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Nutrition of sorghum plants fertilized with nitrogen or inoculated with *Azospirillum brasilense**

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Summary Sorghum plants were inoculated with *Azospirillum brasilense* or received an N-amended nutrient solution. Azospirillum inoculation increased plant dry weight and nitrogen assimilation by 25%. Most plant growth responses to Azospirillum were comparable to application of 2.0 m*M* N. Increased scavenging of nutrients, altered root permeability or nitrogen fixation are possible explanations for these effects.

Introduction

Inoculation of cereal crops with the N_2-fixing bacterium *Azospirillum brasilense* has been reported to improve growth and plant N nutrition[5], although the response to inoculation has been highly variable[8,11,13]. Most studies indicate that the majority of the growth response is attributable to some factor other than N input[14,15]. Physiological studies of plant-bacterial interactions require the determination of the amount of additional N that non-inoculated plants must receive to produce plants of equal size to the inoculated host. Equal growth does not necessarily indicate functional equivalence since there may be nutritional and metabolic differences. This experiment was designed to measure the growth response of sorghum to N fertilization or inoculation with *A. brasilense* and to determine the fertilizer-N input which resulted in growth equivalence between N-amended and Azospirillum-inoculated plants.

Materials and methods

Sorghum [*S. bicolor* (L) Moench cv. Bok 8] seeds (30 ± 5 mg) were surface sterilized and plated in 1.5 L pots containing 1.25 kg of soil[2]. The soil was a slightly acid (pH 5.7) loam that had been limed (10 g $CaCO_3$ kg^{-1}) and sterilized with ethylene oxide, and it initially contained 13 μg NH_4^+-N, 4 μg NO_3^--N and 1.5 mg Kjeldahl N g^{-1} soil. Plants were inoculated with one of two strains of *A. brasilense* (JM 125A2 and Cd ATTC 29729) or were left uninoculated and received one of four N-amended nutrient solutions. Strains of *A. brasilense* were cultured for 72 h in peptone-succinate-salt broth[6]. An inoculum of 5 ml of bacterial suspension (10^9 cells) was applied at planting and again after 7 days. Uninoculated plants were given 5 ml of sterile broth. There were three plants per pot and each treatment was replicated five times.

The nutrient solution used was equivalent to one-quarter strength Johnson's solution[7], except for N. Micronutrients were supplied as previously[2]. Nitrogen was added as 0.0, 0.5, 1.0

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Table 1. Yield and elemental composition for sorghum inoculated with either *Azospirillum brasilense* or fertilized with N*

	Dry weight, g per pot		N concentration, %		Total N	Total P
Treatment	Shoot	Root	Shoot	Root	mg per pot	mg per pot
N concentration (mM)						
0.0 (control)	13.2 c	5.6 c	0.66 e	0.55 d	118 d	32.3 d
1.0	14.6 b	6.6 b	1.03 c	0.61 d	191 c	40.9 bc
2.0	15.5 b	6.9 b	1.20 b	0.83 b	242 b	41.6 bc
4.0	17.9 a	9.0 a	1.44 a	0.95 a	342 a	52.2 a
Azospirillum strains						
125A2	17.0 a	7.4 b	0.76 d	0.77 bc	186 c	44.7 b
29729	16.9 a	7.3 b	0.73 de	0.72 c	174 c	37.3 cd

* Mean values (5 replicates) for each parameter having common letters within a column are not significantly different at 0.05 level[1].

Table 2. Micronutrient concentrations in sorghum inoculated with *A. brasilense* or fertilized with N*

	Fe		Mn		Zn		Cu	
Treatment	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
N concentration (mM)				$\mu g\ g^{-1}$				
0.0 (control)	36.4 a	2915 b	35.9 ab	241 c	10.8 a	20.6 a	3.3 a	8.3 a
1.0	38.1 a	3000 b	30.7 a	197 ab	11.7 ab	29.0 c	3.7 a	9.6 b
2.0	57.0 b	2814 b	38.0 b	208 bc	13.3 b	28.1 bc	4.5 b	8.8ab
4.0	55.4 b	3710 c	34.3 ab	236 c	11.0 a	29.3 c	4.5 b	8.6ab
Azospirillum strains								
125A2	40.4 a	2195 a	33.1 a	155 a	10.2 a	26.0 b	3.5 a	7.6 a
29729	63.3 b	2473 a	32.7 a	182 ab	10.6 a	24.3 ab	3.5 a	9.0 b

* Mean values having common letters within a column are not significantly different at the 0.05 level[1].

or 2.0 m*M* NH_4NO_3, with concentrations chosen to bracket the growth response of sorghum to colonization by Azospirillum. The pH of each solution was adjusted to 6.9 with 0.01 *N* KOH. Pots inoculated with *A. brasilense* received no combined N in solution. Plants were grown in a Sherer Model CEL 36-10 growth chamber and were watered to field capacity 3 to 5 times a week with nutrient solution. Day/night temperature and relative humidity were 32/27°C and 75/40%, respectively. The light/dark period was 16/8 h, and photosynthetic photon flux density varied from 600 to 500 $\mu E\ m^{-2}\ s^{-1}$ on the growth platform. Plants were rotated daily in a split-block design to minimize positional effects.

Plants were harvested over a two day period at week 9, dried at 70°C for 48 hours, and ground to 60 mesh. Plant N content was determined with an ERBA model 1400 automated nitrogen analyzer. Plant P and micronutrients were determined by colorimetry and atomic absorption spectrophotometry[4].

Root segments (2 mm length) were fixed, stained with methylene blue, and examined with a light microscope to observe root morphology. These segments revealed the characteristic root morphology associated with *A. brasilense* colonization in inoculated, but not in uninoculated roots[9].

Results and discussion

Although plants inoculated with Azospirillum were similar in N and P content to the 1.0 m*M* N treatment, their dry weights were comparable to plants supplied with 2.0 or 3.0 m*M* N (Table 1). This implies that N input by *A. brasilense* may have contributed to growth enhancement in sorghum. However, this growth effect may not be due to N alone. Azospirillum-inoculated plants had shoot and root dry weights which were 30% greater than the -N control confirming similar observations by Tilak[16] and Smith *et al.*[13]. The concentration of N in the shoots of inoculated plants was similar to the -N control, but root N content was comparable to the 2.0 m*M* N treatment (Table 1). The lower shoot N contents and higher root N contents for Azospirillum-inoculated plants led Schank *et al.*[12] to suggest that inoculated plants had a faster growth rate which resulted in a lower percent N content, but a higher dry matter yield. Subtraction of seed N (3 mg) from the total N in the —N control (118 mg) indicated that 115 mg N were assimilated from the soil. This amounted to 5.1% of the total soil N pot^{-1}. Plants inoculated with Azospirillum assimilated 20 mg N per plant above the seed and soil contribution even under the suboptimal light conditions of this experiment. The experiment was carried out in sterilized soil and so the establishment of Azospirillum was not subject to any competitive effects of native soil microorganisms.

The P uptake by sorghum inoculated with Azospirillum was greater than the —N control and was higher in plants inoculated with strain 125A2 (Table 1). Enhanced P uptake following *A. brasilense* inoculation[9] suggests that these rhizosphere bacteria increase the availability of nutrients through altering root surface characteristics involved in nutrient uptake[17]. Such a contribution to plant nutrition may offer a better explanation for the growth response following inoculation with Azospirillum than the small increase in N input due to N_2 fixation[12,18]. Rhizosphere changes, pH effects, and the production of plant growth-promoting substances[11,15] by these diazotrophs are alternate explanations for these yield increases[3]. Inoculation with strains of *A. brasilense* produced varied effects on host nutrient uptake (Table 2). Iron concentration in roots of inoculated plants was significantly lower than in uninoculated plants. Shoot Fe concentration in plants inoculated with strain 29729 was equivalent to that found in plants given 4.0 m*M* N. The low level of root Fe in Azospirillum-inoculated plants is one effect not linked to plant N status. The exclusion of Fe in sorghum inoculated with Azospirillum may be due to rhizosphere pH or redux effects. Associative N_2-fixing bacteria have mechanisms which maintain O_2 at low levels[10] which may alter iron uptake by the roots.

Changes in dry weight and N content were influenced by improved N nutrition in Azospirillum-inoculated plants. For these characteristics it was possible to compensate for the *A. brasilense*-assisted N input by increasing fertilizer N. Other plant effects are not as readily explained and may be linked to changes in root function[16]. It is unlikely that adjustments of the pH or micronutrient levels in nutrient solutions given to uninoculated plants would duplicate the effect of inoculation with *A. brasilense.*

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