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Root nodulation in guar: Effects of soils, *Rhizobium* inoculants, and guar varieties in a controlled environment

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ABSTRACT

Guar (*Cyamopsis tetragonolobus* L. Taub.) is a leguminous crop plant that produces a seed containing galactomannan gum, which is used in many applications as a lubricant, binder, thickener, and emulsifier, among other functions. A recent spike in worldwide guar consumption has been driven largely by the industrial uses of the gum. As a legume, the primary ecosystem service agricultural producers expect from guar is nitrogen fixation, but the common perception is that guar does not nodulate effectively. The objective of this replicated and repeated greenhouse study was to test the effects of contrasting soils, representing typical alkaline soils in which guar is cultivated worldwide, and *Rhizobia* inoculants on nodulation and plant growth characteristics in two guar varieties. In the study, abundant nodulation was observed. The soils had contrasting effects on nodulation, with a high nodule number of low weight (497 mg/24.3 nodules) in the clay loam and low nodule number of high weight (583 mg/8.64 nodules) in the sandy loam, but no effect of the inoculants was observed. The difference in nodule characteristics between the soils may have resulted from differences in indigenous *Rhizobium* population sizes and/or species available to associate with guar roots. The lack of inoculant effects may have resulted from high indigenous levels of *Rhizobium* in the soil and/or low concentrations of *Rhizobium* or uncompetitive *Rhizobium* delivered through the seed-applied inoculants. Based on the results of this study, we expect that guar will nodulate and fix nitrogen effectively in field conditions, given that these processes are not inhibited by exogenous factors, such as water stress. Achieving increases in nodulation and nitrogen fixation above baseline levels may require a concerted effort to develop effective inoculants for guar, which may offer a unique opportunity to expand biological nitrogen fixation in agriculture in semi-arid regions.

1. Introduction

Production and consumption of guar (*Cyamopsis tetragonolobus* L.), which is a leguminous crop plant also known as clusterbean, has increased dramatically in recent years (Abidi et al., 2015; Yadav and Shalendra, 2015). Guar is grown in a variety of semi-arid regions around the world, including semi-arid parts of India, Pakistan, the United States, Australia, the Mediterranean region, Sudan, and other regions (Gresta et al., 2013; Mubarak et al., 2015; Trostle and Auld, 2013; Whistler and Hymowitz, 1979; Zamir et al., 2016). The plant produces a seed that contains galactomannan gum, which is used in a variety of food and industrial applications as a lubricant, binder, thickener, or emulsifier, among other functions (Yadav and Shalendra, 2015). The recent spike in guar consumption has been driven largely by the industrial uses of the gum, primarily by the oil and gas industry as a lubricant in the process of hydraulic fracturing or fracking (Mudgil et al., 2014; Yadav and Shalendra, 2015).

In the process of hydraulic fracturing, guar gum derivatives are used to thicken the fluid that is used to fracture subterranean rock. This fluid contains sand, which is more effectively carried into the rock fractures when the gum is present. The presence of sand in the cracks keeps them from collapsing and makes them porous, enabling oil and natural gas to flow through the cracks for pumping to the surface (Mudgil et al., 2014). Oil and gas production from hydraulic fracturing has been in use for more than 60 years at a low rate, but it has only been recently that the technique has provided a significant fraction of total crude oil and natural gas production, while also sharply increasing the level of production (Cook and Perrin, 2016). In 2008, for example, total crude oil production in the U.S. was about 5 million barrels per day with about 10% coming from fracking, but by 2015 production was just over 9 million barrels per day with 51% coming from fracking (Cook and Perrin, 2016). This has made the U.S. the largest consumer and

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importer of guar gum products in the world, while India, Pakistan, and several other nations have been meeting the demand (Abidi et al., 2015; Gresta et al., 2014; Yadav and Shalendra, 2015).

Across the guar-producing countries, understanding the ecosystem services that the crop can provide, and the conditions under which these are provided, has been an increasing focus, to make the benefits of guar clearer to producers and policy makers. The primary ecosystem service that is expected from guar is nitrogen fixation. As a legume, guar has the potential to associate with *Rhizobia* bacteria in the soil to form root nodules in which atmospheric nitrogen is fixed, increasing the nitrogen fertility of the crop and soil. But there is a worldwide perception that guar does not effectively nodulate effectively (Abidi et al., 2015; Khandelwal and Sindhu, 2012), though little scientific work has been conducted on the topic.

In the literature, we were only able to find two studies testing or making observations of root nodulation in guar in the field. In a study of nodulation by indigenous *Rhizobium* species in a saline alkaline soil, Bhardwaj (1974) reported that 32% of guar plants had nodules. The authors characterized this nodulation rate as “very poor” compared to other tested species. Stafford and Lewis (1980) reported that application of a *Rhizobium* inoculant to guar seed in field conditions increased nodules per plant by 36% relative to uninoculated seed in northern Texas, with a concomitant increase in plant productivity. The authors reported that nodule number varied according to observation timing, with more nodules found at 8 weeks after planting compared to 4 or 12 weeks. At 8 weeks after planting, nodule number per plant ranged from 0.5 to 8.9. In addition to these field studies, several controlled-environment studies have addressed the impacts of specific exogenous factors on guar nodulation.

Nodule formation and function in legume crops is affected by environmental and management factors, as well as interactions between nodulating bacteria and the plant variety (Zahran, 1999). High temperature, drought stress, high soil acidity, limiting soil nutrients, and low soil *Rhizobium* populations concentrations are the factors most commonly reported to constrain nodulation and nitrogen fixation (Bell et al., 1989; Gibson, 1971; Hungria and Vargas, 2000). Venkateswarlu et al. (1983) reported that a water stress event reduced nodule fresh weight but not nodule number in guar. The authors found that nitrogenase activity was reduced with water stress, but rapidly recovered upon rewetting. In a study on the effect of temperature, Arayangkoon et al. (1990) reported that a root temperature of 40 °C reduced N fixation by 80% and nodule weight by 50%; nodulation was unimpeded below 37 °C. In solution culture, Bell et al. (1989) found that guar has a high Ca requirement relative to other tested legumes for nodulation of > 50 µM Ca. Khandelwal and Sindhu (2012) isolated 95 *Bradyrhizobium/Rhizobium* strains from guar nodules and tested them for short-term impacts on nodulation and root and shoot elongation in guar plants. They reported highly significant differences among bacterial strains in the number and weight of nodules formed, with greater nodulation corresponding with greater plant productivity.

Guar is an increasingly important leguminous crop in semi-arid regions around the world. Despite the nitrogen fixation potential of guar, we have little understanding of the conditions under which this occurs or how to optimize conditions for nitrogen fixation. Using two commonly grown guar varieties, our objective in this study was to test the effect of two texturally contrasting soils and two *Rhizobium* inoculants on root nodulation, plant nitrogen, and plant productivity. We hypothesized that the soils would differently affect plant nodulation, nitrogen, and productivity and that the tested inoculants would increase these parameters in both soil types and guar varieties.

2. Materials and methods

2.1. Experimental design

This research was fully replicated and conducted twice in a greenhouse at the Texas A&M AgriLife Research and Extension Center in Vernon, Texas, USA. The experimental units were 7.6 L pots. The experimental treatments were soil (2), guar variety (2), and seed inoculation (3). There were four replicate pots per treatment combination, giving 48 total pots, which were arranged in a completely randomized design. The pots were laid out in three long rows on greenhouse benches with approximate spacing of 0.3 m on each side. The first iteration of the study began with guar planting on 23 May 2017 and was terminated 12 July 2017 (50 days); the second iteration was started on 17 July 2017 and terminated on 31 August 2017 (45 days).

2.2. Experimental procedures

Two soils were used in the study: a Miles loamy fine sand (fine-loamy, mixed, superactive, thermic Typic Paleustalfs) from near Lockett, Texas, USA and a Tipton loam (fine-loamy, mixed, superactive, thermic Pachic Argiustolls) from near Chillicothe, Texas, USA. The soils were collected from areas along fence-lines that have no direct modern agricultural history. Fresh soil was collected for each iteration of the study and the pots were sterilized with bleach and rinsed thoroughly before beginning the studies to ensure that there was no *Rhizobium* inoculant contamination. The physical properties and chemical parameters of the two soils used in this study were measured by a commercial lab (Water's Agricultural Lab, Camilla, Georgia, USA).

Two guar varieties were tested: Kinman and Lewis. Seeds were obtained from Texas Foundation Seed Service (Vernon, Texas, USA). Three rhizobial inoculation treatments were tested: no inoculation; seed inoculated with Micronoc (SonoAg, Plainview, Texas, USA); and seed inoculated with *Rhizobia* strain USDA 3385. The inoculants were powders prepared for seed coating. The Micronoc product is a complex mixture of *Rhizobium* and other microorganismal cultures that includes a bacterium isolated from guar nodules that were produced in West Texas (personal communication from company representatives). The USDA 3385 *Rhizobia* strain was chosen for testing as it was known to effectively nodulate guar and fix nitrogen from a preliminary sterile culture experiment (unpublished data). For application of the inoculants to the seed, the seed was wetted slightly with a sugar water solution (10.5 g L^{-1}) in plastic zip bags, inoculant powder was added, and the seeds and inoculant were mixed until the seeds were coated homogeneously. Inoculant was added to the full capacity of the material to be held on the seed surface, which differed between inoculants, or about 50 mg g^{-1} seed for USDA 3385 and 100 mg g^{-1} seed for Micronoc. Seeds were planted immediately following application of the inoculants at a rate of five seeds per pot at 13 mm depth. Seedlings were thinned to one plant per pot in the days following germination.

Throughout both studies, the pots were watered by hand at equal volumes. Soil moisture levels were maintained at adequate levels to ensure that soil moisture deficiency did not inhibit plant growth, root nodulation, or nitrogen fixation in both soils, given their physical and chemical differences. Watering times, which differed in interval depending on atmospheric conditions, were determined visually by observation of dry soil in the top 25 mm of the pot. The greenhouse cooling system was not capable of reducing the greenhouse temperature below outdoor ambient levels, which were often hot in the summer, but was successful at preventing temperature rise above ambient levels. The black pots were wrapped in aluminum foil to prevent radiant heating of the soil above ambient temperatures, which was successful, as verified by periodic soil temperature measurements (data not recorded). Ambient temperature and humidity inside the greenhouse were measured continuously by a OM-92 temperature/humidity sensor and datalogger package (OMEGA Engineering, INC. Norwalk, Connecticut, USA).

Harvest and dismantling of the pots was done 45–50 days after planting on the dates listed previously, shortly after the first pods were set. It is expected that nodule characteristics would change over the