat (1). Seeds were sterilized by fumigation for 4 h in a desiccator jar with chlorine gas generated by adding 5 mL concentrated HCl to 100 mL 5% (vol/vol) NaOCl. Sterilized seeds were sown on square Petri dishes with basal medium [BM; Gamborg's B5 salts, 0.05% 2-(4-morpholino-)ethane sulfonic acid, pH 5.8, 2% (wt/vol) glucose, 0.7% agar]. After a cold treatment for 3 d at 4 °C, the plates were placed vertically in a growth chamber at 22 °C under a 16-/8-h light-to-dark photoperiod (45  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light irradiance from cool white fluorescent tungsten tubes). Shoot regeneration from root explants was as described (2) with modifications: 7-mm-long apical root segments were taken from 7-d-old seedlings and placed on callus-inducing medium (BM supplemented with 2.2  $\mu\text{M}$  2,4-dichlorophenoxy acetic acid and 0.2  $\mu\text{M}$  kinetin) for 4 d. Explants were transferred to shoot-inducing medium (SIM; BM supplemented with 10  $\mu\text{M}$  2-isopentenyl adenine and 0.86  $\mu\text{M}$  3-indoleacetic acid). Hormones were dissolved in DMSO and supplied to the medium after autoclaving.

**Correlation Study.** Shoots, primordia, roots, callus, and greenness were monitored for root explants of 88 *Arabidopsis* accessions at different time points during SIM incubation (7, 11, 14, and 21 d). The number of shoots after 7 d of SIM incubation was not included, because almost no shoots were formed at that time point. Formation of primordia, shoots, and roots was quantified by counting the number of developmental events. Developing structures were counted as primordia when they were green or purple and dome-shaped or spherical with a smooth surface and a clearly organized cellular patterning and eventually had leaf primordia (as represented in Fig. 2A). For a developing structure to be counted as shoot, it had to originate from a primordium and have side structures that were clearly identifiable as trichome-bearing leaves emerging from a single meristem (as represented in Fig. 2B). Explant greening and callus development were defined within five classes ranging from class 0 to class 4 for the absence of the response to the strongest response among all explants of all accessions (Fig. 2 C and D). Pairwise correlations between the different parameters were calculated with the Spearman's rank correlation coefficient. The correlation coefficients were clustered based on Euclidean distances, whereas intercluster distance was measured by complete linkage clustering. These analyses were done with the R software package, version 2.10.1 ([www.r-project.org/](http://www.r-project.org/)).

**Linkage Mapping.** The genotypes for the Nok-3  $\times$  Ga-0 RILs at each of 75 markers are available at <http://www.jic.ac.uk/staff/ian-bancroft/arabidopsis.htm>. From 94 RILs available, NG1, NG33, NG36, NG40, NG49, NG68, NG71, and NG88 were not

used. The regeneration rate (i.e., the number of explants producing at least one shoot) among 20 explants was measured at 14 and 21 d for each of the 86 RILs. The experiment was done in triplicate. Because data were binomial, probit-transformed data were used for quantitative trait loci (QTL) analysis as implemented in GenStat, version 14 (3), comprising a preliminary search for QTL by means of a simple interval mapping followed by a composite interval mapping and a backward selection. Mapping was conducted with an interval size of 5 cM at a genome-wide type I error rate of 5%. Thresholds to identify significant QTL were calculated as described (4).

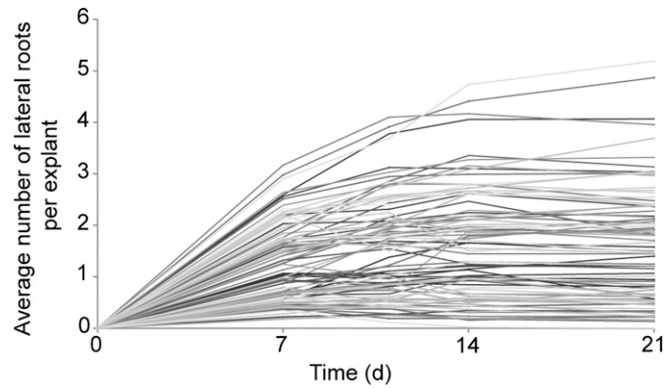
**Association Mapping.** The shoot regeneration rate among 30 explants was assessed after 14 and 21 d of SIM incubation for 88 accessions. Six biological replicates of the experiment were done sequentially in time. Analogously to the linkage mapping, data were probit-transformed. SNP-trait associations for 250,000 SNPs (<https://cynin.gmi.oeaw.ac.at/home/resources/atpolydb>) (5), considering a minor allelic frequency of 5%, were evaluated by fitting a linear mixed model to the probit-transformed data, controlling for the population structure as described (6), and testing the null hypothesis of no association by means of a Wald test. The association mapping with 62 full-sequenced accessions at the time of analysis (January of 2012; <http://signal.salk.edu/atg1001>) was done in a similar way as described above considering a minor allelic frequency of 10%. Fitting linear mixed models using REML and Wald testing was done with GenStat, version 14 (3). The genotype and linkage disequilibrium plots were constructed at the Genome Variation Server (<http://gvs.gs.washington.edu/GVS137/>).

**Quantitative Complementation Test.** The shoot regeneration rate among explants was assessed after 14 d threefold for four genotypes (Nok-3/*rpkl-5*, Ga-0/*rpkl-5*, Nok-3/Col-0, and Ga-0/Col-0) and their reciprocal crosses. The experiment was replicated two times, totaling the number of experimental units to 48. A probit regression model of the form  $y = \beta_0 + \beta_1 EXP + \beta_2 P_1 + \beta_3 P_2 + \beta_4 P_1 \cdot P_2 + \varepsilon$  was fitted to the binomial data, where  $y$  represents the probit-transformed binomial data;  $EXP$  is the experiment (two levels), and  $P_1$  and  $P_2$  are two dummies with Nok-3 and Ga-0 and *rpkl-5* and Col-0 as levels, respectively. One single observation was left out of the analysis because of its large standardized residual. The interaction between the mutated *rpkl* allele and the RPK1 QTL alleles was evaluated by an analysis of deviance, with the dispersion parameter fixed at one, as implemented in the generalized linear model framework in Genstat, version 14 (3).

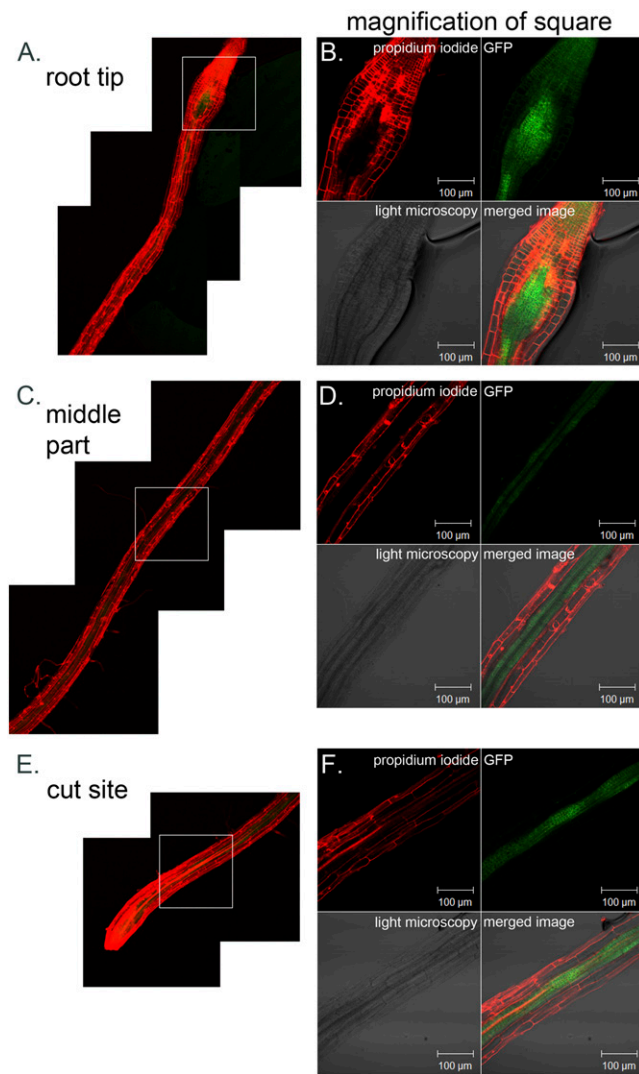
***rpkl* Regeneration.** Data were collected from three (Col-0 and *rpkl-5*) or two (*rpkl-1*) time-independent replicates, with three plates per repeat and genotype and on average, at least 18 (Col-0 and *rpkl-5*) or 10 (*rpkl-1*) explants per plate. A generalized linear model, as implemented in GenStat, version 14 (3), was fitted to probit-transformed data. Differences were assessed by an analysis of deviance.

**Microscopy.** For confocal microscopy, explants were mounted in propidium iodide (10  $\mu\text{g/mL}$ ) or half-strength liquid Murashige and Skoog medium under glass coverslips. Confocal laser-scanning microscopy was done with an inverted Axiovert 100M (Zeiss) microscope and an argon ion laser to generate 488-nm light for GFP excitation and 543-nm light for propidium iodide fluorescence. Images were captured with the LSM510 image acquisition software (Zeiss).

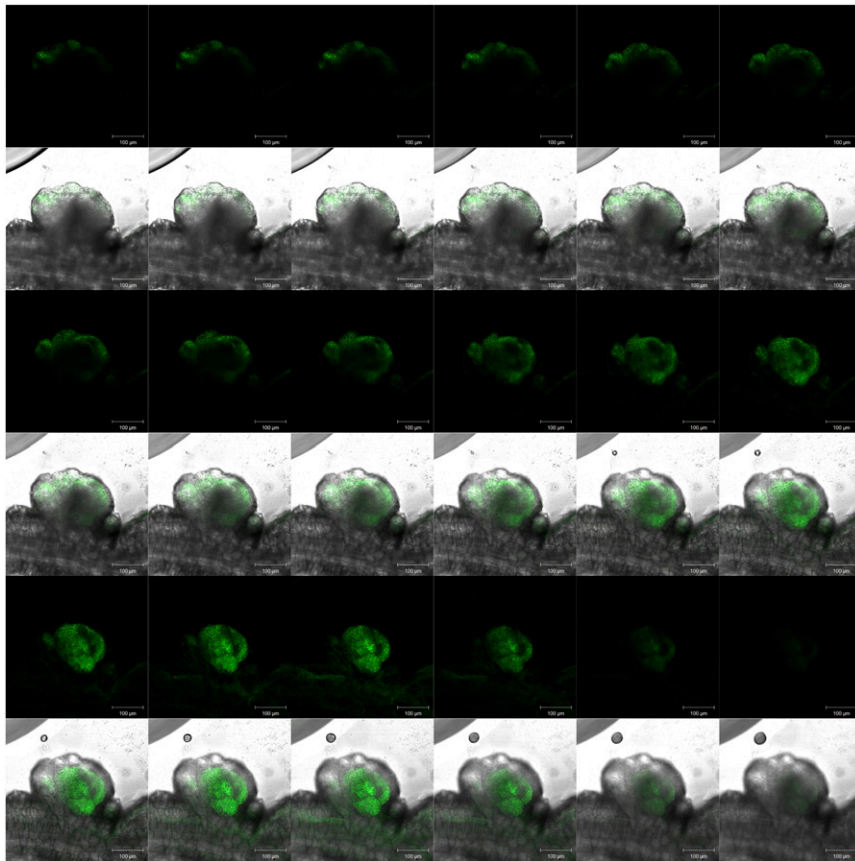
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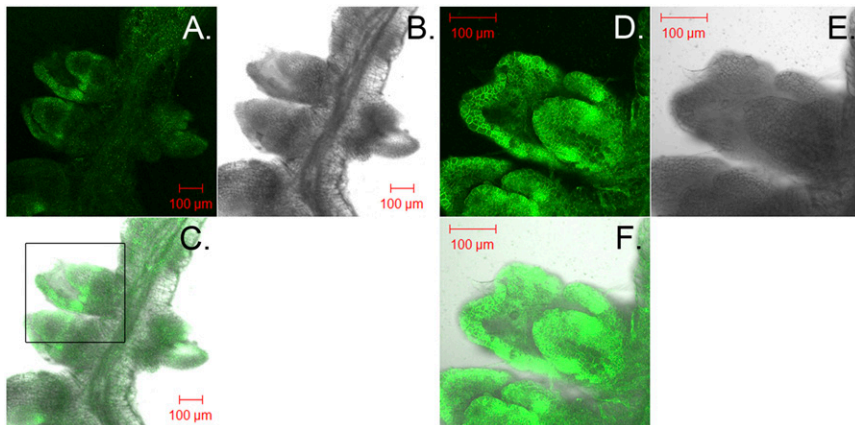
**Fig. S1.** Root formation on root explants of 88 *Arabidopsis* accessions throughout SIM incubation.



**Fig. S2.** Confocal images of *pRPK1::RPK1-GFP* expression in a root explant after 4 d of incubation on callus-inducing medium. Root explant at the (A and B) root tip, (C and D) middle part, and (E and F) cut site. (B, D, and F) Magnification of the areas indicated by the squares in A, C, and E, respectively.



**Fig. S3.** Z stack (2.8- $\mu$ m slices) of the primordium in Fig. 5E (11 d after SIM incubation) showing (Upper) the *pRPK1::RPK1-GFP* fluorescence and (Lower) the merged images with light microscopy.



**Fig. S4.** *pRPK1::RPK1-GFP* expression in developing shoots after 11 d of SIM incubation. (A and D) GFP fluorescence, (B and E) light microscopy, and (C and F) merged images. (D–F) Magnifications of the area indicated by the square in C.





Table S2. Spearman's rank correlation coefficients (r) between different phenotypical responses accompanying shoot regeneration at different time points of SIM incubation

	7 d				11 d				14 d				21 d						
	Roots	Callus	Greenness	Primordia	Roots	Callus	Greenness	Primordia	Shoots	Roots	Callus	Greenness	Primordia	Shoots	Roots	Callus	Greenness	Primordia	Shoots
<b>7 d</b>																			
Roots	1.000	0.049	0.150	0.007*	0.769	0.122	0.163	-0.070	-0.070	0.719	0.049	0.048	-0.088	-0.116	0.679	0.175	0.123	-0.068	-0.073
Callus	0.049	1.000	0.493	0.225	0.032	0.401	0.331	0.143	0.174	0.006*	0.384	0.305	0.104	0.148	0.051	0.340	0.192	0.081	0.036
Greenness	0.150	0.493	1.000	0.289	0.118	0.503	0.539	0.276	0.236	0.082	0.453	0.446	0.163	0.220	0.142	0.392	0.307	0.071	0.050
Primordia	0.007*	0.225	0.289	1.000	-0.036	0.245	0.222	0.316	0.431	-0.088	0.209	0.189	0.176	0.296	-0.010*	0.102	0.086	0.075	0.089
<b>11 d</b>																			
Roots	0.769	0.032	0.118	-0.036	1.000	0.070	0.144	-0.090	-0.102	0.847	0.042	0.068	-0.099	-0.129	0.806	0.147	0.091	-0.063	-0.087
Callus	0.122	0.401	0.503	0.245	0.070	1.000	0.596	0.388	0.325	0.032	0.532	0.454	0.255	0.318	0.099	0.436	0.326	0.076	0.083
Greenness	0.163	0.331	0.539	0.222	0.144	0.596	1.000	0.429	0.297	0.075	0.517	0.608	0.293	0.323	0.157	0.469	0.430	0.113	0.102
Primordia	-0.070	0.143	0.276	0.316	-0.090	0.388	0.429	1.000	0.519	-0.145	0.364	0.406	0.670	0.737	-0.056	0.176	0.266	0.436	0.459
Shoots	-0.070	0.174	0.236	0.431	-0.102	0.325	0.297	0.519	1.000	-0.167	0.252	0.262	0.312	0.583	-0.048	0.049	0.119	0.168	0.280
<b>14 d</b>																			
Roots	0.719	0.006*	0.082	-0.088	0.847	0.032	0.075	-0.145	-0.167	1.000	0.127	0.147	0.020	-0.006*	0.863	0.111	0.068	-0.068	-0.077
Callus	0.049	0.384	0.453	0.209	0.042	0.532	0.517	0.364	0.252	0.127	1.000	0.633	0.374	0.422	0.227	0.529	0.377	0.128	0.137
Greenness	0.048	0.305	0.446	0.189	0.068	0.454	0.608	0.406	0.262	0.147	0.633	1.000	0.434	0.432	0.213	0.426	0.522	0.221	0.197
Primordia	-0.088	0.104	0.163	0.176	-0.099	0.255	0.293	0.670	0.312	0.020	0.374	0.434	1.000	0.618	0.126	0.182	0.296	0.475	0.541
Shoots	-0.116	0.148	0.220	0.296	-0.129	0.318	0.323	0.737	0.583	-0.006*	0.422	0.432	0.618	1.000	0.163	0.127	0.224	0.319	0.586
<b>21 d</b>																			
Roots	0.679	0.051	0.142	-0.010*	0.806	0.099	0.157	-0.056	-0.048	0.863	0.227	0.213	0.126	0.163	1.000	0.084	0.042	-0.106	-0.131
Callus	0.175	0.340	0.392	0.102	0.147	0.436	0.469	0.176	0.049	0.111	0.529	0.426	0.182	0.127	0.084	1.000	0.516	0.105	0.150
Greenness	0.123	0.192	0.307	0.086	0.091	0.326	0.430	0.266	0.119	0.068	0.377	0.522	0.296	0.224	0.042	0.516	1.000	0.228	0.301
Primordia	-0.068	0.081	0.071	0.075	-0.063	0.076	0.113	0.436	0.168	-0.068	0.128	0.221	0.475	0.319	-0.106	0.105	0.228	1.000	0.428
Shoots	-0.073	0.036	0.050	0.089	-0.087	0.083	0.102	0.459	0.280	-0.077	0.137	0.197	0.541	0.586	-0.131	0.150	0.301	0.428	1.000

Numbers of shoots, primordia, and lateral roots and classes for callus and greenness are presented. Data were collected for 88 different *Arabidopsis* accessions using three repeats with each time, 30 explants per accession. Because almost no shoots were observed after 7 d of SIM incubation, this parameter was not included. All correlations were significant at  $P < 0.05$ , except for the indicated correlations. \*Not significant.

**Table S3. Regeneration QTLs identified by using an Nok-3 × Ga-0 inbred population**

QTL	Trait*	Origin positive allele	Chromosome	Position (cM/Mb)	Percent explained variation			
					Single trait		Multitrait	
					14 d	21 d	14 d	21 d
REG-1	14, 21, multi	Nok-3	1	91/25.9	31.8	23.8	30.6	27.8
REG-2	21, multi	Ga-0	2	23/7	—	12.4	4.9	22.2
REG-3	14	Ga-0	2	70/18	14.3	—	—	—
REG-4	21	Nok-3	3	25/7.7	—	7.4	—	—
REG-5	14, multi	Nok-3	3	84/23	12.1	—	11.7	9.6

\*Single trait analysis for regeneration-responsive explants after 14 or 21 d of SIM incubation is indicated by 14 or 21, respectively; multi indicates multitrait analysis for responsive explants after 14 and 21 d of SIM incubation.