Trends in Plant Science

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Forum

Plant–cyanobacterial symbioses: mutualism with emerging translational possibilities

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Engineering rhizobial–plant nitrogen fixation in crops is a major goal in plant synthetic biology. We propose expanding efforts to plant–cyanobacterial symbioses as an alternative path. Targeting the earliest step of this partnership—likely conserved across its independent evolutionary origins—may offer a comparatively simple and promising strategy for enabling nitrogen-fixing crops.

The World Economic Forum in 2025 established 'green nitrogen fixation' as one of the Top Ten Emerging Technologies (https://www.weforum.org/). It is estimated that the Haber–Bosch process for producing synthetic nitrogen fertilizers consumes nearly 2% of global energy while emitting over 2 tons of carbon dioxide per ton of ammonia produced [1]. As such, parts of current agricultural practices are inefficient and highly toxic to the environment.

Biological nitrogen fixation (BNF) offers a potential solution to the global nitrogen challenge. BNF is an energetically demanding process catalyzed by the oxygen-sensitive nitrogenase complex and is thus restricted to only certain bacteria and archaea. A few plant lineages have evolved to form partnerships with these N_2-fixing microbes, with the legume–rhizobia symbiosis being the most widely known and most intensively studied system [2]. However, there is another, largely overlooked N_2-fixing partner for plants: cyanobacteria. Compared with other N_2-fixing symbionts, cyanobacteria are less dependent on the host. In addition to fixing their own carbon through photosynthesis, they possess specialized cells called heterocysts for N_2 fixation and do not require hosts to create a micro-oxic environment. Furthermore, unlike the legume–rhizobia symbiosis, the plant–cyanobacteria endophytic partnership has evolved independently several times —in hornworts, liverworts, ferns (*Azolla*), gymnosperms (cycads), and among angiosperms, such as *Gunnera* (Figure 1). Considering that the organs cyanobacteria can colonize vary widely, ranging from the thallus, leaf, and root, to the stem, these N_2 fixers are likely more amenable to forming symbioses with diverse crop species compared to rhizobia.

In the forum article, we argue that understanding how cyanobacterial symbiosis evolved in these diverse plant lineages can reveal novel strategies to engineer N_2 fixation in crops. With the recent advancements in genetic tools and genomic resources for hornworts, liverworts, cycads, and *Gunnera*, the time is ripe to tackle the genetic basis of cyanobacterial N_2 fixation in plants.

Diversity of plant–cyanobacterial symbioses

Cyanobacteria, in particular Nostocales, can associate with a wide variety of plants in diverse forms, ranging from loose epiphytic interactions to intimate intracellular symbiosis. Recent studies on the ecological roles of epiphytic interactions with feather mosses (e.g., *Pleurozium*), peat mosses (*Sphagnum*), and rice [3–5] have brought significant new insights into cyanobacterial associations. However, the extent to which host plants actively contribute to establishing these interactions remains unclear. Here, we focus on the endophytic interaction whereby the cyanobionts are hosted in specialized structures within the plants (Figure 1). We refer readers to a recent review that has detailed the endophytic cyanobacterial symbioses across land plants [5].

All the known plant cyanobionts are filamentous, heterocyst-forming cyanobacteria and are generally classified under the order Nostocales. Previous studies by us and others have found that the cyanobionts are phylogenetically diverse and overlap substantially with those from cyanolichens [6]. With the exception of *Nostoc azollae* from *Azolla*, cyanobacteria are recruited from the environment and exhibit high levels of promiscuity. For example, *Nostoc punctiforme* ATCC 29133 was originally isolated from the cycad *Macrozamia* and has been shown to reconstitute functional symbioses with the hornworts *Anthoceros punctatus* and *Anthoceros agrestis*, the liverwort *Blasia pusilla*, as well as the angiosperm *Gunnera manicata* [7,8]. This broad compatibility with diverse plant hosts suggests that symbiotic cyanobacteria might be responding to a common set of plant signals.

Chemical communication in cyanobacterial symbiosis

To date, very little is known about how cyanobacteria endosymbiosis is initiated and established. Other than the vertical transmission found in *Azolla*, it is postulated that the symbiosis proceeds through the following general steps [5].

First, under nitrogen-deficient conditions, plants secrete hormogonia-inducing factors (HIFs) to initiate cyanobacteria differentiation into a motile cell type, hormogonia, in preparation for symbiosis [9]. Hormogonia are typically narrower filaments that lack heterocysts, and their gliding mobility is powered by a type IV pilus system [9]. Hormogonia are guided to the site of symbiosis by plant-derived chemoattractants [9]. The chemical nature

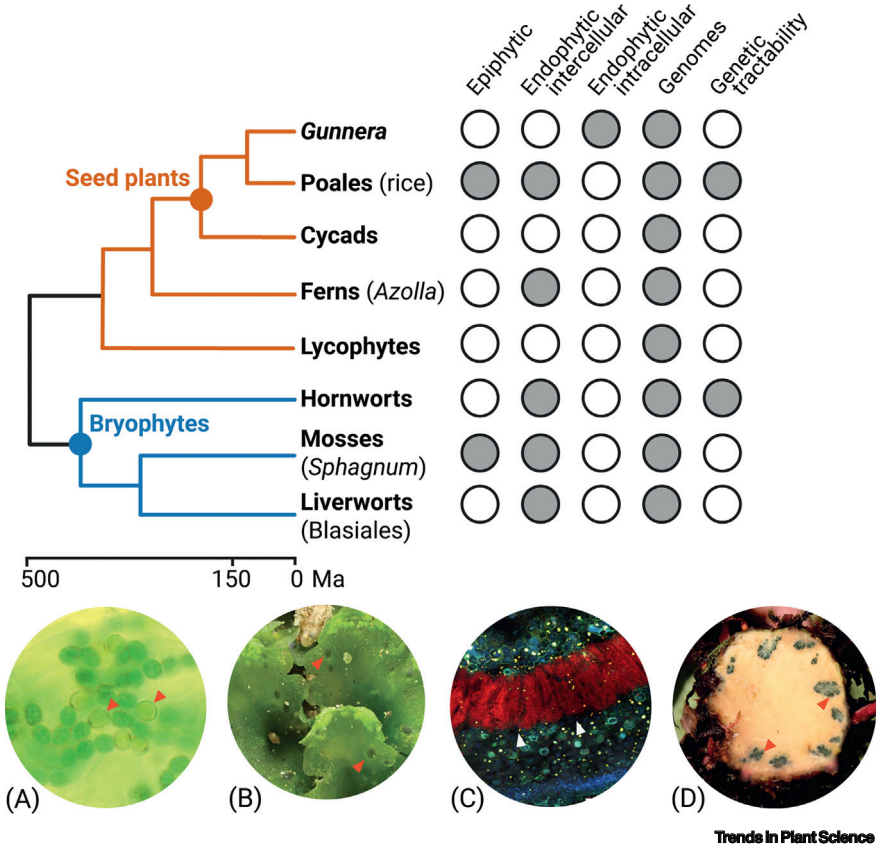

Figure 1. Evolutionary time tree and spectrum of cyanobacterial symbiosis from liverworts to flowering plants. Among mosses and liverworts, epiphytic cyanobacteria are ubiquitous. Despite having abundant genomic resources available for all plant groups, so far only hornworts and rice are capable of genetic manipulation. (A) Cyanobacterial colony inside of a hornwort showing the heterocysts (red arrowheads). (B) Round colonies (red arrowheads) in the hornwort *A. agrestis*. (C) Cyanobacterial ring (white arrowheads) in a cycad coralloid root transverse section. (D) *Gunnera* stem showing the cyanobacterial colonies (red arrowheads). Photos by J.C. Villarreal (A and C) and Fay-Wei Li (B and D).

of HIF has remained elusive. Campbell and Meeks [9] were the first to show that growth media conditioned by nitrogen-starved hornworts (*A. punctatus*) can elicit hormogonia differentiation in multiple *Nostoc* species, while plants grown on nitrogen-replete media had little or no influence. This finding suggests that the production of HIF is strongly induced by nitrogen availability. Campbell and Meeks [9] noted that HIF is less than 12 kDa in mass and that its activity can be abolished by autoclaving. The mucilage from *Gunnera* glands was also shown to have HIF activity [10]. Further characterizations by the authors suggested that *Gunnera* HIF is likely a 0.5–12 kDa molecule sensitive to autoclaving and to proteinase K treatment, the latter suggesting a possible peptide component. However, this study did not present results from mock treatments; considering that the proteinase K treatment involved an additional overnight incubation step at 37°C, it is unclear exactly which factor compromised the HIF activity. Hashidoko *et al.* [11] reported the isolation of diacylglycerols (DAGs) from cycad coralloid roots as candidate HIFs; however, these extracts were derived from macerated whole roots, including resident cyanobionts, rather than from plant exudates alone, and direct evidence that DAGs are secreted by the plant and act as HIF is currently lacking.

Once the cyanobacteria have entered the plant, the plants produce hormogonia-repressing factors to cue cyanobacteria to differentiate back to their vegetative state. In addition, heterocyst formation is stimulated during symbiosis, likely in response to plant signals. When the symbiosis is established, cyanobacteria live endophytically in the host and provide it with fixed nitrogen in exchange for carbohydrates and other essential nutrients [8]. It is clear that plants are capable of manipulating various aspects of cyanobacterial cell differentiation and motility by using a battery of chemical signals. However, as with the HIF, none of the plant genes or secreted compounds involved in the above process are known.

Harnessing the chemical signals to engineer symbiosis

We believe that identifying the relevant chemical signal will enable the rational engineering of a prototype plant–cyanobacteria association. A recent study [12] showed that inoculating N_2-fixing *Nostoc* or *Anabaena* species into the soil of wheat and *Arabidopsis thaliana* resulted in only marginal growth improvement—likely because the cyanobacteria remained near the soil surface and did not migrate toward the roots. By contrast, inoculating high doses of *Nostoc* in hydroponic conditions —which are more permissive for cell motility—yielded clear epiphytic and occasional endophytic interactions on rice [5] and wheat [13] roots. In rice, these associations further translated into significant root growth promotion [5]. We hypothesize that HIFs, and possibly additional chemoattractants, represent the missing cues needed for directed colonization of crop plants in soil (Figure 2).

For instance, introducing the biosynthetic pathways for both HIF and the chemoattractant—under the control of

root-specific, nitrogen starvation-induced promoters—could enable engineered plants to actively recruit and enrich N_2-fixing cyanobacteria in the rhizosphere. The resulting increase in local nitrogen availability from cyanobacterial activity could, by itself, substantially enhance plant growth, particularly in nutrient-poor soils. Notably, because *Nostoc* and related cyanobacteria are autotrophic and possess oxygen-excluding heterocysts for nitrogen fixation, plants may not need to be engineered to form specialized symbiotic structures or provide carbon rewards. Not all plant species, however, may be equally receptive hosts: *A. thaliana* has been shown to mount a defense response against cyanobacterial extracts [14], indicating that pre-screening candidate crop species for compatibility with nitrogen-fixing cyanobacteria is a necessary prerequisite before undertaking engineering strategies.

It remains possible that the strength of chemoattraction alone may not overcome phototropism, or that roots may extend too deep for photosynthetic cyanobacteria to thrive. In such cases, understanding how carbohydrates are exchanged in natural symbiotic systems could inform strategies to shift the interaction toward a more mutualistic one. A homolog of the SWEET transporter was recently proposed to supply sugar to the cyanobionts in hornworts [8]. Beyond the signaling and structural requirements, broader environmental constraints must also be considered for any field application. These include the availability of nitrogenase cofactors such as molybdenum and vanadium, which can limit nitrogen fixation rates even when the symbiosis is established [15], as well as abiotic stresses (e.g., drought and temperature extremes) and biotic factors that may interfere with cyanobacterial establishment or activity in the rhizosphere [15].

In summary, by decoding and harnessing the molecular language of symbiosis—beginning with HIF and its associated attractants—we can start designing plants that actively engage N_2-fixing microbes for their own benefit. The evolutionary breadth of plant–cyanobacterial partnerships—spanning independently evolved symbioses in hornworts, liverworts, *Azolla*, cycads, and *Gunnera*—provides a uniquely powerful comparative framework. Identifying molecular components conserved across these lineages, for example, through comparative transcriptomics or gene family evolution analyses, represents a promising route toward engineering broadly compatible hosts for nitrogen-fixing cyanobacteria. Such an approach could open new frontiers in sustainable agriculture, reducing dependence on synthetic fertilizers while illuminating the fundamental principles that once enabled plants to thrive in nutrient-poor environments.

Figure 2. We propose that the characterization and introduction of HIFs and chemoattractants could enable engineered plants to actively recruit and enrich N_2-fixing cyanobacteria in the rhizosphere without altering the crop's genetic constitution, thereby enhancing growth and yield.

Declaration of interests

The authors declare no competing interests.

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