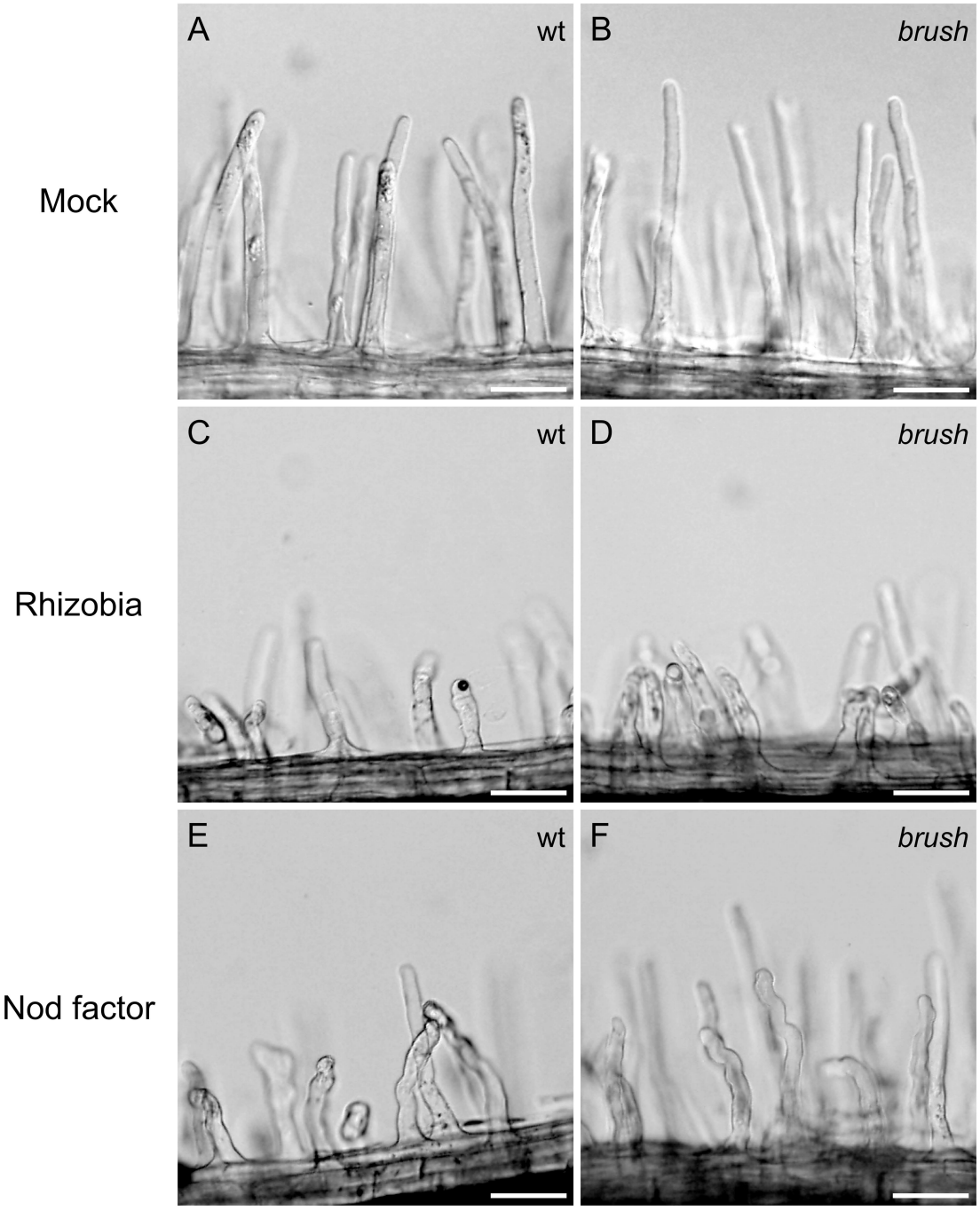
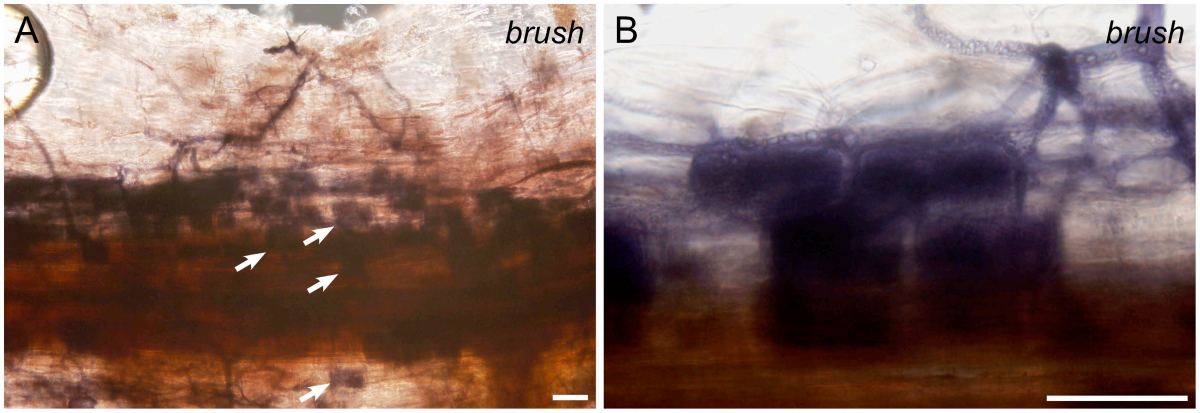
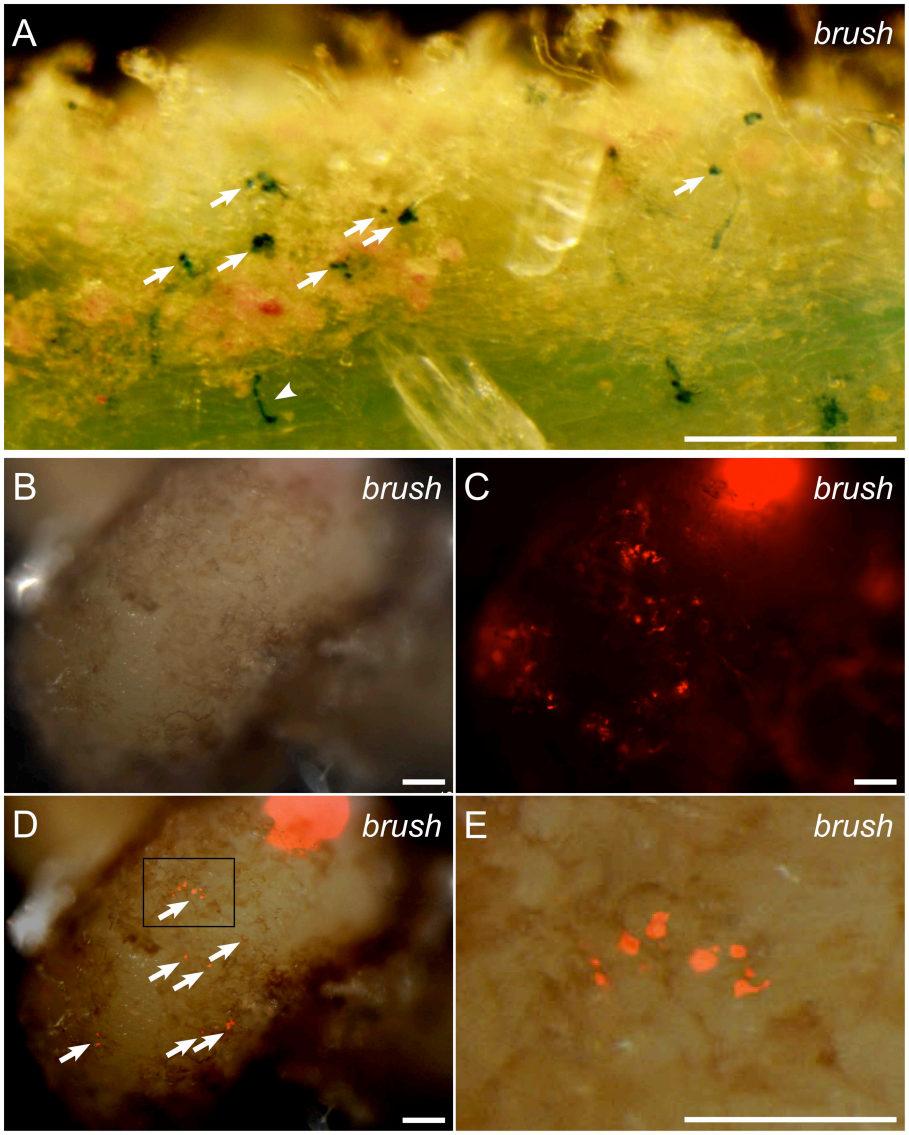


Supplemental Figure S1: Root hair morphology of *brush* and wild type roots.
Wild type (A, C, E) compared to *brush* (B, D, F) root hairs shown after mock (A, B), rhizobial (C, D), or Nod factor (E, F) treatment. All pictures were taken from comparable root regions with not yet fully mature root hairs, which are known to respond best to treatment. After seed sterilization, seeds were immediately germinated on Petri dishes containing half-strength Broughton and Dilworth (B&D) medium in 1% agar for 2 days in the dark. Seedlings were subsequently inoculated with rhizobia (final OD_{600} = 0.01) or 10^{-8} M Nod factor (extracted from *M. loti* 303099 wild type) was applied to the roots. After 24 hours of incubation at 16/8 hr photoperiods at 26°C, plants were carefully removed from the agar plate and mounted on a glass slide with half-strength B&D medium containing 0.01% Triton X-100 to reduce air bubbles entrapped in the root hairs. Images were taken at 10x magnification using a bright field microscope (Olympus BP50). Bars: 50 μm.



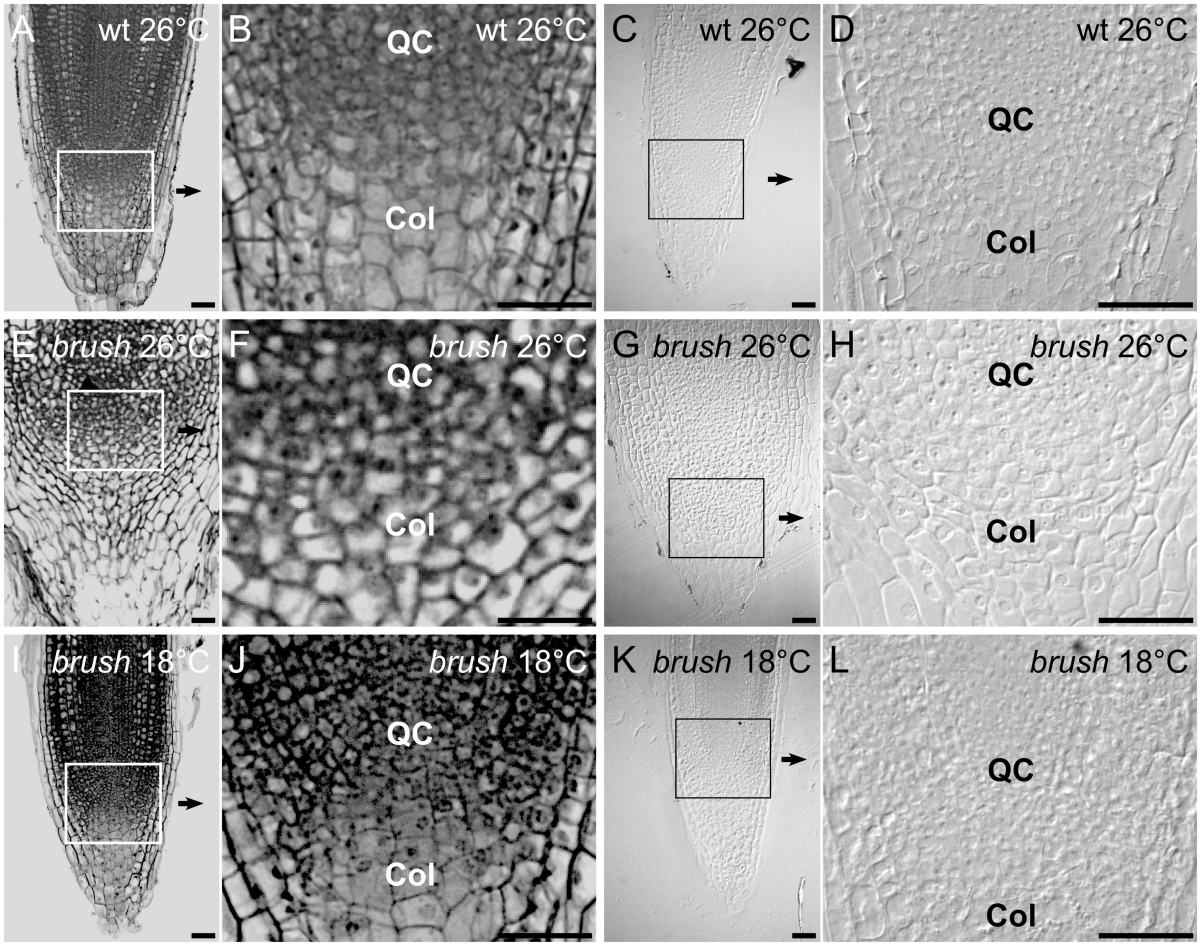
Supplemental Figure S2: Arbuscular mycorrhiza phenotype of *brush*.
A) Arbuscules in *brush* roots. Arrows point to examples of cells containing arbuscules. B) Detailed view of colonization. Arbuscules are apparent inside six neighboring cells. Four-day-old seedlings were planted together with chives as nurse plants infected with *G. intraradices* (strain deposited at the International Bank for the Glomeromycota as BEG195) in pot culture as described by Wegel et al. (1998) and grew under summer greenhouse conditions for 3 weeks. Staining of fungal material was carried out according to Vierheilig et al. (1998). The samples were analyzed with an Olympus BP50 microscope (Olympus, Japan). Bars: 50 µm.

Vierheilig H, Coughlan AP, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol **64**: 5004-5007
Wegel E, Schauser L, Sandal N, Stougaard J, Parniske M (1998) Mycorrhiza mutants of *Lotus japonicus* define genetically independent steps during symbiotic infection. Mol Plant Microbe Interact **11**: 933-936



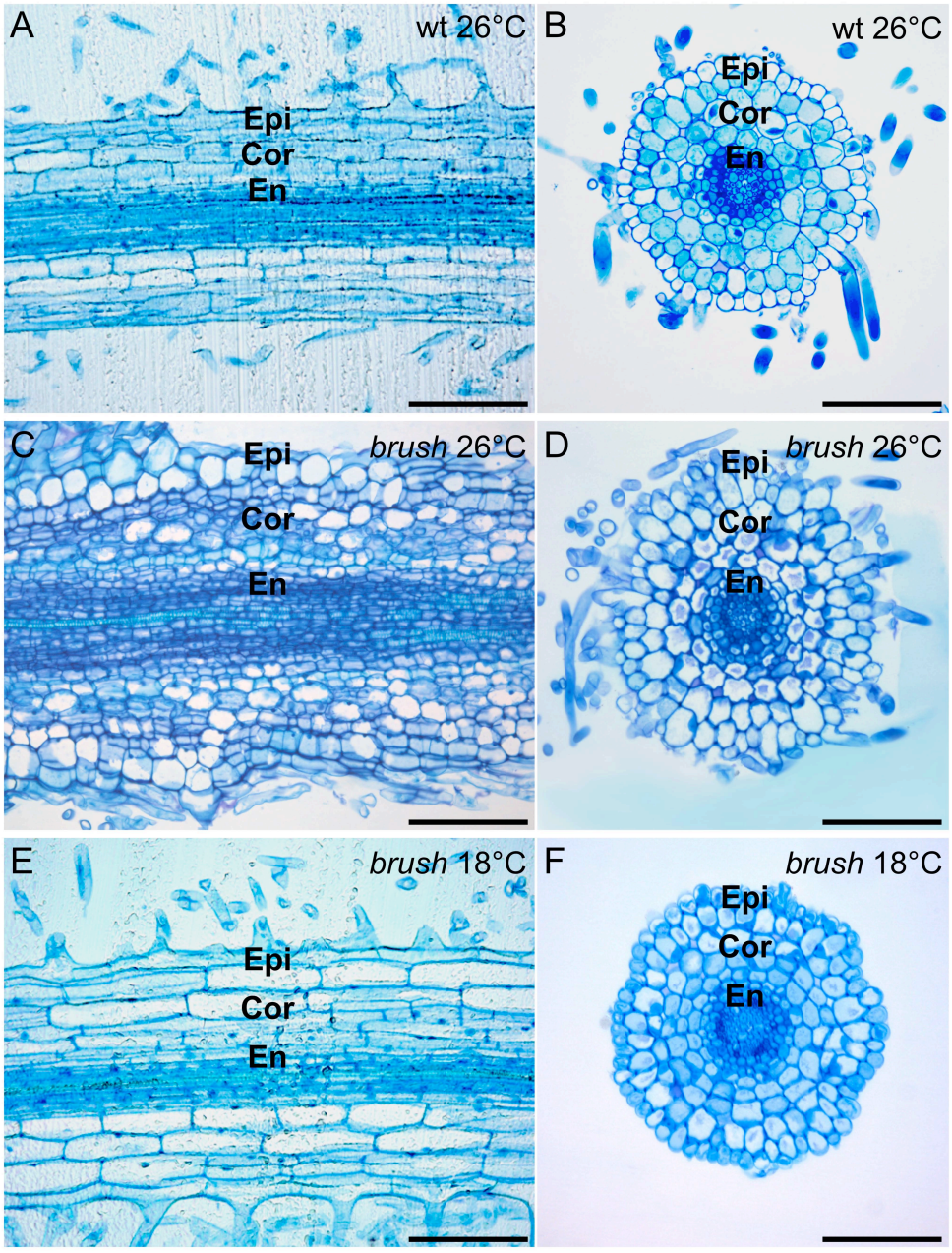
Supplemental Figure S3: Infection thread formation and colonization of root hairs on *brush* plant roots.

A) IT formation and colonization of root hairs in *brush* mutant plant roots after 2 weeks of inoculation with *M. loti* JRL501 expressing *lacZ*. In *brush* root hairs, many rhizobial colonization events were observed (arrows) and in few cases complete infection thread formation was apparent (arrowhead). Four-day-old seedlings were planted in glass jars with half-strength B&D medium and grown at 26°C. B-E) IT formation and colonization of root hairs on *brush* mutant nodules 1 month after inoculation with *M. loti* MAFF303099 expressing *DsRed*. B) Light microscopic and C) fluorescence microscopic view of a nodule. D) Merged view of B) and C). Arrows indicate colonization of the root hairs. E) Enlarged view of the region indicated in D). Root hair curling and colonization is visible but no IT formation. Four-day-old seedlings were planted in glass jars and grown at 26°C as described in Materials and Methods. Bars: 100 μm.



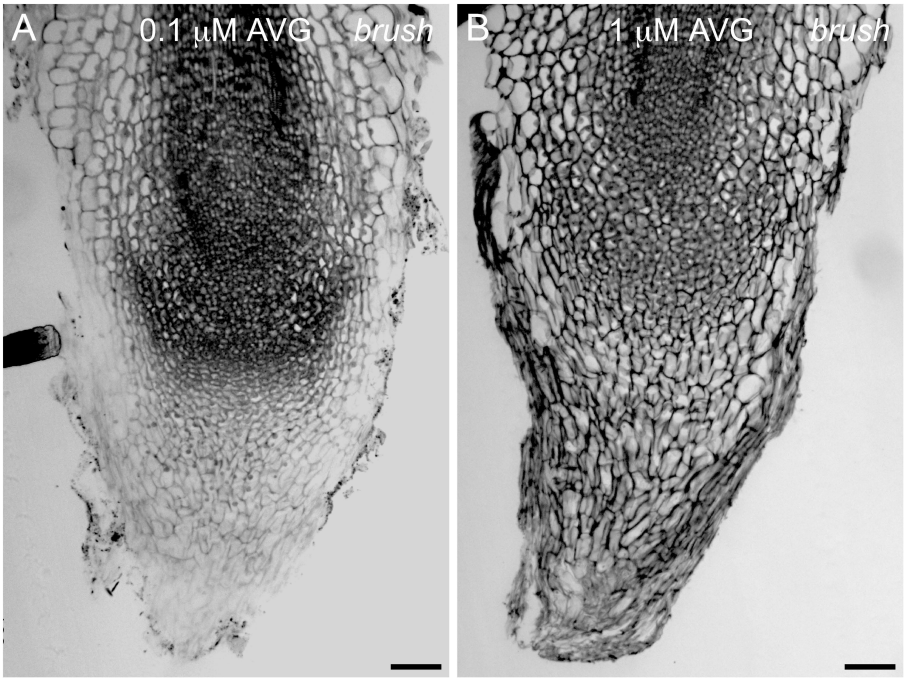
Supplemental Figure S4: Temperature-dependent *brush* phenotype at the root apical meristem.

A-D) Longitudinal sections of wild type roots at 26°C. A) and C) Overview of root tips. B) and D) Enlarged views of RAM with the putative quiescent center (**QC**). Cell layers are regular and columella cells (**Col**) are expanded longitudinally. E-H) Longitudinal sections of *brush* roots at 26°C. E) and G) Overview of root tips. F) and H) Enlarged views of the RAM. Columella cells do not show expansion in longitudinal direction. I-L) Longitudinal sections of *brush* roots at 18°C. I) and K) Overview of root tips. J) and L) Enlarged views of the RAM. Columella cells are lacking longitudinal expansion but all cell layers are present and of uniform appearance. Four-day-old seedlings were planted in glass jars and grown for 4 weeks. Samples were fixed and embedded as described (see Materials and Methods). Section thickness was 5 µm. Bars: 50 µm.

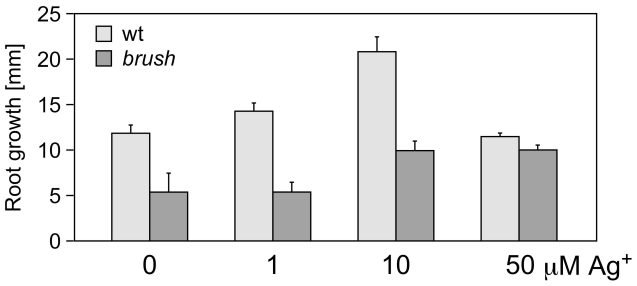


Supplemental Figure S5: Temperature-dependent *brush* phenotype at the root hair zone.

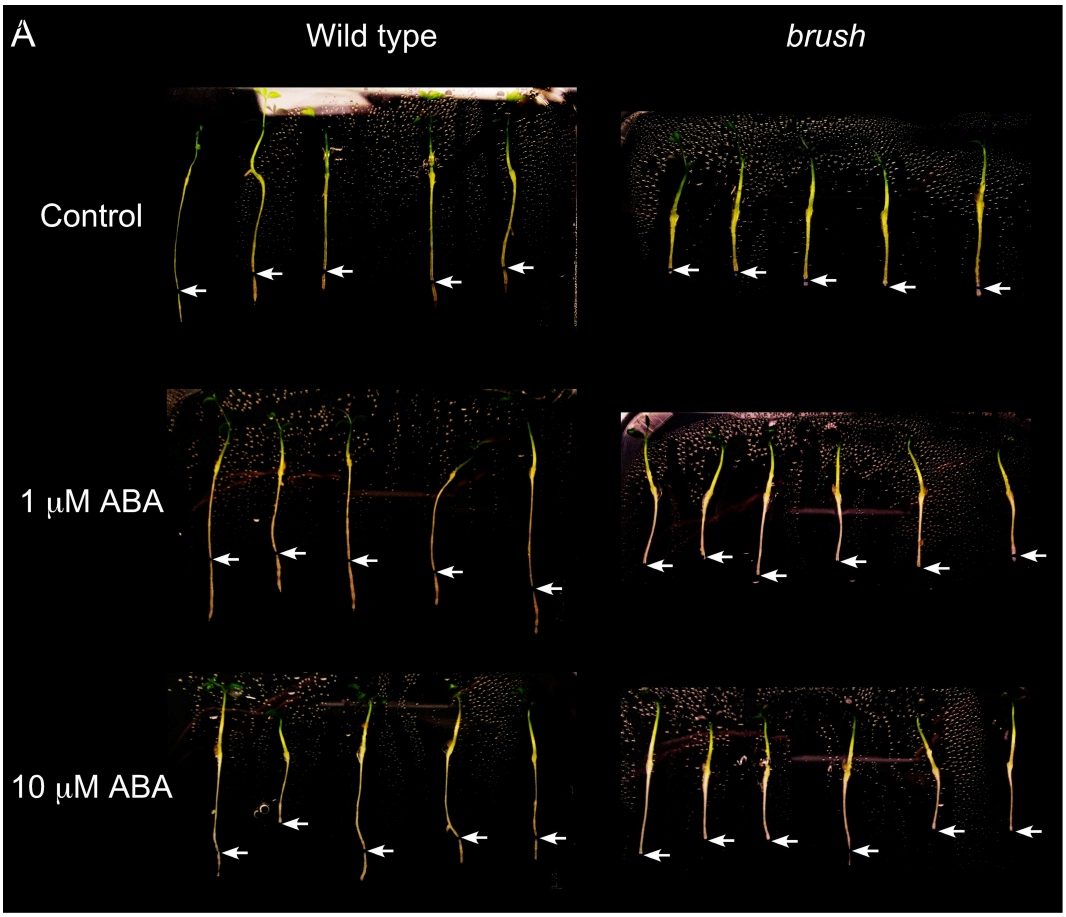
A) Longitudinal and B) transverse sections of wild type roots at 26°C, approximately 900 µm from the root tip. The regular architecture of epidermal cell layer (**Epi**), cortical cells (**Cor**), and endodermis (**En**) is clearly visible. C) Longitudinal and D) transverse sections of *brush* roots at 26°C, approximately 800 µm from the root tip. Cortex and epidermal cells have an irregular shape and show radial expansion. E) Longitudinal and F) transverse sections of *brush* roots at 18°C, approximately 800 µm from the root tip. The root architecture appears wild type-like. Thickness was 10 µm for transverse sections and 5 µm for longitudinal sections. For transverse sections, two-day-old seedlings (germinated in the dark) were transferred to half-strength B5 plates and grown for 10 days. For longitudinal sections, four-day-old seedlings were planted in glass jars and grown for 4 weeks. Samples were fixed and embedded as described (see Materials and Methods). Bars: 100 µm.

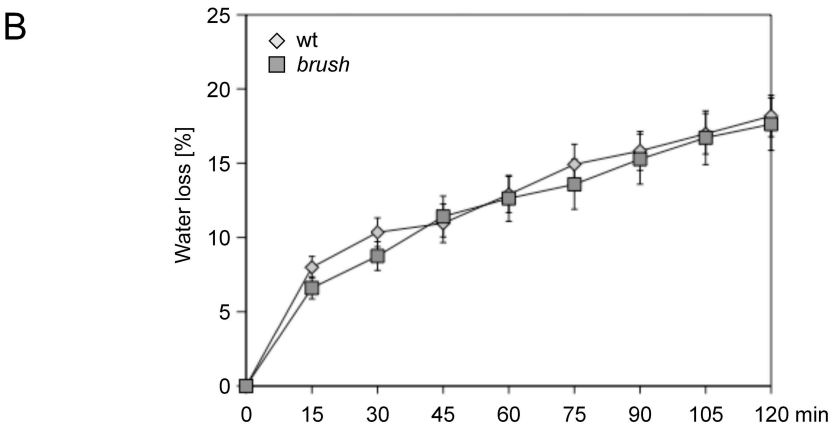


Supplemental Figure S6: AVG treatment of *brush* roots.
Sections of roots treated with A) 0.1 μM AVG and B) 1 μM AVG. Samples were fixed and embedded as described (see Materials and Methods). Four-day-old seedlings were planted in glass jars and grown for 9 days at 26°C with AVG added in the respective concentrations. Bars: 100 μm.

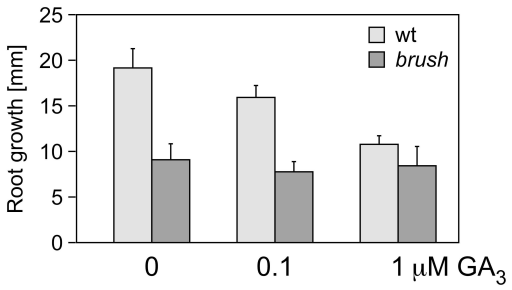


Supplemental Figure S7: Silver ion treatment of *brush* and wild type roots.
Two-day-old seedlings were treated with 1, 10, and 50 µM Ag_2SO_4 on plates with half-strength B&D medium plus 1% agar. Shown are the growth increments of the roots between day 1 and 5 at 16h/8h photoperiods at 26°C in a growth chamber. Values shown are mean ± SE from four to five individual plants each.





Supplemental Figure S8: ABA treatment of *brush* and wild type roots.
A) Root growth of wild type and *brush* mutants at different ABA concentrations. Four-day-old seedlings were transferred into a plate containing half-strength B&D medium in 1% agar with different concentrations of ABA and grown at 24°C for 6 additional days. B) Kinetics of water loss from excised leaves. Four to five young leaves were collected from individual six-month-old plants, and total fresh weight was measured every 15 minutes up to 2 hours in ambient laboratory conditions. The plants had been grown under greenhouse conditions. The loss of water content is indicated as percentage of initial fresh weight. Values shown are mean ± SE from eight individual plants each.



Supplemental Figure S9: GA_3 treatment of *brush* and wild type roots.
Two-day-old seedlings were treated with 0.1 μM and 1 μM GA_3 on plates with half-strength B&D medium plus 1% agar. Shown are the growth increments of the roots between day 1 and 10 at 16h/8h photoperiods at 26°C in a growth chamber. Values shown are mean ± SE from five individual plants each.

Supplemental Table S1: Calcium spiking response in *brush* root hairs.

	Calcium spiking response*	
	per plant root	per individual root hair cell
wild type	11 / 12	22 / 26
brush	7 / 7	10 / 13

* Seedlings of *L. japonicus* Gifu wild type and *brush* mutant were grown in a chamber for 2 days (26°C in the dark) and transferred to BNM medium (Ehrhardt et al., 1992) for microinjection. The injection needle was prepared from a glass capillary (GB150F-8P, Science Product) by a puller (P-97, Sutter instrument). 0.2-0.5 μl of 5 mM calcium indicator dye, Oregon Green 488 BAPTA-1 dextran MW 10,000 (Invitrogen) and reference dye, Texas Red dextran MW 10,000 (Invitrogen) were loaded into the needle and the remaining space was filled with injection buffer (0.15 M KCl, 0.09 M Hepes, pH 7.0). The loaded needle was manipulated with a micromanipulator (PS-7000C, Scientifica) and the dyes were injected by iontophoresis using a bridge amplifier equipped with a timer module (BRAMP-01R, TMR-01, npi electronic). Observation of calcium spiking was performed on a Leica inverted microscope DMI6000B equipped with 40x dry objectives (N.A. 0.6). The injected root hairs were treated with 10 nM Nod factor and the fluorescent images were acquired every 5 sec with filter systems GFP and TX2 (Leica). The acquired images were analyzed with the Leica software package LAS-AF and Microsoft Excel. The left column represents the ratio of the number of plants showing the typical calcium spiking pattern observed upon NF application relative to the total number of plants tested. The right column indicates the ratio of the number of root hairs from this population of plants that show spiking relative to the total number of root hairs analyzed.

Ehrhardt DW, Atkinson EM, Long SR (1992) Depolarization of alfalfa root hair membrane potential by *Rhizobium meliloti* Nod factors. Science **256**: 998-1000