

Abstract

Plant oriented movements, or tropisms allow the plant to actively respond to environmental stimuli to get more light, better access to nutrients and to grow roots deeper into the soil. Gravitropism drives the growth of roots along the gravity vector. Perception of gravity is triggered by the sedimentation of statoliths in columella root cap, but the exact signalling pathway behind this process is not known. Perception of gravity results in an unequal redistribution of the phytohormone auxin in the outer cell layers which leads to different rate of growth on the root's upper and lower side and bending of the root. The changes in auxin redistribution are accompanied by changes in apoplastic pH. Knowing an exact pattern of these pH changes could shed light on the mechanisms laying behind the gravitropic response pathway. While microelectrodes can be used to measure pH precisely, they are not suitable for the long-term imaging of growing roots. In the past few years, several pH sensitive dyes and genetically encoded sensors emerged. These can be used for long-term live *in vivo* imaging of pH changes in growing roots.

In this thesis, I analysed the performance of several published pH sensitive genetically encoded sensors and available dyes in the roots of *Arabidopsis thaliana*. I observed that dyes varied greatly in their sensitivity to pH as well as their ability to penetrate the root apoplast and cells. I discovered one pH sensitive dye that reported pH with a high spatio-temporal resolution and covered the pH range suitable for the rhizosphere. Using this dye, I could visualize the highly dynamic pattern of changes in root surface pH during gravistimulation, similar to pattern previously reported in monocots.

Most of the genetically encoded sensors were not suitable for reporting pH in the root apoplast, apart from one that was not expressed evenly in the root tissues. I recloned the promising sensor to be driven by a set of promoters that improved its expression, and the resulting sensors will be used for further experiments. Finally, based on my observations from previously published genetically encoded sensors used in this thesis, I also designed and cloned a series of new genetically encoded pH sensitive sensors anchored to the cell wall polymers. Genetically encoded pH sensitive sensors and especially dyes can be used in wide range of experiments, not only for determination of pH upon gravistimulation, providing insight into other aspects of plant life.

Key words: apoplast, pH, root, auxin, growth, gravity, *Arabidopsis*, cell wall, apo-pHusion, apo-pHluorin, HPTS, live-cell imaging