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DIFFERENTIAL RESPONSE OF COMMON BEAN GENOTYPES TO MYCORRHIZAL COLONIZATION

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Abstract. **Mycorrhizae are an important symbiotic relationship between fungi and plant roots. Arbuscular mycorrhizae (AM) enhance the growth, development and health of colonized plants. After colonizing plant roots, AM fungi differentiate to form branched tree-like structures (arbuscules). One of the major benefits of AM fungi is to increase P uptake from the soil. The objective of this study was to quantify differences among seven common bean (*Phaseolus vulgaris* L.) genotypes response to AM fungi. Plants were grown for 40 days in mycorrhizal or non-mycorrhizal soil and analyzed for plant height, shoot dry weight, days to flowering, and leaf spectral reflectance. Results indicate that there is substantial variation among bean genotypes for growth in AM soil and low-P. AM fungi significantly increased plant growth in terms of shoot weight and chlorophyll reflectance. This work allowed the selection of bean genotypes DOR 364 and Calima as highly mycorrhizal responsive and G19839 as mycorrhizal unresponsive. Characterization of these bean genotypes and recombinant inbred lines will further improve our understanding of the genetics and physiological mechanisms of AM fungi symbiosis in plant growth and yield.**

Arbuscular mycorrhizal (AM) fungi are the most important root-fungus symbiotic associations found in plants. These associations have been shown to promote plant growth and health in nutrient deficient soils (Smith and Read, 1997). The value of vegetables produced in the state of Florida amounts to approximately 1.5 billion dollars per year (Florida Agricultural Statistics, 1995-96). Of this, $75 million are derived from snap beans sales. A large proportion of the snap beans are grown in low-P soils, and nutrient applications are necessary, adding to production costs. The presence of mycorrhizal fungi would, therefore, prove highly beneficial in enabling the plants to effectively extract the soil for previously unavailable plant nutrients.

Arbuscular mycorrhizal associations have wide-spread application in the horticultural industry (Azcon-Aguilar and Barea, 1997). Early AM inoculation is often important to seedling establishment. AM inoculation can be carried out when the seedling are produced in sterile media where AM propagules are typically absent or in extremely low numbers. AM mycorrhizal associations have particular implications for the plants as biofertilizers and bioprotectors which enhance crop productivity. Appropriate management of the symbiosis allows a reduction of chemical fertilizer and pesticide imputes.

There is a great variability on AM colonization among plant species (DiBonito et al., 1994; Gao et al., 2001). AM fungi have been reported in the roots of many plants including onions (Tawaraya et al., 2001), tomato (Gao et al., 2001), grapes (Linderman and Davis, 2001), and wheat (Zhu et al., 2001). The improvement of nutrient uptake such as N, P, S, Cu, and Zn would be of particular significance in the sandy, nutrient poor, Florida soils. Furthermore, there are reports that mycorrhizae improve drought resistance and tolerance to extreme pH in soil. In a study with *Quercus velutina*, Dixon et al. (1984) showed that plants with AM inoculation significantly improved water balance during mild drought.

Common bean is the world's most important food legume (Pachico, 1993). Beans offer a low cost alternative to beef and milk because bean seed is rich in protein, Fe, fibers, and complex carbohydrates. Adaptation of bean to the range of environmental conditions around the world has resulted in the evolution of extensive genetic variation for nutrient efficiency in this crop (Hacisalihoglu and Kochian, 2003). Consequently, its rich and apparent genetic diversity for stress tolerance makes it an excellent crop model plant.

The objectives of this study were: (1) to investigate the response of bean genotypes to inoculation of arbuscular mycorrhizae under greenhouse conditions, and (2) to identify the most mycorrhizae-responsive and -unresponsive bean genotypes.

Materials and Methods

Seeds of bean genotypes used in the present study were obtained from Dr. E. Vallejos (University of Florida) and USDA-National Plant Germplasm System (Fort Collins, Colo.). Based on the initial screening experiments, seven bean genotypes (Calima, Jamapa, Voyager, Avanti, DOR364, G19839, and Contender) were identified as the most diverse genotypes for mycorrhizal responsiveness.

Plants were grown in 1-gallon plastic pots in a greenhouse maintained at 27 ± 3°C day and 21 ± 3°C night temperature during spring 2005 with natural day length and light intensity in Tallahassee, Florida.

Six seeds were sown in pots filled with a commercial soil medium (85% peat moss, perlite, vermiculate, limestone). Before planting, soil was autoclaved at 65°C for 90 min to destroy indigenous mycorrhizae. Plants in each pot were thinned to three plants 7 d after emergence. All seven genotypes were grown with or without mycorrhizae to compare their responses to inoculation. To produce mycorrhizal plants, pots were inoculated with approx. 2000 spores of *Glomus intraradices* (Reforestation, Monterrey, Calif.), spores were placed in the

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Table 1. Leaf spectral reflectance (SPAD) readout, plant height, shoot biomass, mycorrhizal responsiveness of seven bean genotypes inoculated with mycorrhiza under low P conditions, compared to non-mycorrhizal control. Data are presented as means (S.E.), n = 4 replications.

Bean genotype	Mycorrhizal treatment	Reflectance (SPAD)	Plant height (mm)	Shoot biomass (g)	Mycorrhizal responsiveness (%)
Calima	Non-mycorrhizal	27	570 (98.9)	24.91 (1.61)	
	Mycorrhizal	34	541 (94.3)	50.85 (0.85)	51.00
Jamapa	Non-mycorrhizal	34	572 (70.1)	12.88 (5.56)	
	Mycorrhizal	35	408 (124)	22.84 (1.68)	43.60
Voyager	Non-mycorrhizal	32	851 (13.9)	24.45 (7.28)	
	Mycorrhizal	41	861 (36.7)	31.76 (2.08)	23.00
Avanti	Non-mycorrhizal	24	1195 (95.2)	18.16 (5.97)	
	Mycorrhizal	38	763 (56.3)	20.14 (0.90)	9.83
DOR364	Non-mycorrhizal	29	258 (22.5)	11.09 (4.31)	
	Mycorrhizal	33	679 (191)	26.66 (1.48)	58.40
G19839	Non-mycorrhizal	30	436 (21.2)	35.06 (4.61)	
	Mycorrhizal	34	1086 (50.6)	34.99 (7.28)	-0.20
Contender	Non-mycorrhizal	18	521 (2.41)	27.89 (0.15)	
	Mycorrhizal	37	388 (21.6)	32.02 (5.76)	12.90

root region. Non-mycorrhizal control plants received no spores. Plants were irrigated as needed to avoid water stress. Plants were fertilized with low P(N:P:K; 26:2:13) fertilizer with micronutrients (Scotts, Marysville, Ohio).

Plants were harvested on two different occasions. (1) 20 d after planting (vegetative); and (2) 40 d after planting (anthesis, opening of flowers). On each occasion, four plants were harvested from each pot, shoots were weighed, and roots were collected for mycorrhizal evaluation. Plants were evaluated for plant height and chlorophyll content with a chlorophyll meter (SPAD-502 meter, Spectrum Tech, Plainfield, Ill.) every three days until harvesting. Mycorrhizal responsiveness (MR) was calculated using the following formula:

$$MR\ (\%) = [\text{Mycorrhizal shoot wt} - \text{non-mycorrhizal shoot wt}] / \text{mycorrhizal shoot wt} * 100$$

The experiments were set up in a complete randomized design with four replicates, and the variation within means is presented as the standard error.

Results and Discussion

The mean effect of mycorrhizal inoculation on plant growth response under low P conditions varied among bean genotypes. The use of mycorrhizae induced a statistically significant increase in plant height, reflectance measurement (SPAD readout), shoot biomass and mycorrhizal responsiveness (MR) (Table 1).

Shoot height of mycorrhizal-p-inoculated plants was not always significantly greater than that of non-mycorrhizal plants. Shoot biomass at 40 d harvest increased significantly for most of the bean genotypes except G19839 (Table 1).

Variation among genotypes for growth at low P ranged from 58.4% (DOR 364) to -0.20% (G19839), averaging 28.2% (Table 1). Forty day after planting, colonization increased mycorrhizal responsiveness 108% and 82% in bean genotypes DOR 364 and Calima, respectively; while having no affect on genotype G19839 (Table 1).

As expected, mycorrhizae enhanced vegetative growth and leaf chlorophyll content. Genotype Contender showed the highest SPAD readout increase (two-fold) comparing to non-mycorrhizal control (Table 1).

Leaf spectral reflectance was significantly different for the time of day in mycorrhizal plants (Fig. 1B). Both mycorrhizal and non-mycorrhizal plants showed the highest spectral readout (SPAD) in the afternoon at 17.00 o'clock.

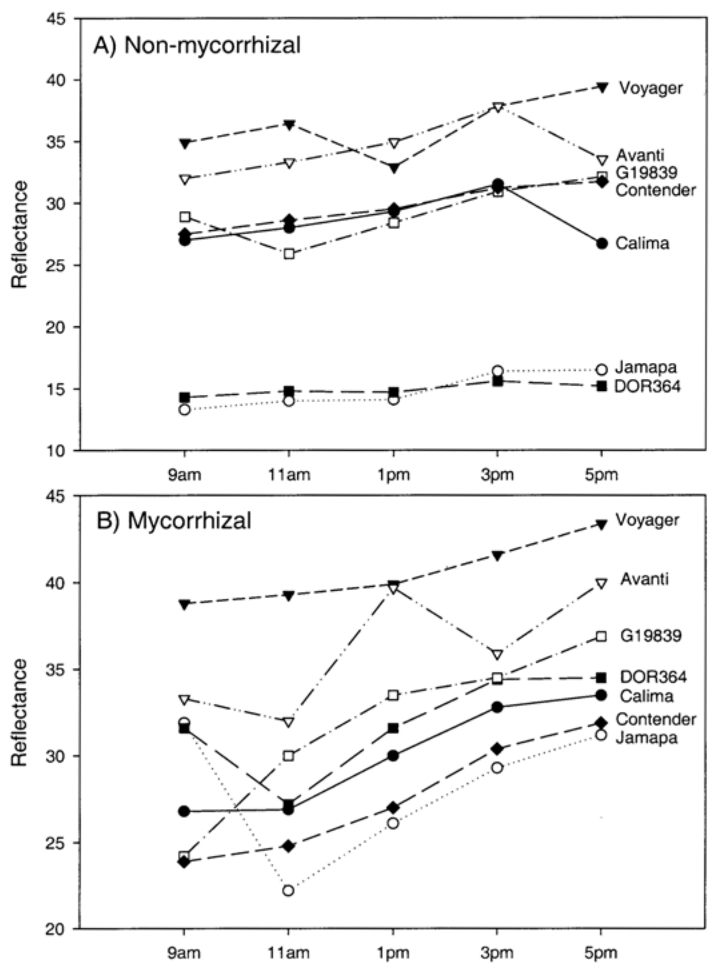

Fig. 1. Hourly leaf spectral reflectance measurements (SPAD readout) collected on non-mycorrhizal (A) and mycorrhizal (B) plants 40 d after planting. Data are presented as means of n-8 replications.

These results have demonstrated the benefits of mycorrhizae fungi on the growth of common bean. There was a substantial genotypic variation in mycorrhizal responsiveness among seven bean genotypes. Mycorrhizal responsiveness was based on final shoot biomass at 40 d after planting. Most genotypes tested showed a positive response to mycorrhizae under low P conditions (Table 1). This is consistent with observations of saving P fertilizer when potatoes were inoculated with mycorrhizae (Sieverding, 1991). Diederichs (1991) showed that application of 1 $g \cdot kg^{-1}$ superphosphate increased the growth of maize plants in low-P soils. Thus, more efficient uptake of P via mycorrhizae increases the plant growth and development.

This study evaluated bean genotypes for mycorrhizal responsiveness. According to present results, the mycorrhizal responsiveness of seven genotypes determined by shoot biomass can be arranged in the following order: DOR 364 > Calima > Jamapa > Voyager > Contender > Avanti > G19839 (Table 1). Furthermore, the greatest spectral readout for leaf reflectance was found at 17:00 o'clock; while the lowest reading was at 13:00 o'clock in both inoculated and non-inoculated plants (Fig. 1).

Common bean is a mycorrhiza dependent plant species and there is a substantial genotypical variation in mycorrhizal response among bean genotypes. Inoculation of *Glomus intraradices* could significantly increase the growth and development of bean genotypes, specifically DOR 364 and Calima. Further investigations are needed to establish the detailed affects of mycorrhiza in bean growth and physiology.

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