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Spotlight

Opening the door to nitrogen fixation in oxygenic phototrophs

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Conveying biological nitrogen fixation (BNF) to photosynthetic species may be the next agricultural revolution, yet poses major engineering challenges. Liu et al. created a diazotrophic strain of a previously non-nitrogen-fixing species, the cyanobacterium Synechocystis sp. PCC 6803, and uncovered critical aspects of nitrogen fixation in an oxygenic species.

With the increasing world population, the demand for cereal grains and other plant-based foods is increasing proportionally. Thus, efficient strategies are required to improve the yield of cereal grains and other plant-based foods. A potential method to improve crop yield is to increase the nitrogen usage efficiency (NUE) of cereal grains, because plants cannot fix their own nitrogen and rely heavily on low-availability nitrates in the soil, as well as synthetic fertilizers, such as urea, for their nitrogen requirements [1]. During the early 20th century, the advent and subsequent improvement of the Haber–Bosch process for converting atmospheric nitrogen (N_2) into synthetic fertilizer (urea) allowed production to meet the demand for synthetic fertilizer and thereby facilitated drastic improvements in crop yield [2]. However, the excessive use of synthetic fertilizers can lead to several environment concerns [3]. This predicament has opened a new avenue of research, focused on harnessing BNF, whereby different nitrogenases fix atmospheric nitrogen to produce ammonia, thereby fulfilling the nitrogen demand of plants [4].

To date, a vast amount of research has been dedicated to exploring, engineering, and improving crop symbiosis with nitrogen-fixing bacteria [4]; however, engineering diazotrophic plants has not been equally developed as an avenue of research. Plants with the ability to fix their own nitrogen could spark the next agricultural revolution by dramatically improving crop yield without the need for synthetic fertilizers [5]. Theoretically, engineering diazotrophic plants could be achieved through the introduction of a nitrogen fixation (nif) gene cluster, and subsequent transfer of nitrogenase expression and activity. However, there are several challenges that need to be overcome to achieve this feat. Many nitrogenases are highly sensitive to oxygen and this, along with the complex nature of its transcriptional regulation and high energy requirements, makes engineering BNF in plants challenging [6]. Interestingly, there are certain non-model species of cyanobacteria with nitrogen-fixation capabilities, which could illuminate strategies to overcome the difficulty of achieving BNF within cereal crops. 'Borrowing' machinery from cyanobacteria could allow for the transfer of nitrogen-fixation capabilities to plants. Ultimately, such a strategy is particularly of interest because cyanobacteria are the progenitors of chloroplasts within plants, and this evolutionary relationship also could increase genetic compatibility. Within this context, seminal work by Liu et al. demonstrated efficient transfer, optimization, and expression of the nif cluster from the photosynthetic and diazotrophic Cyanothece sp. ATCC 51142 (hereafter Cyanothece 51142) to the closely related but natively nondiazotrophic cyanobacterium Synechocystis sp. PCC 6803 (hereafter Synechocystis 6803) [7].

The nif cluster from Cyanothece 51142 contains 35 genes, which encode structural proteins of the nitrogenase, cofactors necessary for electron transfer, various accessory proteins, ferredoxins, and hypothetical proteins [8]. The cluster shows high transcription levels in the dark and low levels in light, which has been attributed to the circadian separation of photosynthesis during the day and nitrogen fixation at night [9]. While such genes are missing in Synechocystis 6803, in their first set of experiments, the authors showed that it was possible to express this entire gene cluster in this cyanobacterium. Interestingly, the entire cluster was maintained over a period of 4 years, demonstrating a stable system. More importantly, all genes were shown to be transcribed and nitrogenase activity was found to reach 2% of Cyanothece 51142 grown under the same conditions. These results show the ability of Synechocystis 6803 to utilize the genes and regulatory mechanisms conveyed by the nif cluster of Cyanothece 51142.

Liu et al. then sought to improve the nitrogen fixation activity by determining a minimal set of genes required for BNF in Synechocystis 6803. To determine the minimal set, a top-down approach was used, in which genes were selectively removed from the original gene cluster, and nitrogenase activity was compared. Through this approach, the authors demonstrated a set of 24 genes, which showed improved nitrogenase activity over the original nif cluster. This reduced cluster of 24 genes was then expressed on a native plasmid: pCA2.4. This resulted in an impressive increase to 31% of the nitrogenase activity of Cyanothece 51142. However, the authors showed that this nitrogenase activity was still extremely sensitive to oxygen and dropped drastically even in microoxic conditions as low as 0.5% oxygen. To overcome this hurdle, structural and regulatory genes encoding the uptake of hydrogenase from Cyanothece 51142 were inserted into Synechocystis 6803 alongside the minimal nif cluster. The presence of the

uptake hydrogenase (believed to supply ATP, remove oxygen from nitrogenases, and provide reducing power) resulted in twofold and sixfold increases in nitrogenase activity under 0.5% and 1% oxygen conditions, respectively. While these levels did not reach the same as in anaerobic conditions, the increase did reveal the importance of uptake hydrogenase in engineering less oxygen-sensitive nitrogen-fixing capabilities.

This work represents a highly successful transfer of nitrogenase activity to a nondiazotrophic cyanobacterium, the implications of which are demonstrated in Figure 1. The ability to do so was made possible by elegant choices on the authors' part to utilize a non-model system to engineer a closely related species. The regulatory mechanisms already in place in Cyanothece 51142 allowed for expression of the nif cluster in Synechocystis 6803 at the proper timing to cooperate with oxygenic photosynthesis and bypassed the need to construct such regulation from scratch. After transferring BNF, the new diazotrophic strain of Synechocystis 6803 served as a platform to explore optimization of nitrogenase activity, and revealed the critical gene set as well as a regulatory mechanism that aids oxygen tolerance. While transfer into a plant species has yet to be achieved, theoretical future applications can be foreseen. For example, transferring the reduced nif cluster to a model plant, such as arabidopsis (Arabidopsis thaliana), and investigating its potential BNF ability could lead to further expansion in cereal grains, such as Zea mays. This could lead to the engineering of diazotrophic plants, thereby dramatically improving food security.

While the potential of this work is exciting, challenges to genetically engineering plants have limited the experimental work done to date. Two major hurdles include the bottleneck imposed by slow and difficult transformation and regeneration of edited plants, which commonly leads to random placement of inserted genes, and also a lack of understanding surrounding the gene pathways, networks, and regulatory mechanisms of many plant species. In recent years, targeted gene insertion into plants has improved, thanks to CRISPR-based technologies, as has been demonstrated in the model organism arabidopsis [10]. Additionally, advances in computational modeling have aided understanding of biological processes by allowing in silico experimentation. Computational tools, such as OptCircuit [11] and EuGeneCiD [12], can be applied to whole-plant models [13] or specific tissue models [14,15] to design the minimum genetic intervention required to achieve the desired genetic functionality and predict outcomes of planned interventions. The combined advances both experimentally and computationally set the stage for the findings of Liu et al. to be applied in plants in the near future.

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Declaration of interests

No interests are declared.

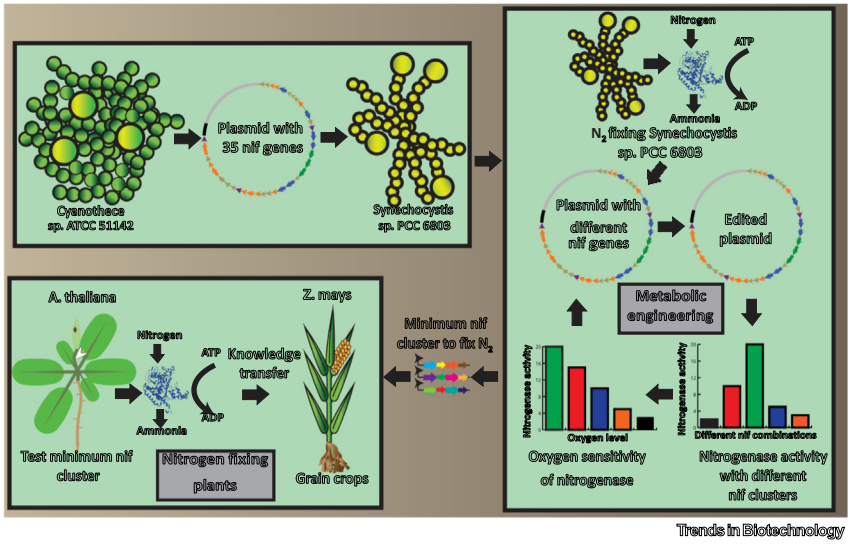

Figure 1. Diagram illustrating the experimental flow developed by Liu et al. [7] to engineer nitrogen fixation in the cyanobacterium Synechocystis 6803, and implications for future research using their protocol.

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References

1. Yan, M. et al. (2020) Rethinking sources of nitrogen to cereal crops. Glob. Chang. Biol. 26, 191–199
2. Melillo, E.D. (2012) The First Green Revolution: debt peonage and the making of the nitrogen fertilizer trade, 1840–1930. Am. Hist. Rev. 117, 1028–1060
3. Erisman, J.W. et al. (2011) Reactive nitrogen in the environment and its effect on climate change. Curr. Opin. Environ. Sustain. 3, 281–290
4. Goyal, R.A.-O. et al. (2021) Molecular biology in the improvement of biological nitrogen fixation by rhizobia and extending the scope to cereals. Microorganisms 9, 2076–2607
5. Good, A. (2018) Toward nitrogen-fixing plants. Science 359, 869–870
6. Curatti, L. and Rubio, L.M. (2014) Challenges to develop nitrogen-fixing cereals by direct nif-gene transfer. Plant Sci. 225, 130–137
7. Liu, D. et al. (2018) Engineering nitrogen fixation activity in an oxygenic phototroph. mBio 9, e01029–18

8. Welsh, E.A. et al. (2008) The genome of Cyanothece 51142, a unicellular diazotrophic cyanobacterium important in the marine nitrogen cycle. Proc. Natl. Acad. Sci. U. S. A. 105, 15094–15099
9. Stöckel, J. et al. (2008) Global transcriptomic analysis of Cyanothece 51142 reveals robust diurnal oscillation of central metabolic processes. Proc. Natl. Acad. Sci. U. S. A. 105, 6156–6161
10. Wolter, F. and Puchta, H. (2019) In planta gene targeting can be enhanced by the use of CRISPR/Cas12a. Plant J. 100, 1083–1094
11. Dasika, M.S. and Maranas, C.D. (2008) OptCircuit: an optimization based method for computational design of genetic circuits. BMC Syst. Biol. 2, 24
12. Schroeder, W.L. et al. (2021) Optimization-based Eukaryotic Genetic Circuit Design (EuGeneCiD) and modeling (EuGeneCiM) tools: computational approach to synthetic biology. iScience 24, 103000
13. Saha, R. et al. (2011) Zea mays iRS1563: a comprehensive genome-scale metabolic reconstruction of maize metabolism. PLoS ONE 6, e21784
14. Chowdhury, N.B. et al. (2022) Dissecting the metabolic reprogramming of maize root under nitrogen-deficient stress conditions. J. Exp. Bot. 73, 275–291
15. Simons, M. et al. (2014) Assessing the metabolic impact of nitrogen availability using a compartmentalized maize leaf genome-scale model. Plant Physiol. 166, 1659–1674