Structure–function analysis of the GmRIC1 signal peptide and CLE domain required for nodulation control in soybean

Dugald E. Reid*, Dongxue Li*, Brett J. Ferguson and Peter M. Gresshoff†

Australian Research Council Centre of Excellence for Integrative Legume Research, School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia

*These authors contributed equally to this work.
† To whom correspondence should be addressed. E-mail: p.gresshoff@uq.edu.au

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Abstract

Legumes control the nitrogen-fixing root nodule symbiosis in response to external and internal stimuli, such as nitrate, and via systemic autoregulation of nodulation (AON). Overexpression of the CLV3/ESR-related (CLE) pre-propeptide-encoding genes GmNIC1 (nitrate-induced and acting locally) and GmRIC1 (Bradyrhizobium-induced and acting systemically) suppresses soybean nodulation dependent on the activity of the nodulation autoregulation receptor kinase (GmNARK). This nodule inhibition response was used to assess the relative importance of key structural components within and around the CLE domain sequences of these genes. Using a site-directed mutagenesis approach, mutants were produced at each amino acid within the CLE domain (RLAPEGPDPHHN) of GmRIC1. This approach identified the Arg1, Ala3, Pro4, Gly6, Pro7, Asp8, His11, and Asn12 residues as critical to GmRIC1 nodulation suppression activity (NSA). In contrast, none of the mutations in conserved residues outside of the CLE domain showed compromised NSA. Chimeric genes derived from combinations of GmRIC1 and GmNIC1 domains were used to determine the role of each pre-propeptide domain in NSA differences that exist between the two peptides. It was found that the transit peptide and CLE peptide regions of GmRIC1 significantly enhanced activity of GmNIC1. In contrast, the comparable GmNIC1 domains reduced the NSA of GmRIC1. Identification of these critical residues and domains provides a better understanding of how these hormone-like peptides function in plant development and regulation.

Key words: Autoregulation of nodulation, CLE peptides, legumes, nodulation, soybean, symbiosis.

Introduction

In agricultural systems, reduced nitrogen is often limiting and thus requires application of nitrogen fertilizer, which has both cost and environmental concerns (Jensen et al., 2012). Most legume species develop a symbiotic relationship with soil rhizobia that reduces the need for this input due to biological nitrogen fixation. Rhizobia undergo differentiation to bacteroids and are housed in a complex organ, known as a nodule, which maintains the conditions required for nitrogen fixation to occur. The nodule develops on the roots through a re-initiation of cell divisions and concurrent infection events (reviewed by Ferguson et al., 2010; Desbrosses and Stougaard, 2011).

The development of nodules is regulated by the plant in response to internal and external cues, including available reduced nitrogen, and through a systemic regulatory mechanism known as the autoregulation of nodulation (AON; first proposed by Gresshoff and Delves, 1986). AON is established in response to early nodulation signalling events through long-distance signals between the root and shoot (Delves et al., 1986; Li et al., 2009; Reid et al., 2011b) and is maintained by the nodulation autoregulation receptor kinase (GmNARK) in soybean (Scarle et al., 2003). GmNARK is structurally similar to the CLAVATA1 (CLV1) receptor kinase of Arabidopsis (Clark et al., 1997), and is functionally...