Investigating the role of MYB and ARABIDILLO proteins in root development

Jessica Finch[1,2]* and Juliet Coates[1]

[1] School of Biosciences, University of Birmingham, Edgebaston, B15 2TT. [2] MIBTP, School of Life Sciences, University of Warwick, CV4 7AL. * j.finch@warwick.ac.uk

Introduction, background and aims

Involvement of MYB93 and ARABIDILLO proteins in lateral root formation

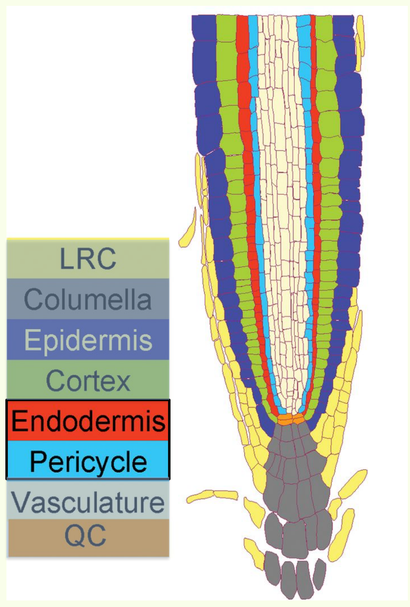

Fig. 1 Layered structure of a primary root. The endodermis plays a key role in regulating LR initiation in the pericycle. Adapted from Kerr & Bennett (2007)[4].

Lateral roots (LR) are an essential structure in vascular plants for the uptake of key nutrients from the soil. LR primordia arise from cells in the pericycle layer of the primary root and the developing LR emerges through the cells of the overlying endodermal layer (Fig.1). The mechanical properties of the endodermis control the rate of LR formation and the cells undergo structural remodelling to accommodate the protrusion[1].

AtMYB93 is a negative regulator of LR development, as knockout mutants show increased LR density, while plants expressing 35S::*MYB93* show decreased LR density[2].

Furthermore, GUS-staining and qRT-PCR have shown MYB93 mRNA to be spatially and temporally localised to the endodermal cells directly overlying LR primordia initiation in the pericycle, immediately before and during the process (Fig. 2)[2].

ARABIDILLO proteins are F-box proteins homologous with the Armadillo/β-catenin proteins crucial to development in fungi and animals[3]. ARABIDILLO-1 and -2 have the opposite effect on LR density to MYB93[3], and interact with MYB93 protein[2]. It is proposed that MYB93 and ARABIDILLOs are antagonistic interaction partners involved in the regulation of LR formation[2].

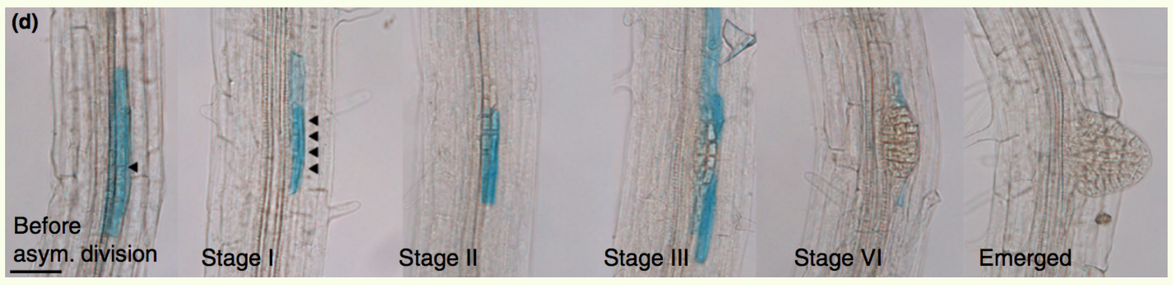

Fig. 2 Stages of LR formation in roots expressing *pAtMYB93::GUS*, showing localisation of MYB93 to endodermis immediately overlying LR-forming pericycle cells. From Gibbs *et al* (2014)[2]

MYB93, ARABIDILLO and stress: interacting influences on LR density

- Root architecture is known to adapt in response to stress: e.g. salt stress negatively regulates lateral root formation[5].
- The Coates lab has shown root architecture adaptations to salt stress to be altered in MYB93 and ARABIDILLO mutants, suggesting that these mutants have an altered barrier between the root and soil.
- One of the key main barriers to diffusion in roots is suberin in the endodermal layer (see Fig. 1). The process of suberisation has been shown to be flexible and reversible in response to nutrient stress[6].
- Co-expression analysis from the Coates lab suggests that MYB93 may upregulate components of the suberin biosynthesis pathway. The link between MYB93 and suberin has also been shown by a recent paper[7].

Hypothesis and experimental approach

Hypothesis: MYB93 and ARABIDILLO mutants and overexpressors will show altered responses to S stress during root development compared with wild type plants (see Fig. 3 for rationale)

To test this hypothesis, we carried out a phenotypic analysis of *Arabidopsis* root growth in MYB and ARABIDILLO transgenic plants (mutants and overexpressor lines) with and without sulfur in the growth medium.

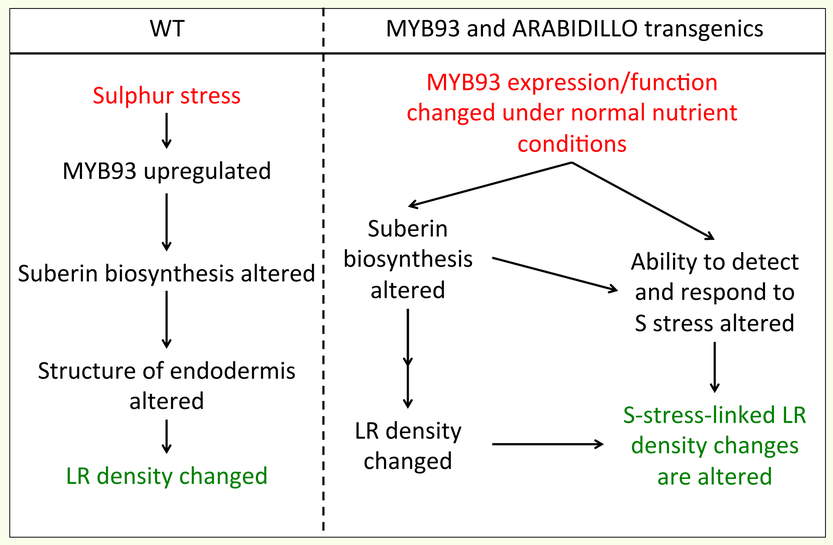

Fig. 3 A summary of our hypothesised link between S stress, MYB93, ARABIDILLO, suberin biosynthesis and LR density, based on the information presented above.

Results: Root assay with normal or low sulfur

30 seeds per genotype (distributed equally between 3 plates) were grown on 'normal' MS media (S concentration: 1.73 mM), or 'low-S' MS media (S concentration: 0.16 µM, ~11,000 times less S than in normal media).

Data was collected from 9-day-old seedlings in the form of manual LR counts and measurement of primary root length from photographed plates using ImageJ, in order to calculate LR density (Fig. 4).


Example root assay *J. Finch*

The key findings were:

- There was no *statistically* significant difference between primary root length on normal media and on low-S media for any of the genotypes (t-test, p = NS), although there was a consistent *trend* towards longer primary roots on low-S media for all genotypes, consistent with Kutz *et al.* (2002)[10]
- The WT seedlings and *myb92, myb93* double mutants both had higher LR density on low-S media than on normal media, in contradiction of the findings of Dan *et al.* (2007)[8], but in accordance with the findings of Kutz *et al*. (2002)[10]
- In normal media, 35S::*MYB93* has higher LR density than WT (ANOVA, post-hoc multiple comparison test, $p < 0.05$) at odds with previous studies which find higher lateral root density in *myb93* mutants and lower in 35S::*MYB93*[2].
- The 35S::*ARABIDILLO1* seedlings were distinctly less healthy on both media, but especially on the low-S media, than any of the other genotypes (possibly accounting for the low recorded LR density and large standard error).

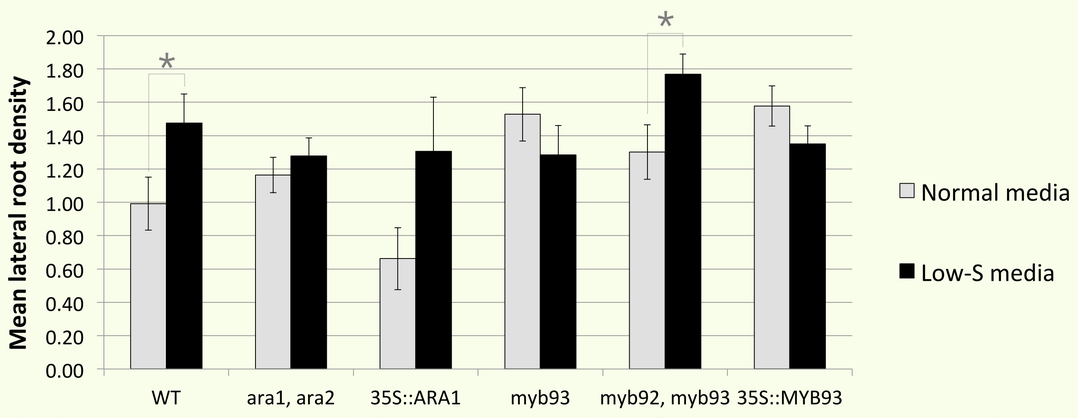

Fig. 4 Mean lateral root density of 9-day-old seedlings of the 6 genotypes on normal and low-S media. Error bars represent ±1 standard error of the mean. * = $p < 0.05$ (two-tailed t-test).

Conclusions and Future Directions

Conclusions

Little research has previously been carried out into the effect of sulfur stress on lateral root density. Dan *et al.*[8] and Kutz *et al.*[10] base their contradictory findings on small sample sizes, thus our finding that LR density increases under low-S stress (in WT plants) represents the most strongly data-supported evidence of S-stress-linked LR density changes to date.

However, our comparison of S-stress-related changes in LR density between genotypes proved inconclusive. Unexpectedly, the *myb93* mutants and the MYB93 overexpressing plants displayed very similar behaviour with respect to their LR density on normal and low-S media, somewhat undermining the validity of our results.

In general, the LR density of each transgenic genotype on normal media, compared with WT plants, did not show agreement with previously published findings, suggesting possible confounding factors such as seed quality played a role. This means that we cannot draw any firm conclusions about the change to each genotype's LR density between normal and low-S conditions.

Suggested further study

- A large scale, high-throughput study of WT LR responses to sulfur-stress should be carried out to establish the baseline behaviour against which transgenic LR S-stress-linked responses can be compared.
- A larger scale repeat of the current experiment but with fresh and verified seed (i.e. genotype confirmed) and with media made from fresh chemicals.
- A study of suberin distribution in WT plants and MYB93 and ARABIDILLO transgenics under normal and S-stressed conditions.

References

1. Vermeer, J. E. M. *et al.* (2014) *Science (80-.).* **343,** 178 LP-183
2. Gibbs, D. J. *et al.* (2014) *New Phytol.* **203,** 1194–207
3. Coates, J. C., Laplaze, L. & Haseloff, J (2006) **103**
4. Kerr, I. D. & Bennett (2007) M. J. *Biochem. J.* **401,** 613–22
5. Duan, L. *et al.* (2013) *Plant Cell* **25,** 324–41
6. Barberon, M. *et al.* (2016) *Cell* **164,** 447–459
7. Legay, S. *et al.* (2016) *New Phytol.*
8. Dan, H., Yang, G. & Zheng, Z. L. (2007) *Plant Mol. Biol.* **63,** 221–235
9. Bielecka, M. *et al.* (2014) *Front. Plant Sci.* **5,** 805
10. Kutz, A. *et al.* (2002) *Plant J.* **30,** 95–106

Acknowledgements: Thanks are given to the members of the Coates group for useful discussion and guidance. This work is funded by the BBSRC through the Midlands Integrative Biosciences Training Partnership (MIBTP).