Polar auxin transport determines adventitious root emergence and growth in rice

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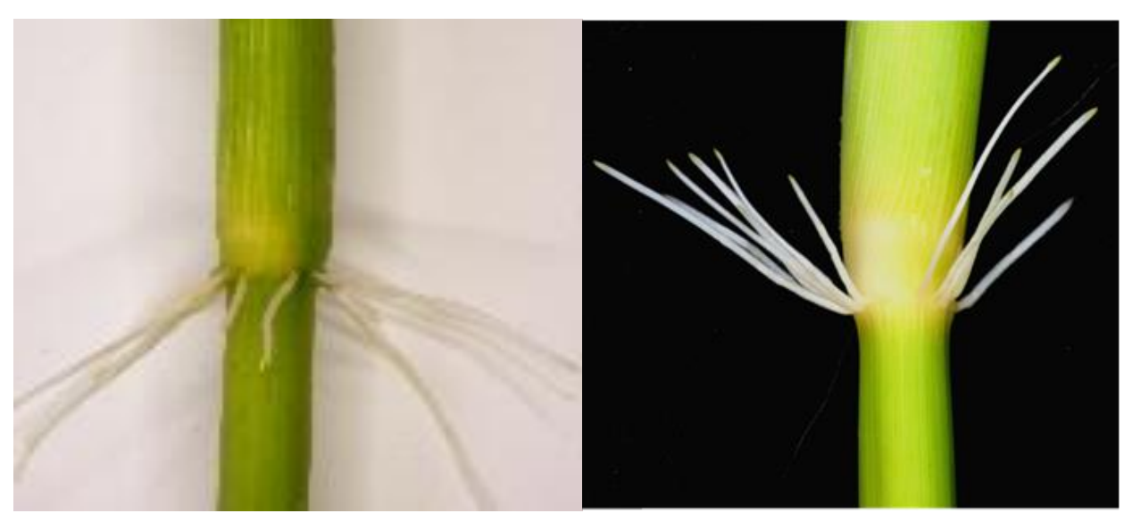
Introduction

Flooding poses a severe and increasing threat on crop production worldwide. Unlike other crop plants, rice (*Oryza sativa L.*) is well adapted to partial submergence. Among the many adaptive responses is the restructuring of the root system. Adventitious roots (ARs) emerge and grow during flooding to support and replace the main root system. In rice, AR emergence is induced by darkness and by ethylene. In the dark, roots grow upward(1) whereas in the light they grow downward. Tropic response are mediated by auxin gradients that are generated through polar auxin transport via auxin efflux carriers(2). In this study, we investigated the contribution of polar auxin transport in establishing the AR growth direction. We analyzed expression of PIN auxin efflux carrier genes with *OsPIN:β-glucuronidase* reporter lines and auxin activity with the *DR5:VENUS* reporter(3) in ARs and in epidermal cells above ARs that were shown to undergo programmed cell death prior to AR emergence(4). The results indicate that local auxin gradients determine penetration, growth rate and growth direction of ARs revealing a deep impact of controlled auxin transport on the overall architecture of the AR system.



white light
+ ethephon

dark

1

Inhibition of auxin transport reduces AR penetration and the root growth angle

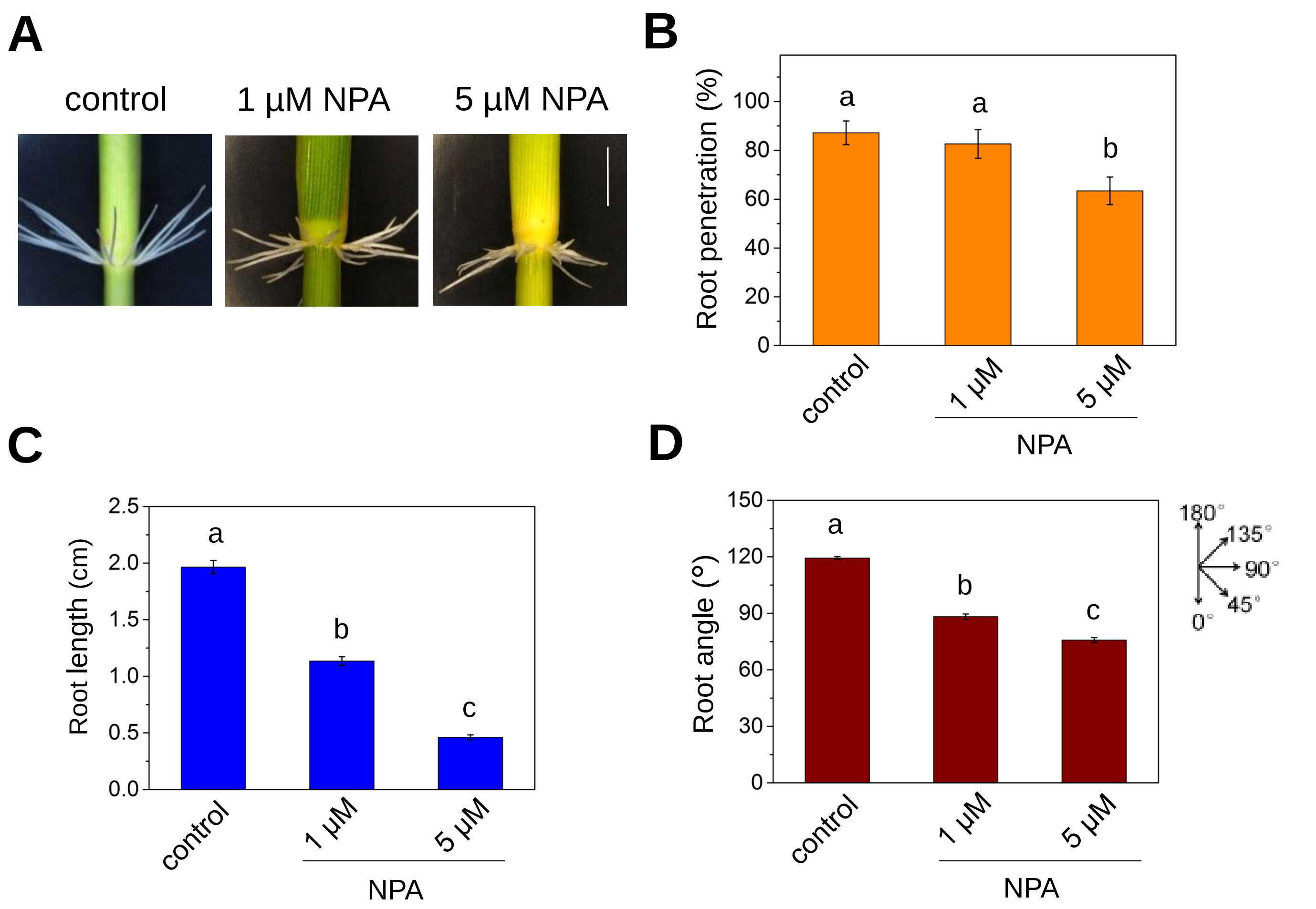


Figure 1: Auxin transport inhibition alters emergence, elongation and growth angle of ARs in rice.
ARs were analysed at the third node of stem sections kept in the dark for 3 d in the presence of the auxin transport inhibitor NPA as indicated. **(A)** Phenotypes of ARs; bar=1 cm. **(B)** Percentage of penetrated ARs. **(C)** Average lengths of penetrated ARs. **(D)** Mean growth angles of ARs. Values are means ±SE, n=9. (P<0.05; ANOVA with Tukey test)

2

Auxin activity changes in the epidermis and in ARs after growth induction

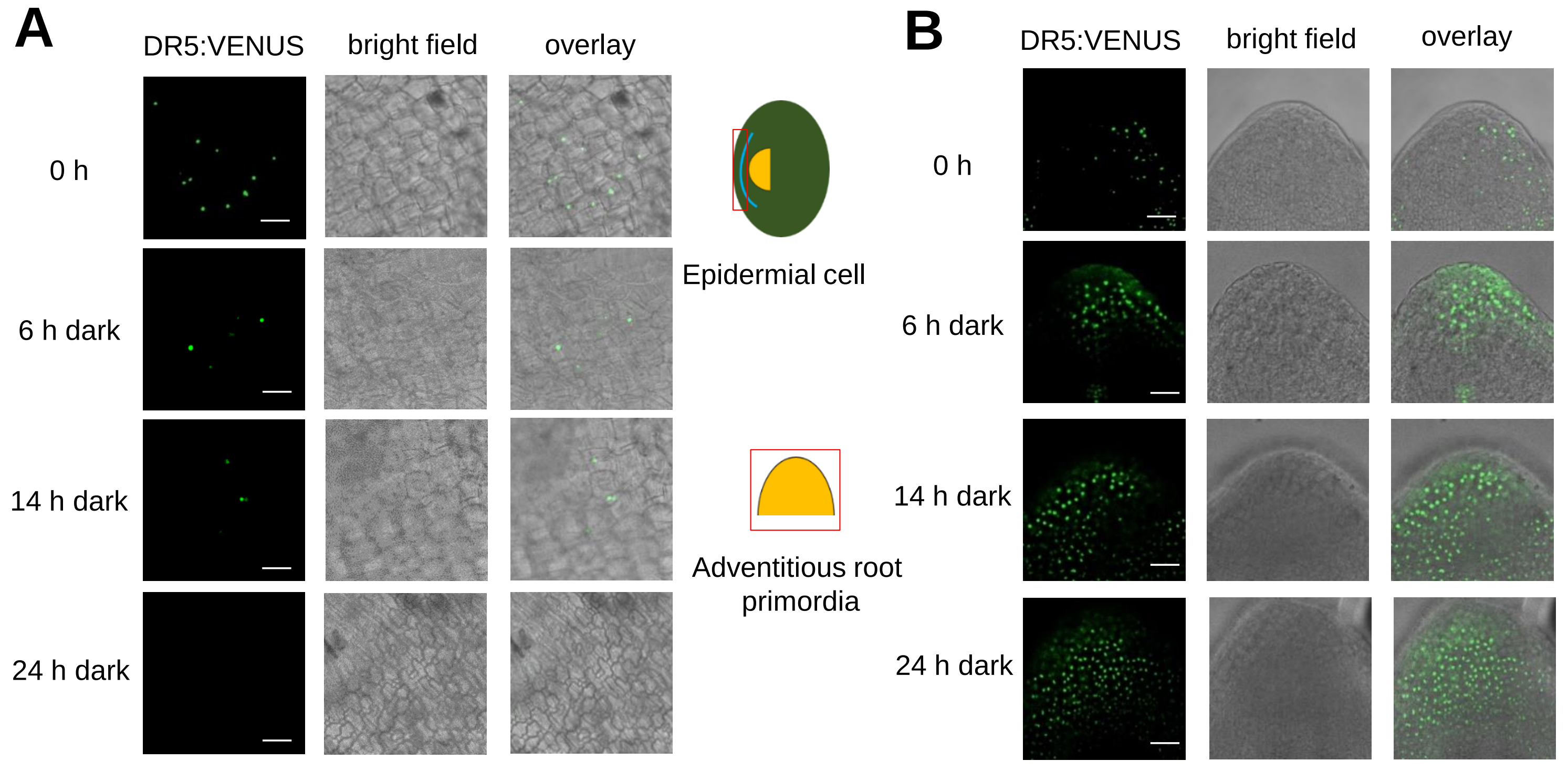


Figure 2: Auxin activity decreases in the epidermis above primordia and increases in AR primordia after induction of AR growth in the dark. (A) Epidermal peels above a root primordium. Auxin activity was detected by CLSM as DR5:VENUS signal. **(B)** Isolated AR primordia from the DR5:VENUS reporter line; bar=25 µm.

3

NPA reduces auxin activity in ARs

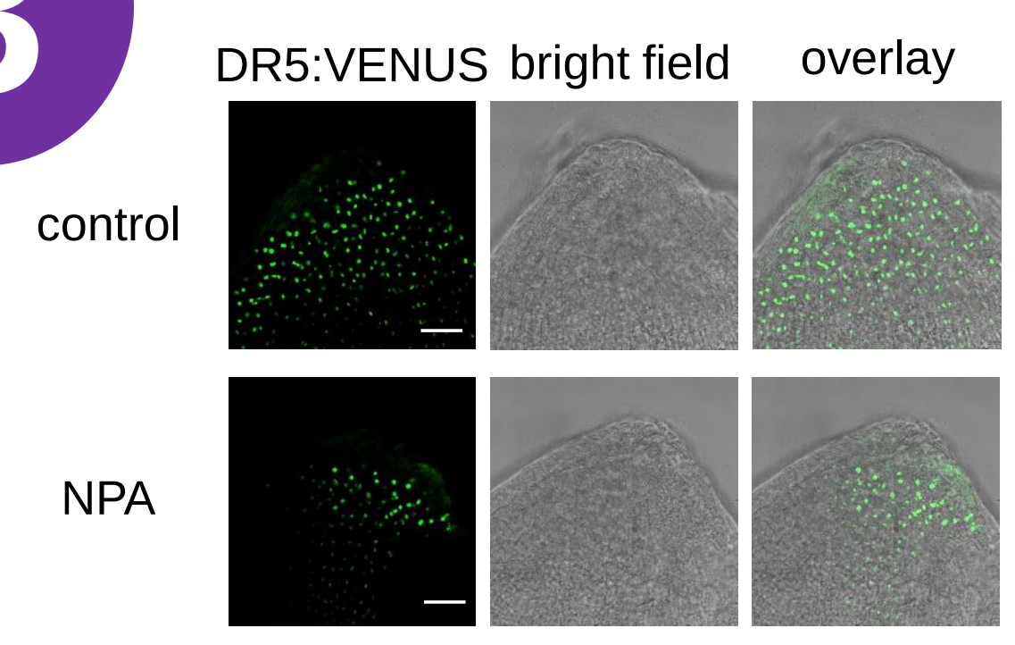


Figure 3: Effect of auxin transport inhibition by NPA on auxin activity in ARs.
Auxin activity in AR primordia treated with or without 5 µM NPA for 1 d was detected with DR5:VENUS; bar=25 µm.

4

OsPIN genes are differentially expressed in ARs and in epidermal cells above ARs

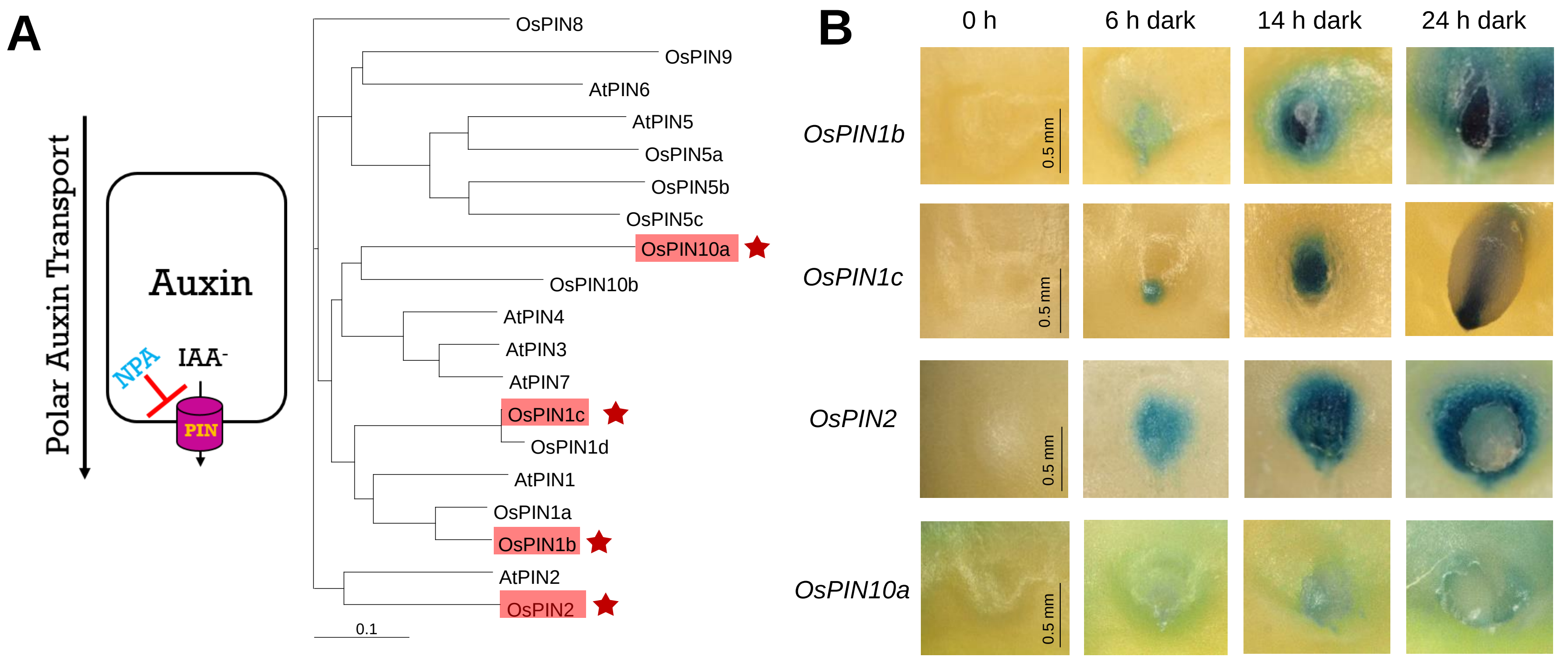


Figure 4: *OsPIN2* expression is induced in epidermal cells above AR primordia after growth induction in the dark.
(A) Phylogenetic analysis of PIN family members in rice and Arabidopsis. Multiple sequence alignment was performed with Clustal Omega and a phylogenetic tree was generated by Treeview; bar=0.1. Red stars indicate PIN genes that were analyzed with GUS reporter lines. **(B)** Top view on emerging ARs in *OsPIN1b:GUS*, *OsPIN1c:GUS*, *OsPIN2:GUS* and *OsPIN10a:GUS* reporter lines reveals induction of *OsPIN2* expression in the epidermis above AR primordia.

5

Auxin distribution changes when ARs redirect growth

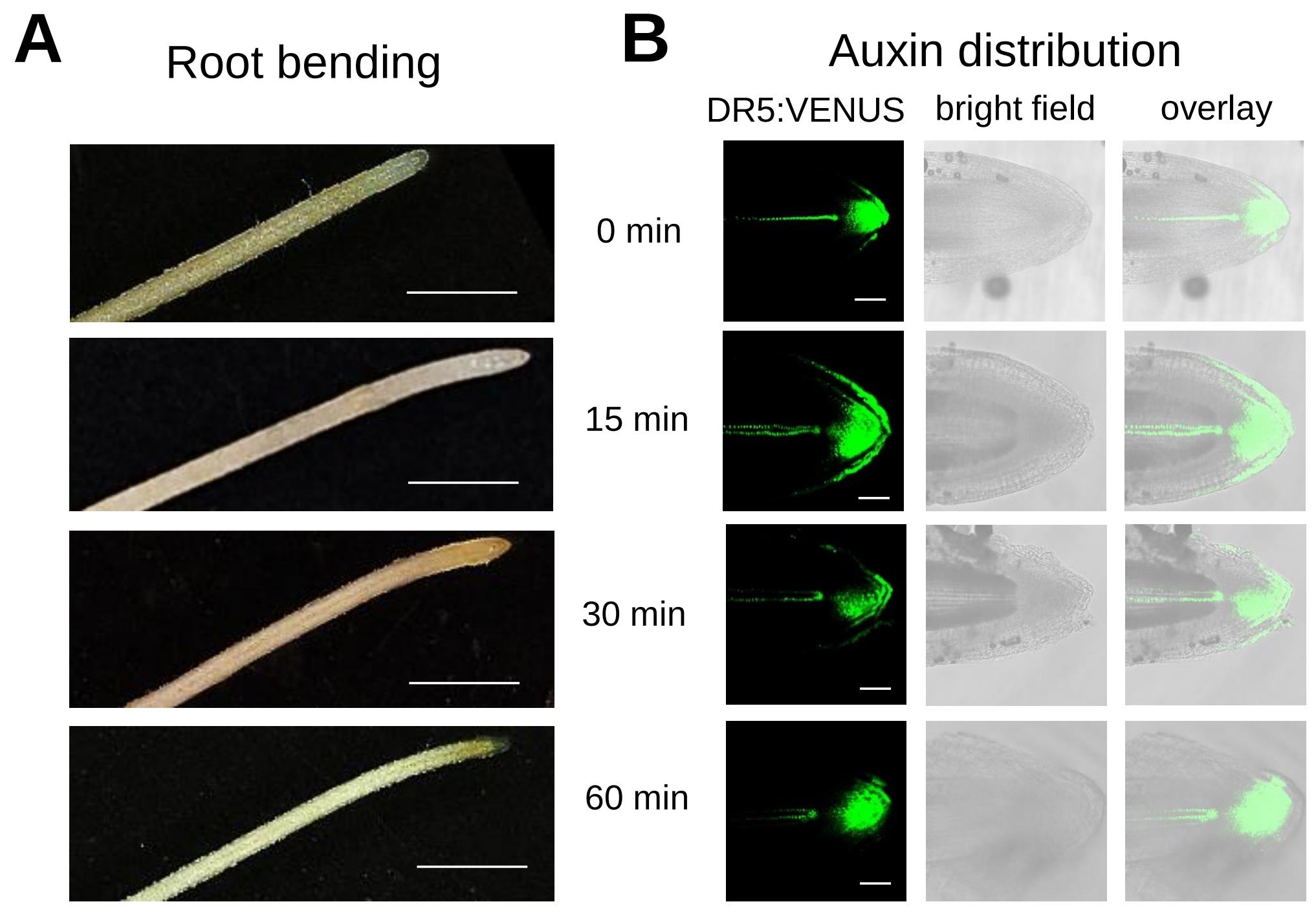


Figure 5: Light exposure triggers higher auxin activity at the lower side of AR tips.
(A) Rice stem sections were kept in the dark for 3 d to induce upward AR growth and afterwards exposed to the light for up to 60 min; bar=2 mm.
(B) Stem sections of the *DR5:VENUS* reporter line were kept in the dark for 5 d and subsequently transferred to the light for 15 min, 30 min or 60 min. Auxin activity in ARs was detected by CLSM; bar=100 µm.

Conclusion

- Polar auxin transport through efflux carriers controls auxin activity in AR primordia and in epidermal cells above ARs.
- *OsPIN2* may mediate auxin depletion in the epidermis above AR primordia and induction of epidermal cell death.
- Polar auxin transport regulates the angle at which ARs grow.
- Auxin shapes the AR system architecture in rice.

References:

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(2) Tanaka H, Dhonukshe P, Brewer PB. and Friml J (2006) Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cellular and Molecular Life Sciences CMLS 63(23): 2738-2754.
(3) Yang J, Yuan Z, Meng Q, Huang G, Périn C, Bureau C, Meunier AC, Ingouff M, Bennett MJ, Liang W, Zhang D (2017) Dynamic regulation of auxin response during rice development revealed by newly established hormone biosensor markers. Frontiers in Plant Science 8: 256.
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