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Soybean miR172c Targets the Repressive AP2 Transcription Factor NNC1 to Activate *ENOD40* Expression and Regulate Nodule Initiation[C][W]

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MicroRNAs are noncoding RNAs that act as master regulators to modulate various biological processes by posttranscriptionally repressing their target genes. Repression of their target mRNA(s) can modulate signaling cascades and subsequent cellular events. Recently, a role for miR172 in soybean (*Glycine max*) nodulation has been described; however, the molecular mechanism through which miR172 acts to regulate nodulation has yet to be explored. Here, we demonstrate that soybean miR172c modulates both rhizobium infection and nodule organogenesis. miR172c was induced in soybean roots inoculated with either compatible *Bradyrhizobium japonicum* or lipooligosaccharide Nod factor and was highly upregulated during nodule development. Reduced activity and overexpression of miR172c caused dramatic changes in nodule initiation and nodule number. We show that soybean miR172c regulates nodule formation by repressing its target gene, *Nodule Number Control1*, which encodes a protein that directly targets the promoter of the early nodulin gene, *ENOD40*. Interestingly, transcriptional levels of miR172c were regulated by both *Nod Factor Receptor1*α/*5*α-mediated activation and by autoregulation of nodulation-mediated inhibition. Thus, we established a direct link between miR172c and the Nod factor signaling pathway in addition to adding a new layer to the precise nodulation regulation mechanism of soybean.

INTRODUCTION

Through a symbiotic relationship with nitrogen-fixing rhizobial bacteria, most legume plants can use atmospheric dinitrogen gas to help satisfy their nitrogen needs. The process of symbiotic nitrogen fixation takes place in specialized lateral organs called nodules (Ferguson et al., 2010). Nodulation is a complex developmental process involving a direct interaction between rhizobium and legume signals (Desbrosses and Stougaard, 2011; Oldroyd, 2013; Ferguson and Mathesius, 2014). Understanding the mechanisms underlying nodulation and nitrogen fixation could aid in improving nitrogen use efficiency of crops, with the aim of reducing nitrogen fertilizer inputs and improving agricultural and environmental sustainability (Salvagiotti et al., 2008; Peoples et al., 2009; Jensen et al., 2012; Gresshoff et al., 2014).

The process of nodulation is initiated by legume roots secreting flavonoid molecules into the surrounding rhizosphere, which attracts compatible rhizobium strains and stimulates them to synthesize lipochitin oligosaccharide signals, called Nod factors (NFs; e.g., in soybean [*Glycine max*]) (Sanjuan et al., 1992). NFs are perceived by root LysM Nod Factor Receptors (NFRs), which activate signaling cascades that promote root hair deformation and microsymbiont infection as well as cortical and pericycle cell division (nodule primordium formation) (Broghammer et al., 2012; Moling et al., 2014). The NFRs of soybean are encoded by *NFR1*α and *NFR5*α (Indrasumunar et al., 2010, 2011).

Downstream components of NF perception have been thoroughly reviewed (Ferguson et al., 2010; Desbrosses and Stougaard, 2011; Oldroyd, 2013). Among them is *ENOD40*, which is upregulated at the onset of nodulation (Yang et al., 1993; Crespi et al., 1994; Mathesius et al., 2000; Compaan et al., 2001) and is expressed in pericycle cells of root vascular bundles, dividing cortical cells, the nodule primordium, and developing nodules (reviewed in Ferguson and Mathesius, 2014). In soybean roots, *ENOD40* expression is upregulated following either *Bradyrhizobium japonicum* inoculation or NF treatment (Kouchi and Hata, 1993; Yang et al., 1993; Minami et al., 1996; Hayashi et al., 2012). Alterations in *ENOD40* expression can significantly influence legume nodule numbers, suggesting that it plays a pivotal role in nodule organogenesis (Charon et al., 1999; Kumagai et al., 2006; Wan et al., 2007). ENOD40 may function in nodulation as a cell-cell signaling molecule; it lacks an open reading frame but does encode two small peptides (Sousa et al., 2001; Röhrig et al., 2002). Notably, ENOD40 peptides can bind to, and enhance the stability of, sucrose synthase (Hardin et al., 2003; Röhrig et al., 2004). Thus, *ENOD40* may promote nodule organogenesis by increasing the carbon sink strength of the dividing cells. Despite

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