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Auxin metabolite profiling

The homonal profiling methods use modern analytical tools based on fast chromatopraphic separation and mass spectrometric quantitative analysis. The major problem associated with plant hormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. On the other hands, the robustness, sensitivity, selectivity, through-put and cost-effectiveness of analysis are also critical factors for convenience of developed method.

The plant hormone auxin is believed to influence almost every aspect of plant growth and development. Auxin transport and metabolism combine to form gradients and maxima/minima in developing cells and tissues, and these dynamic changes in auxin levels are believed to influence specific developmental processes. In order to understand these processes, measurements of steady state auxin levels and biosynthesis rates can give valuable information, and recent advances in instrumentation and method development makes it now possible to quantify auxin at cellular resolution. There is abundant genetic evidence for the existence of multiple pathways for auxin biosynthesis and degradation. This redundancy makes it difficult to understand the relative importance of specific metabolic pathways during development.

We have now developed a method for profiling the majority of known auxin precursors and conjugates/catabolites in small amounts of *Arabidopsis* tissue. Our method includes trace analysis of 21 compounds with different polarity, acidity and basicity as well as stability and abundance in crude plant extract. We found that a using of polymer based (Oasis HLB) sorbents was the best tool in the one-step purification, giving the average total extraction yields close to 85%, 60% and 50% for 0, 10, and 20 mg of fresh plant weight, respectively. The process was completed by a single chromatographic analysis of axin metabolites in 12 minutes using an analytical column packed with sub-2-microne particles. In multiple reaction monitoring mode, the detection limit for most of analytes ranged from 1.0 to 5.0 fmol and achieved linear range was at least five orders of magnitude. Finally, the accuracy and precision of the method was validated using spiked samples of 10-day-old *Arabidopsis thaliana* seedlings.

We have then used this method to profile different tissues from *Arabidopsis* ecotypes and mutant lines, and can show that there are substantial differences in metabolite levels. We hope that by using this method, we will have a tool to get a better understanding of the regulation of auxin metabolism during plant development.