

The soybean (*Glycine max*) nodulation-suppressive CLE peptide, GmRIC1, functions interspecifically in common white bean (*Phaseolus vulgaris*), but not in a supernodulating line mutated in the receptor PvNARK

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Summary

Legume plants regulate the number of nitrogen-fixing root nodules they form via a process called the Autoregulation of Nodulation (AON). Despite being one of the most economically important and abundantly consumed legumes, little is known about the AON pathway of common bean (*Phaseolus vulgaris*). We used comparative- and functional-genomic approaches to identify central components in the AON pathway of common bean. This includes identifying *PvNARK*, which encodes a LRR receptor kinase that acts to regulate root nodule numbers. A novel, truncated version of the gene was identified directly upstream of *PvNARK*, similar to *Medicago truncatula*, but not seen in *Lotus japonicus* or soybean. Two mutant alleles of *PvNARK* were identified that cause a classic shoot-controlled and nitrate-tolerant supernodulation phenotype. Homeologous over-expression of the nodulation-suppressive CLE peptide-encoding soybean gene, *GmRIC1*, abolished nodulation in wild-type bean, but had no discernible effect on *PvNARK*-mutant plants. This demonstrates that soybean *GmRIC1* can function interspecifically in bean, acting in a *PvNARK*-dependent manner. Identification of bean *PvRIC1*, *PvRIC2* and *PvNIC1*, orthologues of the soybean nodulation-suppressive CLE peptides, revealed a high degree of conservation, particularly in the CLE domain. Overall, our work identified four new components of bean nodulation control and a truncated copy of *PvNARK*, discovered the mutation responsible for two supernodulating bean mutants and demonstrated that soybean *GmRIC1* can function in the AON pathway of bean.

Keywords: autoregulation of nodulation, legume nodulation, nitrogen fixation, plant signalling and development, rhizobia, symbiosis.

Introduction

Legumes account for a large source of biologically available nitrogen through a highly specialized symbiotic relationship with nitrogen-fixing soil bacteria termed rhizobia. The relationship is signified by the formation of novel organs on the legume root, called nodules (reviewed in Ferguson *et al.*, 2010). Nodules act to house the rhizobia, providing them with the conditions necessary to generate useable forms of reduced nitrogen. This process is frequently exploited in agriculture, where legumes are used in rotation or alongside other crops to increase yields and improve the nitrogen content and structure of soils (Jensen *et al.*, 2012).

Forming and maintaining nodules require a complex interaction of various plant hormones and signals (e.g. Ding *et al.*, 2008; Ferguson and Mathesius, 2003; Ferguson *et al.*, 2005, 2011; Hirsch and Fang, 1994; Ryu *et al.*, 2012). This is costly to the host plant in terms of resources. As a result, legumes have developed mechanisms that enable them to optimize nodule formation (and hence nitrogen acquisition) under an array of growing conditions. This includes regulating their nodule numbers in response to

environmental factors, such as the nitrogenous content of the soil (e.g. Carroll *et al.*, 1985a,b; Reid *et al.*, 2011a), soil acidity (Ferguson *et al.*, 2013; Lin *et al.*, 2012) and stress (e.g. ethylene) (Gresshoff *et al.*, 2009; Guinel and Geil, 2002).

Legumes also control their nodule numbers via an inbuilt signalling mechanism known as the Autoregulation of Nodulation (AON) (Delves *et al.*, 1986; Kossak and Bohlool, 1984; Reid *et al.*, 2011b). AON is triggered following the first nodulation events, and is predicted to begin with the production of a root-derived CLAVATA/ESR-related (CLE) peptide(s) (Lim *et al.*, 2011; Mortier *et al.*, 2010, 2012; Okamoto *et al.*, 2009, 2013; Reid *et al.*, 2011a,b, 2013). In soybean, two AON CLE peptide-encoding genes have been identified, called *Rhizobia-Induced CLE1* (*GmRIC1*) and *Rhizobia-Induced CLE2* (*GmRIC2*) (Reid *et al.*, 2011a). *GmRIC1* expression is induced early following nodule initiation (within 12 h), whereas *GmRIC2* expression is triggered later (approximately 72 h) (Hayashi *et al.*, 2012; Reid *et al.*, 2011a, 2013).

GmRIC1 and *GmRIC2* are predicted to be transported via the xylem to the shoot, where they are thought to be perceived by a

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