

Spotlight

Soybean breeders can
count on nodulesDefeng Shen ^{1,*} and
Ton Bisseling^{2,3,*}

Soybean, the most important legume crop, plays a crucial role in food security and sustainable agriculture. Recently, Zhong *et al.* demonstrated that a moderate increase in nodule number in soybean improves field yield and protein content. Their findings propose a potential strategy to enhance yield performance in other legume crops.

To ensure global food security, food demand is expected to increase by 35–56% between 2010 and 2050 [1]. Meeting this demand heavily relies on increasingly expensive synthetic nitrogen fertilizers, which place significant pressure on the environment and threaten sustainable food production [1]. Consequently, there is growing interest in legume crops due to their inherent capacity for biological nitrogen fixation. However, to meet future food demands, breeding for legumes with increased yield, compared with the current elite cultivars, is essential [2].

Since the start of agriculture during the Neolithic Revolution, legumes have been cultivated as crops. These included soybean (*Glycine max*) in China, lentil (*Vicia lens*) in the Middle East, and common bean (*Phaseolus vulgaris*) in South America. In addition to these, legumes encompass numerous other economically important crops, such as pea (*Pisum sativum*), chick pea (*Cicer arietinum*), and alfalfa (*Medicago sativa*). Unlike cereals, legumes can develop lateral root organs, known as nodules, through a symbiotic interaction with rhizobium bacteria [3]. These bacteria are intracellularly accommodated

in nodule cells, where they find optimal conditions to fix atmospheric nitrogen into ammonia [3]. The fixed nitrogen is delivered to host plants in the form of either amides or ureides, which sustain plant growth under nitrogen-deprived conditions. In return, rhizobia acquire photosynthetic products from the host to sustain nitrogen fixation [3]. Due to this symbiotic relationship, grain legume seeds are rich in protein and serve as important nutrient sources for humans and livestock [2]. Grain legumes naturally replenish soil nitrogen, leaving it available for the subsequent crops and reducing the need of synthetic fertilizers. Therefore, legume crops play a vital role in sustainable farming practices [2].

Despite the benefits of biological nitrogen fixation, the demand for higher yields often necessitates the use of synthetic nitrogen fertilizers [4]. There have been several attempts in legume breeding to improve yield [5]. However, there is a long-established negative correlation between grain yield and protein content [6]. Therefore, there is a still great need to breed for increased yield without compromising protein content, the Holy Grail in practical legume breeding (see: <https://www.seedworld.com/canada/2020/01/14/is-high-protein-high-yielding-soy-possible/>). This is particularly important for soybean, as it is a primary protein source for many humans and animals [2].

A simple solution seems to be to enhance biological nitrogen fixation by increasing nodule number. However, this approach negatively affects plant growth due to the highly energy-demanding nature of the nitrogen fixation process. To fix one molecule of N₂, rhizobia consume 16 ATP molecules. Since all of the consumed energy is provided by the host plants, nodule number is tightly controlled by the host via a systemic regulatory pathway known as autoregulation of nodulation (AON) [3]. In soybean, CLAVATA3/EMBRYO-SURROUNDING REGION RELATED (CLE) peptides, known as Rhizobially Induced CLE (RIC), are

synthesized when nodules are formed and these are translocated to the shoot (Figure 1A) [3]. There, they are recognized by the receptor kinase NARK, which activates a descending signal to the roots to repress further nodulation (Figure 1A) [3]. Consequently, knocking out of *NARK* leads to an excessive number of nodules, known as supernodulation (Figure 1B) [3]. However, supernodulation mutants are often associated with stunted shoot growth and yield penalties in soybean [7]. This is due to the fact that excessive nodules consume too much energy, leaving less photosynthate available for the growth of the shoot, and the increased nitrogen supply cannot be used to improve shoot growth. Additionally, nodulation traits are difficult to observe in field-grown plants [8]. Thus, the strategy of enhancing nodulation to increase grain yield and protein content has long been shelved by legume breeders.

In a recent study, Zhong *et al.* aimed to solve this conundrum in soybean by optimizing nodule numbers to balance increased nitrogen fixation with higher carbon production [5]. Soybean is a palaeopolyploid, harboring four genes encoding RIC peptides (*RIC1a/1b/2a/2b*) and two genes encoding Nitrate Induced CLE (NIC) peptides (*NIC1/2*), which are induced by nitrate and act locally to inhibit nodulation in response to nitrate. The authors took advantage of the gene redundancy in soybean and created a series of mutants defective in AON genes using a multiplexed CRISPR/Cas9 mutagenesis system. These mutants included *ric1a/2a*, *ric1b/2b*, *ric1a/1b/2a/2b nic1/2* (designated *ric-6m*), and *nark* mutants. They found that *ric1b/2b*, *ric1a/2a*, *ric-6m*, and *nark* mutants all formed more nodules (~1.2 times, ~two-fold, ~threefold, and ~sixfold, respectively) than their elite parental cultivar. The excessive nodule numbers and increased carbon allocation to nodules in *ric-6m* and *nark* mutants resulted in reduced shoot growth. However, *ric1a/2a* and *ric1b/2b*, with a moderate increase in nodule numbers, showed an increased shoot dry weight

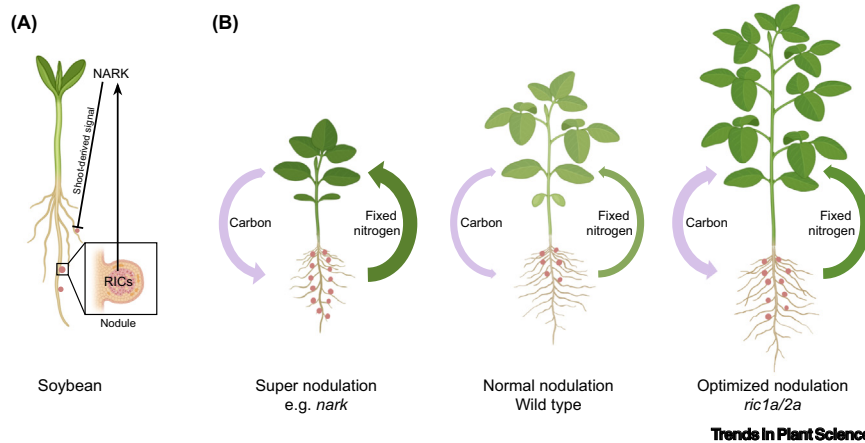


Figure 1. Optimized nodulation enhances carbon and nitrogen allocation to promote shoot growth. (A) Autoregulation of nodulation in soybean: Rhizobially Induced CLAVATA3/EMBRYO-SURROUNDING REGION RELATED (RIC) peptides are synthesized during nodule formation. These peptides are translocated to the shoot, where they activate NARK. NARK induces a shoot-derived signal that moves to the root, inhibiting further nodulation. (B) A proposed model of optimized nodulation promoting soybean yield. While supernodulation mutants fix more nitrogen, excessive nodules consume excessive carbon, and the reduced availability of photosynthates (carbon) for the shoot results in dwarfism and low yield. By contrast, optimized nodulation results in increased nitrogen supply to the shoot. This enhances carbon and nitrogen assimilation, thereby improving shoot growth and allowing balanced carbon allocation between the shoot and root. Figure 1B is modified from Figure 2m in [5]. Figure 1 was created with BioRender.com and Adobe Illustrator.

allowing increased carbon allocation to nodules. Moreover, *ric1a/2a* mutants demonstrated increased nitrogen and chlorophyll content, along with improved carbon assimilation in leaves. So unlike dwarf supernodulation mutants, *ric1a/2a* mutants displayed enhanced shoot growth due to balanced carbon allocation and improved carbon and nitrogen assimilation (Figure 1B).

To test whether the increased shoot mass can result in increased yield, the authors performed extensive multiyear and multi-site field trials to assess the agronomic benefits of the *ric1a/2a* mutants. They tested two independent alleles of these mutants, and these displayed a 10–31% increase in grain yield compared with their wild-type elite cultivar HC-6 across all trials. This improvement was achieved using standard agricultural practices in China, including synthetic nitrogen fertilization and the use of indigenous rhizobia in native soils. Remarkably, *ric1a/2a* mutant seeds also exhibited higher protein

content (+1.77–4.42%) than their already high-protein wild-type cultivar, without any reduction in oil content. Additionally, field-grown *ric1a/2a* seeds had increased nitrogen and carbon content, which was accompanied with higher carbon content in their leaves due to higher net photosynthetic rate and stomatal conductance than their wild-type cultivar. Therefore, the simultaneous increase in both the nitrogen and carbon allocation to the *ric1a/2a* seeds seems to decouple the traditionally negative relationship between protein content and yield or oil content – the Holy Grail in soybean breeding.

Zhong and colleagues provided experimental evidence that a moderate increase in nodule number can enhance both nitrogen and carbon assimilation by balancing source–sink relationships (Figure 1B) [5]. This approach leads to improvements in yield and protein content in soybean, demonstrating that optimization of nodule number, without excessive increase, can be beneficial for crop productivity. It

meets the five criteria recently proposed by [9] for robust examination of whether genetic modification improves crop yields, representing a promising approach to improve soybean yield and quality. However, it remains to be tested whether this finding can be applied to other important grain legumes. Zhong *et al.* exploited the high redundancy of genes in the palaeopolyploid soybean genome. However, for diploid legume crops such as common bean and pea, the numbers of CLE genes are limited and a similar approach is challenging [5]. There is only one NARK ortholog in diploid legumes like common bean and pea [10,11]. An alternative strategy could involve obtaining structural insights into these NARK orthologs to identify key amino acids responsible for the recognition of CLE peptides. This information could guide the design of point mutations via base editing to reduce, but not entirely eliminate, the affinity between the ligand and receptor. Such adjustments could help to fine-tune nodule numbers. This method of modifying receptor kinases has been successfully applied in other contexts, suggesting that it might also be effective for optimizing nodule numbers in these legumes [12].

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Declaration of interests

The authors declare no competing interests.

¹Department of Plant–Microbe Interactions, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany

²College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China

³Department of Plant Sciences, Cluster of Plant Development Biology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

*Correspondence:

dshen@mpi-pz.mpg.de (D. Shen) and ton.bisseling@wur.nl (T. Bisseling).

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