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RESEARCH PAPER

Structure–function analysis of the *Gm*RIC1 signal peptide and CLE domain required for nodulation control in soybean

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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 (*Gm*NARK). 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 *Gm*RIC1. 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 *Gm*RIC1 significantly enhanced activity of *Gm*NIC1. In contrast, the comparable *Gm*NIC1 domains reduced the NSA of *Gm*RIC1. 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.*, 2011*b*) and is maintained by the nodulation autoregulation receptor kinase (GmNARK) in soybean (Searle *et al.*, 2003). GmNARK is structurally similar to the CLAVATA1 (CLV1) receptor kinase of *Arabidopsis* (Clark *et al.*, 1997), and is functionally