MolCALM: molecular and computational analysis of the leaf margin

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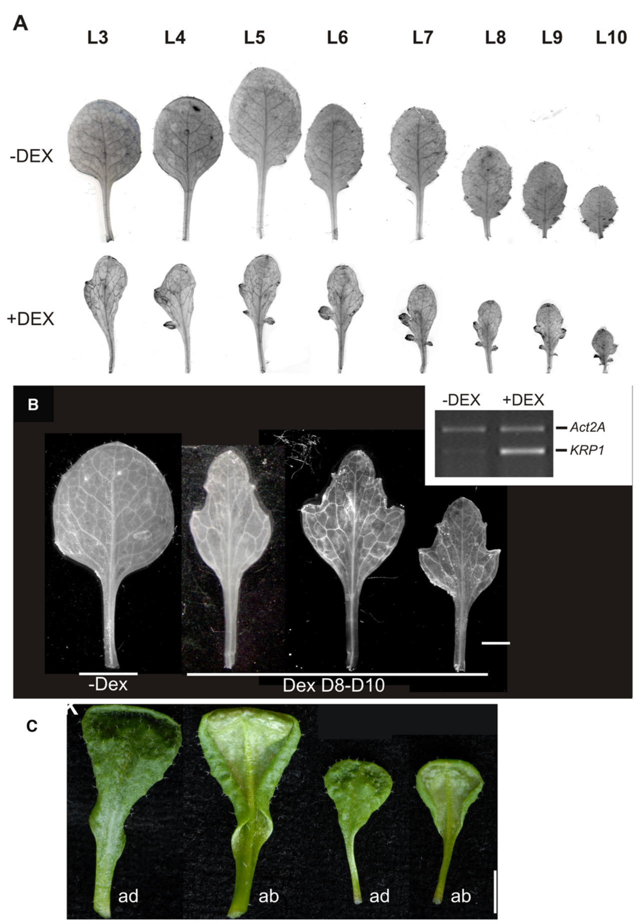
Introduction

The plant leaf is the major organ involved in photosynthesis and thus plays a major role in controlling crop yield. Recent years have seen significant advances in our understanding of the major events underpinning leaf initiation and the subsequent process of leaf development. However, the mechanisms controlling leaf size and shape remain obscure. The main aim of this project was to investigate the role of a specific region of the leaf (the margin) in controlling leaf size and shape. This involved a combination of molecular approaches with novel tools to manipulate gene expression during leaf development. The project provides insight into a basic problem in plant development (with potential linkage to novel approaches to increasing crop yield).

Manipulation of the leaf margin

In a first series of experiments, leaves of the model plant *Arabidopsis thaliana* were analysed using various light microscopy techniques (Nomarski imaging and confocal imaging) to create an atlas of the differentiation of a specific cell type (the margin) which arises around the periphery of the developing leaf. Previous data, including those from the host lab, had suggested that this cell type plays an important role in the control of leaf size and shape. Having described the system, we then targeted repression of growth in cells around the leaf margin, either uniformly in all margin cells or in small groups of margin cells. This involved the use of an enhancer trap line to target the expression of a gene whose product represses cell division (AtKRP1) around the margin or the use of an inducible promoter system (pOpON) linked to elements which targetted gene expression to localised regions at the leaf periphery (CUC2 promoter element) after supply of the Dex inducer.



Following the targetted repression of cell division in the punctate CUC2 domain, a strikingly altered leaf form was observed. Instead of the relatively smooth, slightly serrated form which characterises normal Arabidopsis leaf development, a highly lobed, pseudo-compound leaf form was generated (Fig. 1A). These results show that localised repression of growth at points around the leaf periphery can be used to generate lobed leaf forms.

In a complimentary set of experiments, we exploited the fact that specific miRNAs play a role in controlling gene expression patterns in the developing leaf. By tagging our effector gene (cell division inhibitor KRP1) with a miRNA sequence, we could generate leaves with a novel form for Arabidopsis, reminiscent of a goosefoot (DEX D8-D10 in Fig. 1B). Although Arabidopsis does not form leaves with this shape, some close relatives do, suggesting that alteration of growth patterns at the leaf perimeter might play a role in leaf shape evolution.

When the same cell division repressor was expressed uniformly around the leaf perimeter, a smoother circumference was generated. However, as the leaf grew there was a dramatic change in the 3D form of the leaf. Instead of forming a typically planar structure, the leaf formed a distinct cup-shape (Fig. 1C). This is the form expected if a growing plane of cells is surrounded by a periphery of cells showing a relatively low rate of growth (as predicted if cell division is inhibited). This morphology supports previous ideas on the role of tissue mechanics in the control of leaf form.

Overall, these data demonstrate the potential importance of local growth repression in the control of leaf form and the potential for manipulating leaf shape in a targeted fashion. These results have been published (Malinowski, Kasprzewka & Fleming (2011) Plant J. 66, 941-952).

In a final piece of research (still ongoing), we have looked at the potential role of auxin in the leaf margin in the control of shape. A number of papers have suggested that the directed flow of auxin around the leaf periphery plays an important role in the generation of vascular patterns within the leaf but the outcome on leaf shape has been less well characterised. To do this we have incorporated a modelling element to simulate patterns of auxin flux around the leaf and we are now comparing these model simulations with experimental data on the patterns of auxin transport and the morphology that results from interfering with these patterns. These results will form the basis of a second publication as a scientific output of this research project.

In addition to the research achievements summarised above, the Marie Curie Fellow has undertaken a number of training activities, including an advanced confocal microscopy course. She has attended international meetings and presented posters describing her work, allowing her to build networks with other European scientists. The work will be of interest to both academics interested in a deeper understanding of the mechanism of plant morphogenesis and to researchers in the area of crop science and agriculture aiming to modify and improve plant performance by the adaptation of plant morphology.

In summary, the project achieved its goals. Excellent science was performed and the Fellow gained in technical and associated training which will prepare her for the next stage in her scientific career.