Systemic Regulation of Soybean Nodulation by Acidic Growth Conditions[1[OA]]

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Mechanisms inhibiting legume nodulation by low soil pH, although highly prevalent and economically significant, are poorly understood. We addressed this in soybean (*Glycine max*) using a combination of physiological and genetic approaches. Split-root and grafting studies using an autoregulation-of-nodulation-deficient mutant line, altered in the autoregulation-of-nodulation receptor kinase GmNARK, determined that a systemic, shoot-controlled, and GmNARK-dependent mechanism was critical for facilitating the inhibitory effect. Acid inhibition was independent of aluminum ion concentration and occurred early in nodule development, between 12 and 96 h post inoculation with *Bradyrhizobium japonicum*. Biological effects were confirmed by measuring transcript numbers of known early nodulation genes. Transcripts decreased on both sides of split-root systems, where only one side was subjected to low-pH conditions. Our findings enhance the present understanding of the innate mechanisms regulating legume nodulation control under acidic conditions, which could benefit future attempts in agriculture to improve nodule development and biological nitrogen fixation in acid-stressed soils.

Most legume plants are able to facilitate biological nitrogen fixation through a symbiosis with soil bacteria, commonly called "rhizobia." This relationship results in the formation of novel root organs, called nodules, which are critical for establishing an environment suitable for symbiotic nitrogen fixation (for review, see Ferguson et al., 2010; Desbrosses and Stougaard, 2011; Ferguson, 2013). Nodule development is stringently controlled by internal plant mechanisms (for review, see Reid et al., 2011a) and can also be significantly affected by external factors, including nitrate and ethylene (Ferguson and Mathesius 2003; Gresshoff et al., 2009).

One external factor that can diminish nodulation is low soil pH. It is estimated that about 30% of the world's land surface is acidic (pH < 5.5), including an extensive 40% of arable land (Von Uexküll and Mutert, 1995). Low soil pH reduces nutrient availability, increases Al^{3+} toxicity, and is generally detrimental to crop yields. It has been estimated that Al^{3+} toxicity represents the greatest constraint on plant productivity in 67% of the world's acidic soil regions (Eswaran et al., 1997). These poor growth conditions lead to reductions in root development and nodulation and compromise nutrient transport (Horst, 1983, 1987; Marschner, 1991). This results in yield losses of more than 50% in grain crops, such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), and in many legume crops, including common bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*), and pea (*Pisum sativum*; Mahler and McDole, 1987).

Low soil pH reduces the nodule numbers of legumes such as common bean, lentil, and pea by more than 90% and nodule dry weight by more than 50% (Lie, 1969; Mohebbi and Mahler, 1989; Vargas and Graham, 1989; Alva et al., 1990; Evans et al., 1990). However, certain legume species, such as some *Lupins* spp. and *Mimosa* spp. found in the highly acidic Brazilian Cerrado and Caatinga biomes, can exhibit acid-tolerant nodulation (dos Reis et al., 2010; Sprent, 2009). Both determinate- and indeterminate-forming nodule types are affected by low pH, suggesting a common target. The loss of nodulation and nitrogen fixation caused by low soil pH is reflected by the fact that 75% of the 3.6 million tons of nitrogen fertilizer used worldwide each year is applied in major soybean (*Glycine max*) production regions, where acidic soils are prevalent (Fig. 1; http://www.fertilizer.org/). Attempts to combat this significant problem are now being enhanced by establishing a more detailed understanding of the molecular mechanisms regulated by soil acidity.

The reduced nodulation observed in acidic soil is not solely attributed to hindered plant development, as low pH also negatively affects rhizobia growth, persistence, and function. This is primarily caused by increased proton concentration and a resulting increased metal ion solubility in the growth substrate, which causes intracellular pH instability and inhibits cell function (Bhagwat and Apte, 1989; Graham et al., 1994).

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