AZG2 transporter plays a key role in auxin/cytokinin lateral root development regulation

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Abstract

AZG (AZa-Guanin resistant) is a gene family first described in fungi as purine transporters. Two members of this family, Azg1 and Azg2, are encoded in the Arabidopsis genome. Although they are able to transport purines when functionally expressed in yeast, their physiological role has not been addressed. In the present work, we found evidences suggesting that Azg2 is involved in lateral root (LR) development regulation. The function of Azg2 as a cytokinin (CK) transporter could be demonstrated *in vivo*. AZG2 is expressed in a limited number of cells surrounding the LR primordia (LRP). Azg2 promoter activity is present since stage one of LRPs and persist even when the LR is emerged. Moreover, initial approaches to AZG2 subcellular localization revealed that the transporter is localized at the plasma membrane. We also found that Azg2 expression is induced by auxins being the transcription factor ARF7 necessary for gene activation. Analysis of transgenic lines showed differences in root architecture. Azg2 KO lines showed more LR density than Wt plants, while over-expression lines (OEs) presented a conditional phenotype with lower LR density. Furthermore, when plants were treated with CKs, the differences between phenotypes increased significantly. KOs lines showed resistance to CKs, whereas an enhanced toxicity in the OEs was observed. All this data encourage us to propose Azg2 as a key factor in cytokinin inhibition of LR development.

1 Azg2 is expressed surrounding LRP and micropilar endosperm

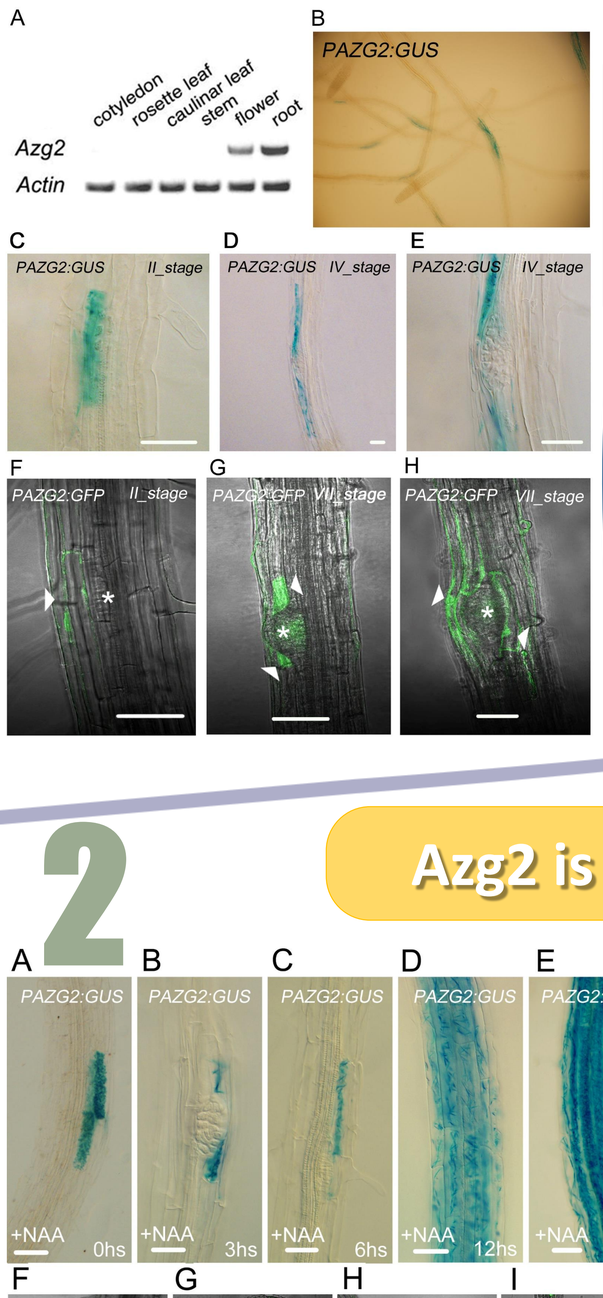

Figure 1.- Azg2 expression is detectable in roots and flowers by RT-PCR. Two different reporter lines, pAzg2::GUS (B-E, I,J) and pAzg2::GFP (F-H) show expression within the root in a reduced number of cells (presumably cortex) surrounding LRP. Azg2 is present in LRP since stage I of LR development (C) . Besides, Azg2 is expressed in seed. After 8 hours of light exposition Azg2 is expressed in the micropylar endosperm where testa is opened (I and J).

2 Azg2 is induced by auxins

Figure 2.- Two different reporter lines (GUS and GFP) were treated with 1uM Naphthalene acetic acid (NAA). After 24hs the reporter signal was spread through out the root (A-I). Furthermore, different lengths of the Azg2 promoter (2000, 1400, 1000 and 240bp) were analyzed revealing that 240bps are sufficient for Azg2 expression in its characteristic cell pattern (H). To determine if ARF7 and ARF19 transcription factors mediate auxin induction of Azg2, KOs lines for both ARFs were transformed with pAzg2::RFP. After treating plants with NAA for 24hs, RFP signal was detectable in Col-0 and arf19 background plants. This result suggests that Azg2 is induced by auxin in ARF7 dependent manner.

3 Azg2 is localized at the plasma membrane

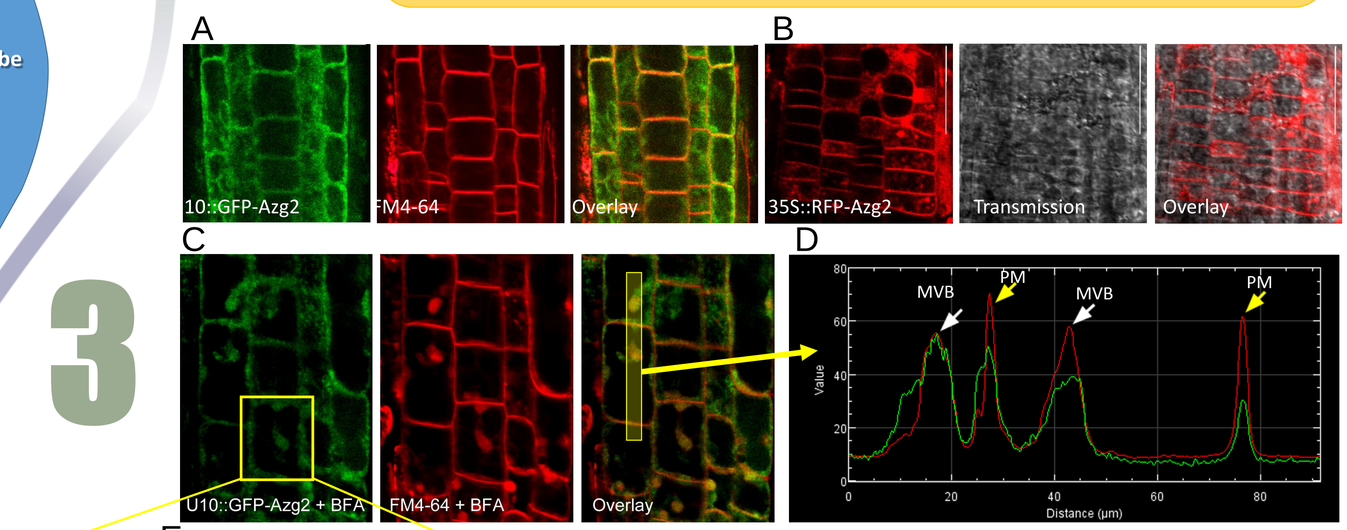

Figure 3.- GFP-and RFP-Azg2 fusions were constitutively expressed in plants. First approaches suggest AZG2 localizes at the plasma membrane. This affirmation is support by the co-localization with the membrane dye Fm4-64 and formation of multi-vesicular bodies containing Azg2 after the treatment BFA. The multichannel plot (J) shows co-localization in plasma membrane (PM) and MVB multi-vesicular bodies.

4 Azg2 is part of a regulatory mechanism for lateral root emergence

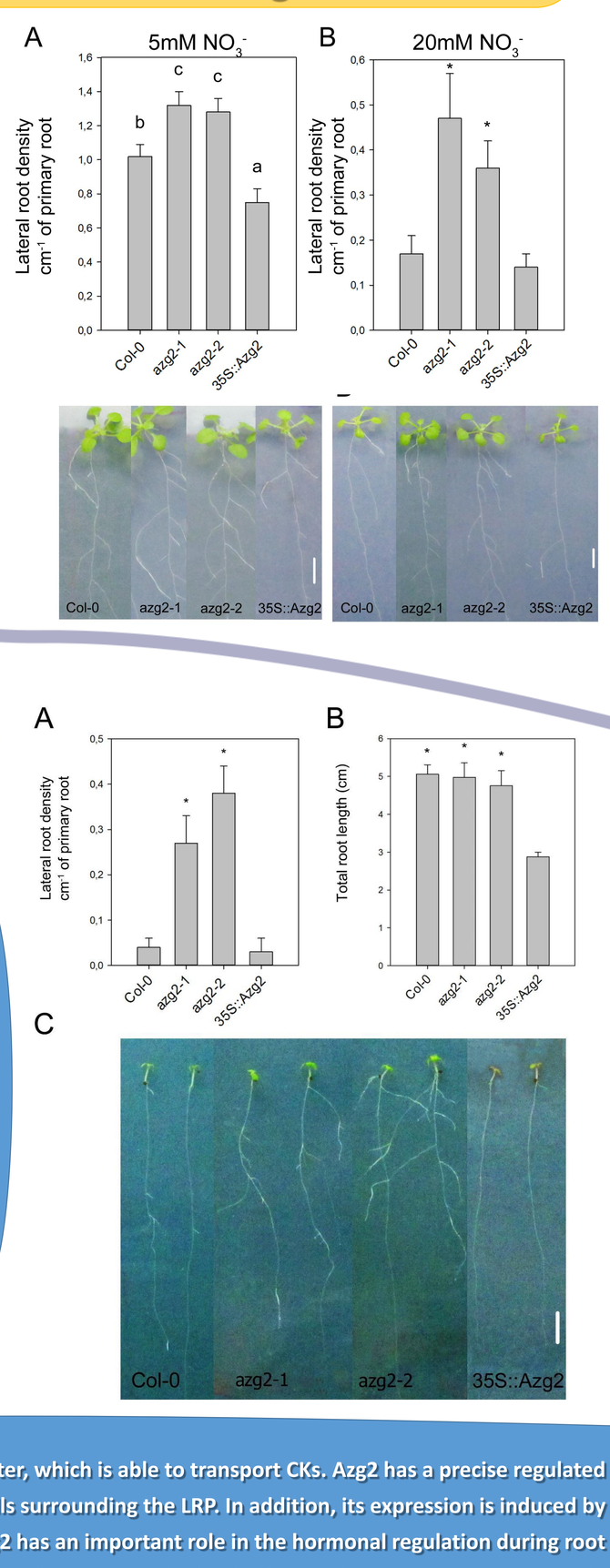

Figure 4.- Azg2 KOs and OEs lines do not show a clear phenotype under normal growth conditions. Nevertheless, when the root architecture was analyzed, we observed higher lateral root density in Azg2 KOs than Col-0 plants. Moreover, under low Nitrogen conditions (5mM NO3-) OE plants showed even less LRs than Wt.

5 Exogenous CKs have differential effect in Azg2 KOs and OEs

Figure 5.- When Col-0 plants are treated with exogenous CKs, they show a strong diminishment in LR emergence. Interestingly, KOs plants of Azg2 developed many lateral root despite of the CK treatment. Azg2 KOs seem to be partially insensitive to the CKs LR emergence inhibition (A-C). For OE lines we observed enhanced CK toxicity effect. We studied main root length to determine that effect and the OE line showed clear differences compared to other lines.

Conclusion

AZG2 is a plasma membrane transporter, which is able to transport CKs. Azg2 has a precise regulated expression within reduced number of cells surrounding the LRP. In addition, its expression is induced by auxins. All this features suggest that Azg2 has an important role in the hormonal regulation during root system development. Lastly, the differences between Azg2 KOs, OE lines and Col-0 phenotypes strongly support this hypothesis.

Acknowledgement

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