

Decoupling Sugar and Spice in Soybean Rhizosphere Depends on BGLU Activity

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Secondary metabolites are central determinants of plant–microbe interactions. They can attract or deter microbes, promote or inhibit their growth and protect the plant from microbe-derived stress (Pascale et al. 2020). Secondary metabolites called flavonoids play a central role in the formation of symbiosis between leguminous plants, such as soybean, and nitrogen (N₂)-fixing bacteria. Plant-produced flavonoids can attract N₂-fixing bacteria and stimulate them to produce Nod factors. Upon perceiving Nod factors, plants alter their tissue development and produce nodules, where these bacteria find an oxygen-low environment conducive to N₂ fixation (Layzell et al. 1993). Isoflavones, the main secreted flavonoids in soybean, are stored inside the plant vacuole in their glycosylated form, a common way for plants to protect themselves from reactive metabolites (Le Roy et al. 2016). When secreted from the roots into the rhizosphere, these metabolites are deglycosylated, which allows them to act as *Nod* gene inducers or chemo-attractants. This deglycosylation is achieved through β -glucosidases (BGLUs), which have been shown to be involved in the secretion of coumarins, another class of plant–microbiome communication signals (Stringlis et al. 2018). However, the extent to which BGLUs influence rhizosphere-secreted metabolites and their potential role in regulating plant–microbe interactions remain unanswered in the field.

In this issue, Matsuda et al. (2023) investigate the role of isoflavone conjugate–hydrolyzing BGLU (ICHG) of soybean in determining the root secretion profile of isoflavone and its conjugates. The authors investigate isoflavone profiles in the rhizosphere, the soil area under the direct influence of the root presence, and the endosphere, the area inside the root, and what the effects of its mutation are on the microbiome of those compartments. The authors identified a nonsense and a missense *ichg* mutant from a library of soybean EMS mutants, whose protein extracts had both lost the ability to deglycosylate malonyldaidzin. These mutant plants showed a reduced accumulation of aglycone daidzein in the root apoplastic fraction, where ICHG is located, while glycosylated isoflavone content was increased. The level of isoflavones inside the root tissue remained stable, indicating that ICHG's effects are limited to the apoplast and function on secreted glycosylates. Since the concentrations of

isoflavones in the apoplast were changed, the authors hypothesized that root exudation of isoflavones might be altered in the *ichg* mutants as well. Using a hydroponics setup, the authors assessed changes in the secretion of isoflavones and their glycosides, comparing their abundance in roots and liquid medium. Counter-intuitively, there seems to be no decrease of isoflavone aglycones in the growth medium, while a decrease was found inside roots. A strong increase of isoflavone glycosides was seen in root exudates of the mutants after 1 week, but not after 5 weeks. To exclude feedforward and feedback effects of the mutation on the regulation and biosynthesis of isoflavones by soybean itself, the authors performed a transcriptomic analysis, confirming no apparent effects on these processes. Since hydroponics growth conditions can affect plant morphology and physiology (Sgherri et al. 2010), the authors opted to assess exudation patterns of the mutants in soil conditions. In a natural soil setup, the soybean *ichg* plants showed the expected decreased levels of isoflavone aglycones in both the rhizosphere and the root tissue, which is in line with the ICHG function and the earlier apoplastic fraction experiment (Fig. 1).

Earlier work showed that isoflavone aglycones alter the composition of the rhizosphere microbiome, enhancing the abundance of *Comamonadaceae* and *Microbacteriaceae*, while repressing that of other families (Okutani et al. 2020). Since the *ichg* mutant showed decreased levels of isoflavone aglycones in the rhizosphere, Matsuda et al. (2023) investigated whether the microbial communities of the *ichg* mutants differed from those of wild-type plants. In rhizosphere samples, no striking differences were observed between the mutants and wild-type plants. In the endosphere, however, an increase of *Yersiniaceae* was observed in both their mutant lines (Fig. 1). Since isoflavone biosynthesis is increased in low N₂ conditions, the authors assessed isoflavone exudation of the mutants as well as the influence on the microbiome in low N₂ conditions, as this theoretically would exacerbate the phenotype of the mutant. The difference between wild-type and mutant plants in contents of isoflavone aglycones and glycosides was similar to their previous experiment, albeit stronger through enhanced isoflavone biosynthesis, showing a robustness of their experimental setup. Endosphere bacterial communities of both

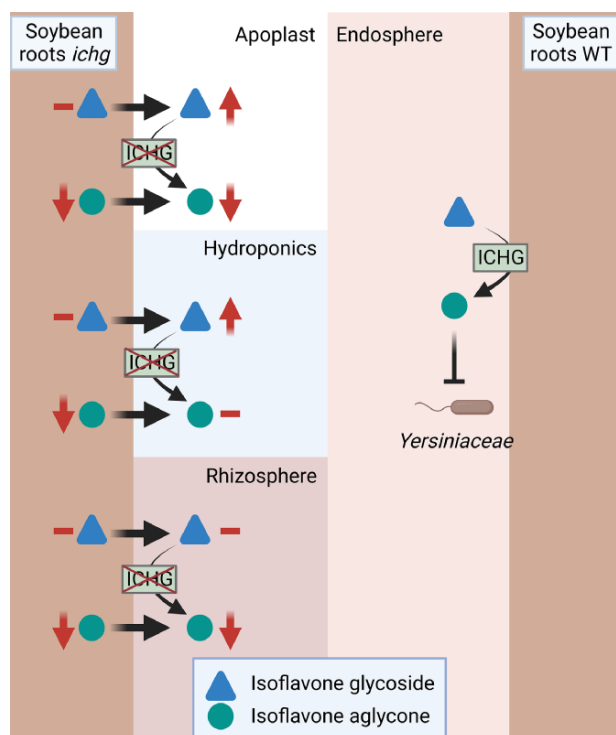


Fig. 1 Effects of the *ichg* mutations on root internal and external amounts of isoflavones and their glycosides. In all conditions, the mutations lower aglycone concentrations in the roots. In the apoplast, the mutations increase glycoside and decrease aglycone contents. In a hydroponics system, the mutations increase glycoside contents, while in the rhizosphere, they decrease aglycone amounts. The mutations had no effect on rhizosphere microbial communities but enhanced the presence of *Yersiniaceae* in the endosphere. The red upward arrow indicates increased accumulation, the red downward arrow indicates reduced accumulation and the red dash indicates no effect. The black arrows indicate movement/conversion between compartments or isoflavone status (glycoside and aglycone). Glycoside is depicted as triangle and aglycone as circle. The lines with a blunt ending indicate inhibition. This figure was created with BioRender.

wild-type and mutant plants showed a clear difference between N_2 -sufficient and N_2 -deficient conditions, but there were no ICHG-dependent changes in microbial abundance. The authors thus conclude that while ICHG clearly modulates the abundance of isoflavone aglycones in the rhizosphere of soybean, these changes might not be enough to influence the bacterial endosphere microbiome, indicating that low amounts of isoflavones could already be sufficient to bring about their effects.

Since isoflavone aglycones are also involved in the establishment of nodule-forming symbioses in soybean, the authors investigated the potential of the *ichg* mutant to engage in symbiosis with *Bradyrhizobium diazoefficiens* USDA 110, a well-known nodule-forming bacterium. More nodules were detected in one of the *ichg* mutants, but nodule weights and infection thread morphology were unaltered across all genotypes, indicating that ICHG has no pivotal role in the establishment of this symbiosis. A recent paper showed a

novel link among overexpression of a soybean isoflavone malonyltransferase, increased levels of isoflavone glycosides in soybean exudates and higher nodulation after infection by *B. diazoefficiens* USDA 110 (Ahmad et al. 2021). Interestingly, Matsuda et al. (2023) in their in vitro experiment found that only daidzein, an isoflavone aglycone, and not the tested isoflavone glycosides could induce *Nod* genes of *B. diazoefficiens* USDA 110, not only suggesting that the aglycone levels in the rhizosphere of the *ichg* mutant are sufficient to initiate this symbiosis but also highlighting the complexity of this interaction.

The authors have identified a role for the BGLU ICHG in defining the isoflavone exudate patterns of soybean, a function previously only demonstrated to be mediated by ABC and Multidrug And Toxic Compound Extrusion (MATE) transporters (Sugiyama et al. 2007, Biala-Leonhard et al. 2021). Their work raises exciting follow-up questions about the biological roles of ICHGs and other deglycosylating enzymes in the rhizosphere. For instance, isoflavones, also known as phytoalexins, could potentially function in resistance to soil-borne pathogens, while different nodulating partners might require specific mixes of isoflavone aglycones and glycosides, and so it would be important to understand how ICHG could manipulate such processes. In general, BGLUs have been implicated in plant defense responses and as general mediators of secondary metabolite balance and storage. Now, a previously underexposed role of BGLUs mediating metabolites in the rhizosphere is slowly beginning to be uncovered by works such as the current paper by Matsuda et al. (2023).

Data Availability

Data sharing not applicable as no datasets were generated or analysed for this commentary.

Disclosures

The authors have no conflicts of interest to declare.

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