

## PERSPECTIVE

# In preprints: hormonal stepping stones to diverging root organogenesis

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Legumes form a symbiotic relationship with bacteria called rhizobia. The rhizobia fix atmospheric dinitrogen, which is used by the plant as a nitrogen resource, and take up molecules, including carbon compounds, from the plant. Rhizobia are housed in nodules that develop on roots after a series of orchestrated stages that are triggered by the perception and entry of rhizobia at root hairs (reviewed by Luo et al., 2023). Study of this relationship and the mechanisms that govern nodule formation and inhibition may reveal targets to promote enhanced nodulation efficiency and increased nitrogen acquisition for the plant. Phytohormones have been shown to play a key role in both nodule formation and inhibition; therefore, a better understanding of hormonal regulatory activity could provide options for such enhancement. To address this, Drapek and colleagues (2023 preprint) focus on the dynamics of gibberellin (GA), a phytohormone previously identified as both a positive and negative regulator of nodulation (Rizza et al., 2017; Fonouni-Farde et al., 2016).

Transcription factor nodule inception (NIN) activates C-terminally encoded peptides (CEPs), which act as signals leading to downstream activation of early nodulation genes. Alongside this, NIN activates CLAVATA3-like (CLE) peptides, which act as signals in the autoregulation of nodulation pathway that causes inhibition of early nodulation genes downstream (Laffont et al., 2020). NIN also has links with the phytohormone GA through activating expression of the GA biosynthesis enzyme gibberellin 3-oxidase (GA3ox). Drapek and colleagues aimed to visualise how the cellular location of GA changed over the course of nodule development through use of a high-sensitivity GA biosensor developed in other recent work (Rizza et al., 2017; Griffiths et al., 2023 preprint). They prepared this GA biosensor for deployment in *Medicago truncatula* using Golden Gate cloning, introducing a mScarlet-I red fluorescent marker to allow them to screen for transformants. They used a traditional callus induction strategy for generating stable lines of *Medicago truncatula* that express the biosensor. Their methodological approach and generated lines raise the possibility of adapting this sensor system for multiple phytohormones.

Using this biosensor, Drapek and colleagues tested the hypothesis that GA3ox catalyses a rate-limiting step in GA biosynthesis. Surprisingly, they found that GA accumulation could also be influenced by post-transcriptional regulation of GA3ox activity or depletion of GA. To determine the impact of lost GA3ox function, they isolated *M. truncatula* lines with insertions of the Tnt1 transposon in the *ga3ox1* gene. These seedlings had short internodes compared with nodules, which aligns with previous

GA-related mutant phenotypes (Kim et al., 2019). GA3ox1 and GA20ox were both found to be expressed in the mutant lines and, although there was no increase in nodule number, nodule size did increase. Expressing the *MGAOL1* gene, which encodes an enzyme that breaks down GA, under different cell type-specific promoters pinpointed the effects of GA on nodule development to the cortical cells. The authors concluded that GA was a positive regulator in nodule organogenesis, and also found evidence for a feedforward loop involving cytokinin, then NIN-mediated regulation of local GA accumulation.

To further characterise the GA molecular interactions involved in nodule formation, the authors went on to target *NODULE ROOT (NOOT)* and *LIGHT SENSITIVE SHORT HYPOCOTYL (LSH)*, key genes that contribute to nodule identity (Schiessl et al., 2023 preprint; Magne et al., 2018). The double mutants *lsh1/lsh2* and *noot1/noot2* were transformed with the GA reporter and imaged, and both were found to have significantly reduced GA in the nodule. These GA-depleted nodules were converted into root-like structures, suggesting that GA accumulation may delineate lateral root versus nodule formation. It was hypothesised that addition of GA may revert mutant phenotypes, and, although nodule size was reverted, nitrogen fixation levels were not reverted. The authors therefore concluded that NOOT and LSH play other roles in nodule formation beyond modulating GA levels.

Interplay between the phytohormones auxin and cytokinin has also been raised as a factor regulating the differentiation between lateral roots and nodules. Auxin and cytokinin both positively regulate nodule formation but, in lateral root formation, auxin acts as a positive regulator and cytokinin as a negative regulator (Azarakhsh and Lebedeva, 2023). Applying the transformation techniques used in this work with biosensors may allow spatial and temporal characterisation of other phytohormones predicted to be involved in nodulation. Although this study focused on GA in legume nodules, the sensor will also be useful for investigating the development of other specialised organs in other plants.

This study used *M. truncatula* lines expressing a GA biosensor. However, it was noted there were limitations in using the biosensor in the central zone of the nodule; nitrogenase enzymes present in the central zone require a hypoxic environment to function, whereas the sensor requires oxygen. Future work may involve generating a GA sensor that is not dependent on oxygen, and this may be required for other hormonal sensors designed for research in this complex and active tissue type.

As rhizobia are able to produce GA, Drapek and colleagues suggested that rhizobial GA production could offer the microbe a fitness advantage. Different rhizobia have been shown to have differing nitrogen fixation efficiencies, leading to altered nitrogen accumulation and yield benefits (Lagunas et al., 2023); increased GA levels could underpin this (although this would require further research). Other bacteria present in the endosphere of the root may also produce GA. For example, *Sphingomonas* sp. LK11, an

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endophytic bacteria originally isolated from legumes, was found to synthesize physiologically active GA<sub>4</sub>, and inactive GA<sub>9</sub> and GA<sub>20</sub> in culture media (Khan et al., 2014). Future studies could test whether the presence of this bacteria can enhance nodule phenotypes.

In conclusion, this study analysed the spatial and temporal accumulation of GA, the interactions between GA and key nodule identity regulatory genes, and tested the importance of GA biosynthesis enzymes in nodule formation. The work provides strong evidence that GA is a potential determinant between lateral root formation and nodulation, and helps to explain previous contradictory findings related to the importance of GA, as well as cytokinin, in these processes.

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