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Plant responses to a grafting process on the example of *Arabidopsis thaliana* hypocotyl during *in vitro* culture

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Micrografting of *Arabidopsis* hypocotyl is an elegant tool for studying many developmental and signaling processes, and a few approaches for efficient grafting procedures have been presented so far (Turnbull et al., 2002). These methods focus on gaining a stabile, functional graft union. Using the method described by Yin et al. (2012), 4 day old *Arabidopsis thaliana* hypocotyls were grafted, with no excision of cotyledons or adventitious roots. The aim of the current study was to analyze various reactions of grafted hypocotyls on tissue and cellular levels as a response to, among others, the mechanical stress that triggers morphological changes.

The stress reactions of grafted hypocotyls were: 1) reduced growth of scion part of the graft in comparison to control plants; 2) occurrence of anthocyanins in cotyledon and hypocotyl epidermis; 3) disappearance of chlorophyll from stock; 4) callus overproduction in the graft union area with cells of different size and shape; 5) emerging of adventitious roots. In transgenic lines (DR5rev::GFP and PIN1::GFP) extraordinary adventitious roots were formed, which had different phenotype (an increased number of root hairs and development of peculiar lateral roots) from those formed by Columbia wild type grafted hypocotyls.

A histological analysis of grafted hypocotyls revealed that some TEs (tracheary elements) developing within the callus tissue do not take part in vascular reconnection. Their organization and orientation is variable, forming different vortices which look very similar to the vortices observed in the woody plants during both *in vitro* and *in vivo* conditions, as an expression of circular polarity (Kurczyńska and Hejnowicz, 1991) and occur only in the scion, the part of the graft where accumulation of auxin takes place.

Nile Red stain showed occurrence of lipid substances in periclinal cell walls of endodermis next to the vascular cylinder in control hypocotyls. In endodermis of grafted plants lipid substances are deposited in all cell walls, not only the periclinal ones. Polyphenolic substances were present in outer periclinal cell walls of endodermis. We also found that some cells of ground tissue exhibit hypertrophic growth and eventually die, leading to the formation of large empty spaces, mostly within the stock.

The results from an immunocytochemical study show a more abundant occurrence of two arabinogalactan protein epitopes, recognized by JIM8 and JIM13 antibodies in endodermal cells and disappearance of galactan epitope recognized by LM6 antibody, in comparison to control hypocotyls. JIM16 epitope, representing an arabinogalactan protein, occurs abundantly in degenerated stock, coating the borders of dead cells. LM8 epitope (xylogalacturonan) was found in vessels and between cells of ground tissue or between scion and stock cells of grafted seedlings, whereas in control plants this epitope did not occur at all.

In conclusion: in stressed by grafting Arabidopsis cotyledons in *in vitro* conditions cells response involves changes in the direction of the auxin flow and the chemical composition of cell walls in tissues engaged in response to stress conditions.

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