Using a chemical genetics approach to dissect the nitrogen signalling pathway in Arabidopsis

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Abstract

Nitrate is an important nutrient and signalling molecule to plants. As it is taken up and assimilated, reduced forms of N accumulate and the expression of many genes associated with nitrate assimilation are repressed. Little is known about the mechanisms involved in this N repression. This project, for the first time, adopts a chemical genetics approach to investigate the feedback regulatory pathway that links the plant's N status to expression of the *NRT2.1* nitrate transporter gene. A novel chemical screening platform was developed that was designed to be used in conjunction with Arabidopsis lines expressing luciferase reporter genes in roots. This semi-hydroponic platform allows roots to be exposed to a variety of nutrient treatments in a 96-well plate format suitable for chemical genetic screens. This was combined with a newly developed 'ice capture' method that provided a rapid and efficient way to harvest root material for the luciferase assay. Using this screening platform in conjunction with a nitrate-inducible luciferase reporter line, *pNRT2.1::LUC*, three chemical libraries, containing 7420 bioactive molecules were screened in duplicate for compounds that antagonise N repression of luminescence. The screen identified a plant-derived alkaloid, camptothecin, that enhanced *pNRT2.1::LUC* expression under N-repressive conditions. The positive effect of camptothecin on expression of the endogenous *NRT2.1* gene was confirmed using real-time PCR and shown to extend to other N-repressed genes of the nitrate assimilatory pathway. Camptothecin is known to target topoisomerase I, an enzyme that is increasingly being linked to a role in chromatin re-modelling, in addition to its more familiar roles in DNA replication and repair. The possible epigenetic role of topoisomerase I in repression of *NRT2.1* and other genes of the nitrate assimilatory pathway is discussed. It was also observed that an arginine treatment strongly stimulated *pNRT2.1::LUC* in the luciferase assay, in a nitrate-dependent manner. Since this effect was not observed at the mRNA level, it is hypothesised that arginine was acting on *pNRT2.1* expression at a post-transcriptional level.