

Shade induced hypocotyl elongation in Arabidopsis seedlings: an imaging approach and Identification and analyses of D27 splice variants in *Petunia axillaris* and *Arabidopsis thaliana*

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This research took place in an effort to clarify the roles and factors involved in growth in Arabidopsis plants. The hypocotyl is often used in growth studies, including growth induced by shade avoidance, hormone treatments, and cell wall studies. In the first week after germination, hypocotyl growth depends solely on elongation of cells as no cell division take place. Epidermal cells have a thicker cell wall and are considered the limiting force of growth. Auxin biosynthesis predominately takes place in the cotyledons and auxin is transported rootward. Biosynthesis is upregulated upon shade and auxin flow is redirected through PIN (PINFORMED) exporters towards the hypocotyl epidermis where it induces growth. The use of image analysis software allowed for more precise, multidimensional, measurements compared to manual measurements. This research showed that shade and exogenous auxin treatments have similar effect on growth patterns, during which growth was predominantly observed in cells in the middle of the hypocotyl. Strong vascular staining of the auxin marker DR5::GUS was observed after IAA treatment. Staining was strongest at the root-hypocotyl junction and weakened going shootwards. Since auxin and (changes in) auxin flux have such an important role in shade avoidance, the triple PIN mutant pin3pin4pin7 was investigated. This mutant is insensitive to shade and here we show that growth only occurs under prolonged exposure to high auxin. Interestingly, this growth is strongest at the bottom of the hypocotyl under certain treatment conditions. Coincidentally, these cells also have an enlarged width, suggestion more isotropic growth than their wild-type counterparts.

Among the many genes induced by shade there are cell wall biosynthesis genes. In previous studies, both CGR2 and CGR3 (cotton Golgi-related) were found to be upregulated during shade avoidance, raising the question if shade can induce structural changes to the cell wall. To address this question cgr2cgr3 mutant was tested for its response to shade treatment. CGR2 and CGR3 are pectin methyltransferases with important roles in rigidity of the cell wall and can therefore influence cell elongation. Here we show that cgr2cgr3 it is impaired in its elongation upon shade. The growth pattern is similar as that in WT plants. Surprisingly though the width of the cell is similar to the width of WT. Together this indicates other cell wall changes involved in elongation and possible compensation measures to the introduced mutations. Together the results indicate that there is a consistent pattern of growth of which its prominence is based on overall length. Proper transport is required to distribute auxin appropriately along the hypocotyl. Since the growth patterns do not correlate with DR5 expression it is yet to be determined how these two facts relate to each other.

This thesis also reports on a second additional project, unrelated to hypocotyl elongation. Dwarf27 (D27) is an isomerase of β -carotene, involved in the first step of strigolactone synthesis. D27 has a domain of unknown function 4033 (DUF4033). Here we report on that D27 has a previously unknown splice variant that potentially disrupts this DUF besides the previously reported longer transcript. While both splice variants locate in plastids, they appear to have separate tissue expression patterns. The question we tried to answer is if these splice variants have separate functionality. This research will be discussed in a single separate chapter.

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