Plant Biotechnology Journal

Plant Biotechnology Journal (2012), pp. 1–10 doi: 10.1111/j.1467-7652.2012.00706.x

Identification of systemic responses in soybean nodulation by xylem sap feeding and complete transcriptome sequencing reveal a novel component of the autoregulation pathway

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Received 5 March 2012; revised 11 April 2012; accepted 12 April 2012.
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Keywords: nodulation, autoregulation of nodulation, legume, RNAseq, systemic signaling, *Glycine max*.

Summary

Establishment of the nitrogen-fixing nodulation symbiosis between legumes and rhizobia requires plant-wide reprogramming to allow infection and development of nodules. Nodulation is regulated principally via a mechanism called autoregulation of nodulation (AON). AON is dependent on shoot and root factors and is maintained by the nodulation autoregulation receptor kinase (NARK) in soybean. We developed a bioassay to detect root-derived signalling molecules in xylem sap of soybean plants which may function in AON. The bioassay involves feeding of xylem extracts via the cut hypocotyl of soybean seedlings and monitoring of molecular markers of AON in the leaf. Transcript abundance changes occurring in the leaf in response to feeding were used to determine the biological activity of the extracts. To identify transcript abundance changes that occur during AON, which may also be used in the bioassay, we used an RNA-seq-based transcriptomics approach. We identified changes in the leaves of bioassay plants fed with xylem extracts derived from either *Bradyrhizobium japonicum*-inoculated or uninoculated plants. Differential expression responses were detected for genes involved in jasmonic acid metabolism, pathogenesis and receptor kinase signalling. We identified an inoculation- and NARK-dependent candidate gene (*GmUFD1a*) that responds in both the bioassay and intact, inoculated plants. *GmUFD1a* is a component of the ubiquitin-dependent protein degradation pathway and provides new insight into the molecular responses occurring during AON. It may now also be used in our feeding bioassay as a molecular marker to assist in identifying the factors contributing to the systemic regulation of nodulation.

Introduction

Symbiotic relationships play critical roles in plant adaptation and survival. Legume plants and soil bacteria known as rhizobia engage in a symbiotic relationship that results in the development of specialized root organs known as nodules. As the nodules develop, the infecting rhizobia undergo differentiation into bacteroids that use a nitrogenase complex to fix atmospheric dinitrogen into ammonia. The ammonia is then provided to the plant in exchange for photoassimilates, principally malate (Udvardi *et al.*, 1988).

Nodule development commences in response to interspecific signalling events (reviewed by Ferguson *et al.*, 2010). The number of nodules that form is predominantly regulated by a systemic process called autoregulation of nodulation (AON), which is mediated by unknown long-distance signalling factors acting between the roots and the shoots (Delves *et al.*, 1986; Reid *et al.*, 2011b). A root-derived signal (Q) is transported to the leaf and initiates the nodule regulation responses that occur via the shoot-derived inhibitor (SDI) signal, which is transported down to the roots to inhibit nodulation.

Root-derived CLE peptides capable of nodule regulation (Lim *et al.*, 2011; Mortier *et al.*, 2010, 2011; Okamoto *et al.*, 2009; Reid *et al.*, 2011a; Saur *et al.*, 2011) are candidates for the mobile Q signal, which is perceived by nodulation autoregulation receptor kinase (GmNARK) in soybean (Searle *et al.*, 2003) and its orthologues in other legumes (Krusell *et al.*, 2002; Nishimura *et al.*, 2002; Schnabel *et al.*, 2005). NARK comprises an extracellular LRR domain that likely forms a semicircular shape, which is the proposed binding site for a CLE ligand (Reid *et al.*, 2011a). An intracellular kinase domain is also required for NARK activity, presumably for the propagation of downstream signals. *GmNARK* is also expressed in the root (Nontachaiyapoom *et al.*, 2007) where it likely plays a related role in locally regulating nodulation in response to nitrate-induced CLE peptides (Reid *et al.*, 2011a). In addition to NARK, it has been demonstrated that the CLV2 receptor protein functions in the shoot as part of the AON mechanism in *Lotus japonicus* and