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Promotion of Leaf Area Development and Yield in *Sorghum Bicolor* Inoculated with *Azospirillum brasilense**

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Abstract

This work studies the effect of inoculation with *Azospirillum brasilense* on growth and yield of *Sorghum bicolor* in hydroponic systems.

Significant enhancement of dry matter content, leaf area development and grain yield was observed in inoculated plants as compared to controls, mainly at 24 to 28 days after emergence.

At later stages, leaf senescence was delayed in inoculated plants, thus favouring dry matter accumulation and grain filling. In plants subject to an osmotic stress potential of −2.0 bar (produced by the addition of polyethylene glycol [PEG 6000]), inoculation diminished the adverse effects caused by osmotic stress such as reduction of leaf senescence. The use of the ^{15}N Natural Abundance Method in a pot experiment provided clear evidence for the absence of biological N fixation in sorghum inoculated with *Azospirillum*.

Keywords: *Azospirillum brasilense*, *Sorghum bicolor*, plant growth promotion, ^{15}N Natural Abundance, osmotic stress, roots

1. Introduction

Experiments using *Azospirillum* inoculants were carried out in commercial fields in Israel during 1978–85. Inoculation was generally beneficial to growth and significantly increased yields of several grain, forage an legume crops by 10–35% (Okon et al., 1988).

In glasshouse-grown grain sorghum (*Sorghum bicolor*), leaf length, plant weight, dry weight and total N content of shoots were significantly increased by inoculation with

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Azospirillum (Kapulnik et al., 1981a; Wani, 1988; Pakowsky et al., 1985). Inoculated sorghum showed improved nutrient utilization, and inoculated plants reached the flowering stage significantly earlier than did non-inoculated controls (Lin et al., 1983).

In the above experiments carried out under dryland conditions in Israel, inoculation of sorghum lead to an average increase of 15–20% in total stover dry matter yield and in grain yield (Kapulnik et al., 1981b; Sarig et al., 1984; Sarig et al., 1988).

The water status of sorghum plants was improved by inoculation as evidenced by higher leaf water potential, lower canopy temperatures and greater stomatal conductance and transpiration (Sarig et al., 1988).

Proposed mechanisms by which *Azospirillum* improves the growth of plants include morphological changes in the root system (Okon and Kapulnik, 1986) and possible involvement of phytohormones (Tien, et al., 1979).

In this work we studied the effects of *Azospirillum* inoculation on shoot development and vegetative and reproductive yield of *Sorghum bicolor* plants grown under conditions of osmotic stress hydroponically or in pots.

2. Materials and Methods

Azospirillum brasilense strain Cd (ATCC 29729) was grown on liquid malate medium (Okon et al., 1977). The cultures were washed with 60 mM sterile phosphate buffer at pH 6.8 three times by centrifugation for 10 min at 3000 g and were resuspended in phosphate buffer to a cell concentration of 10^8 colony forming units $(CFU)/ml^{-1}$.

Grain sorghum hybrid RS-610 (Hazera Seed Co., Haifa) was used for all experiments. For reduction of contaminants and pathogens, seeds were disinfected in 0.75% sodium hypochlorite for 15 min and washed with sterile water. The seeds were allowed to germinate for 24 hr in sterile water at 25°C.

Greenhouse experiments were carried out in 1L or 5L hydroponic systems adapted for sorghum growth (Sullivan and Ross, 1979). Nutrient solution for hydroponics was as described by Blum et al. (1977).

Healthy-looking germinated seeds with roots 1 mm in length were transferred to 48 ml PVC pots which were filled with washed sea sand. The sand was watered to saturation with tap water. Germinated seeds were sown at a depth of 2 cm and each pot was inoculated with 1 ml suspension (10^8 CFU/ml) of *Azospirillum*. The controls were treated with 1 ml of autoclaved bacterial suspension. The sand was covered with a thin layer of vermiculite to reduce evaporation.

Three days after emergence (DAE), seedlings were removed from the pots and transferred to the hydroponic systems. The plants were grown at $27° \pm 2°C/18° \pm 2°C$ day/night temperatures with a photoperiod of 13.5 hr, which was supplemented by fluorescent light to a peak rate of 1000–1200 $\mu E\, m^{-2} sec^{-1}$ (photosynthetic active

reaction at the plant level). The position of the plants on the benches was changed twice weekly.

Osmotic stress was created by adding polyethylene glycol (PEG-6000) (−2.0 bar at 25°C) to nutrient solution (Michel and Kaufmann, 1973). PEG was added gradually during a 2-day period in order to avoid an osmotic shock to the plants. PEG was not found to be toxic to cultures of *A. brasilense*. Pure nutrition solution had a water potential greater than −0.2 bar.

The 5L hydroponic experiments were repeated 3 times with 8 plants per treatment. Plants were grown for 120 days. Plant dry matter, total leaf area and green leaf area were determined during plant growth. Final grain yield per plant and 1000 kernel weight were also measured.

The 1L hydroponic experiments were repeated 3 times with 16 plants per treatment. Plants were grown for 35 days.

Growth of sorghum in pots

20L pots were filled with clay soil (flannagan silt loam Aquic agriudolls). Three surface-sterilized seeds were sown at a depth of 2.5 cm and each seed was inoculated with 1 ml suspension of *Azospirillum*. The pot surface was covered with a thin layer of perlite to avoid cross contamination. One week after plant emergence the number of plants was reduced to one per pot. They were grown for 76 days under the same conditions described for hydroponics. Accumulation of dry matter, leaf area, time of leaf appearance and % N were determined during growth. Biological N_2 fixation was estimated by measuring the ratio of ^{14}N:^{15}N and comparing it to the natural abundance of ^{15}N in the atmosphere (Shearer and Kohl, 1988).

At the end of all the experiments, *Azospirillum* was isolated and identified from the root systems of inoculated plants according to the method described by Okon et al. (1977). No *Azospirillum* could be detected from the roots of control plants.

3. Results

Plant dry matter

Inoculation with *Azospirillum brasilense* in 5L hydroponic systems significantly increased the total plant dry weight at full maturity by 18% above that of non-inoculated controls. Inoculation did not change the shoot/root ratio although there was a significant increase of 15% in the dry matter of leaves as shown in Table 1.

In 1L hydroponic systems, dry matter in sorghum shoots and roots was significantly higher in inoculated plants 28 days after emergence (DAE) and remained so to the end of the experiment at 35 DAE (Fig. 1).

Osmotically stressed plants showed a 14% decrease in dry matter as compared to 25% in the control (Fig. 1).

Table 1. Effect of inoculation with *Azospirillum brasilense* on dry matter yield in *Sorghum bicolor* in 5 liter hydroponic system (120 DAE, full maturation)

	Total dry weight (g/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Shoot/root ratio
Inoculated	70.6a	63.5a	7.1a	5.9
Control	59.6b	53.4b	6.2b	5.7

Numbers followed by the same letter in each column do not differ at P=0.05.

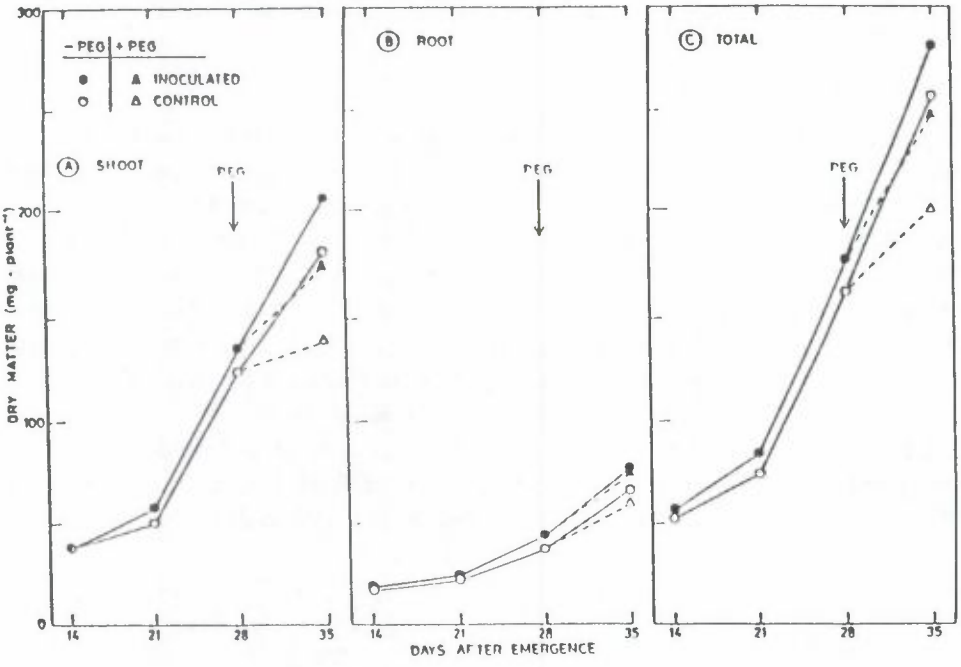

Figure 1. Effect of *Azospirillum brasilense* inoculation on dry matter accumulation in *Sorghum bicolor* in 1L hydroponic system. An osmotic stress of −2.0 bar was obtained by adding PEG 6000 to the medium at 28 DAE.

In pot experiments (Table 2) inoculation led to a 33% increase in dry matter content at 76 DAE and a 30% increase in the total N content of the plant. Percent nitrogen in the leaves was not affected by inoculation. Results of ^{15}N determination were similar in inoculation and control plants indicating that biological N_2 fixation did not contribute to the N content of *Azospirillum*-treated plants (Table 2).

Table 2. Effect of *Azospirillum brasilense* Cd inoculation on dry matter, % N and N_2-fixation (^{14}N:^{15}N) in *Sorghum bicolor* grown in pot (76 DAE)

	Dry weight (g/plant)	% N in shoots	N content (mg/plant)	Atom % ^{15}N
Inoculated	6.0a	0.81	48a	0.7548
Control	4.5b	0.83	37b	0.7447

Numbers followed by the same letter at each column do not differ at P=0.05.

Plant growth and leaf area

At almost all the sampling dates in the various experiments, the leaf are of inoculated plants was greater than that of non-inoculated controls. In the 1L hydroponic system, there was a significant leaf area increase of 37% at 24 DAE and 22% at 28 DAE (Fig. 2). In another similar experiment, a constant 12% increase was observed (Fig. 3), and in the pot experiment, leaf area increased by 52–60% at 24–25 DAE (Fig. 4).

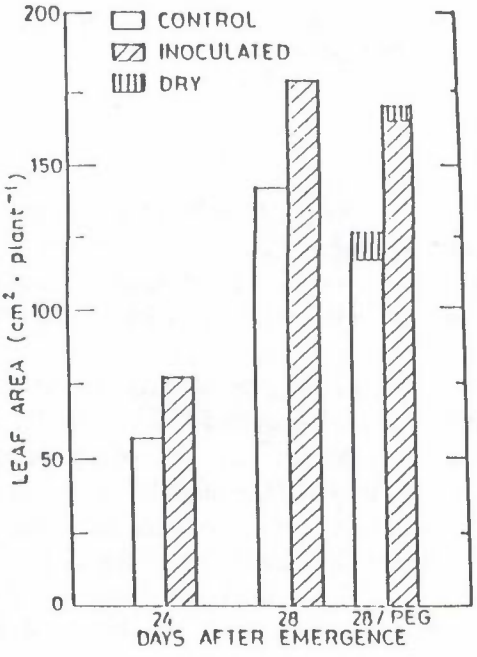

Figure 2. Effect of *Azospirillum brasilense* inoculation on leaf area of *Sorghum bicolor* in 1L hydroponic system. An osmotic stress of −2.0 bar was obtained by adding PEG 6000 to the medium at 24 DAE.

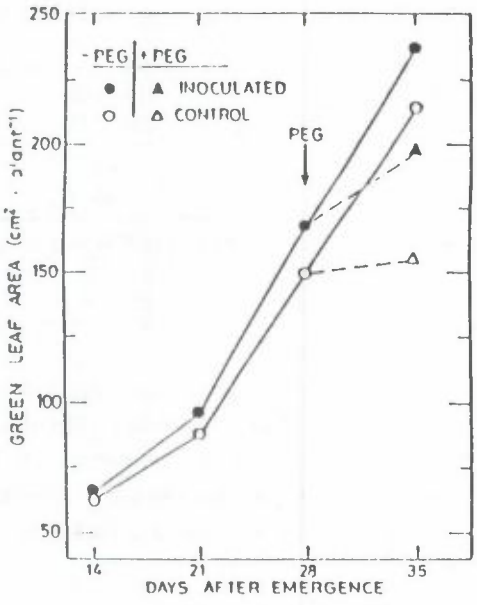

Figure 3. Effect of *Azospirillum brasilense* inoculation on green leaf area of *Sorghum bicolor* in 1L hydroponic system. An osmotic stress of −2.0 bar was obtained by adding PEG 6000 to the medium at 28 DAE.

The increase in leaf area in the pot experiment was due to a significantly greater rate of leaf development. During the period of 17–24 DAE, leaf area increased by 14 cm/day in inoculated plants as opposed to 8 cm/day in controls. From 24–33 DAE, the rate of leaf area increase was 29 cm/day in inoculated plants and 20 cm/day in controls (Fig. 4).

In 5L hydroponic experiments, the control leaves reached a maximum area at 60 DAE; the leaves of inoculated plants reached this level 10 days later (Fig. 5). From 70 DAE until the end of growth, the green leaf area of inoculated plants exceeded that of controls by 23 and 74% at 110 DAE and 120 DAE, respectively (Fig. 5). Leaf appearance and the elongation rate of leaves were monitored in the pot experiment (Fig. 6). There was no difference between the treatments in the date of leaf appearance. Inoculation clearly increased leaf elongation rate (Leaves no. 4–8: Fig. 6). The elevated rate of elongation was more pronounced during the first 4 days of leaf elongation (Fig. 6).

In 1L hydroponic systems, the addition of PEG led to a reduction in leaf area and promoted leaf senescence. These effects were less pronounced in inoculated plants than in controls (Figs. 2,3).

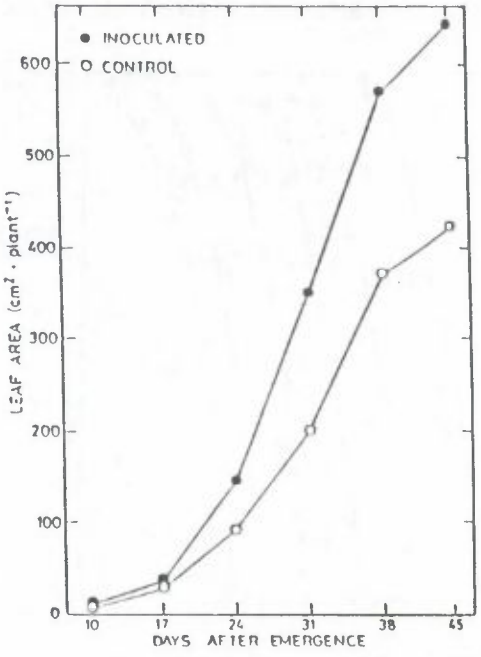

Figure 4. Effect of *Azospirillum brasilense* inoculation on leaf area of *Sorghum bicolor* grown in pots.

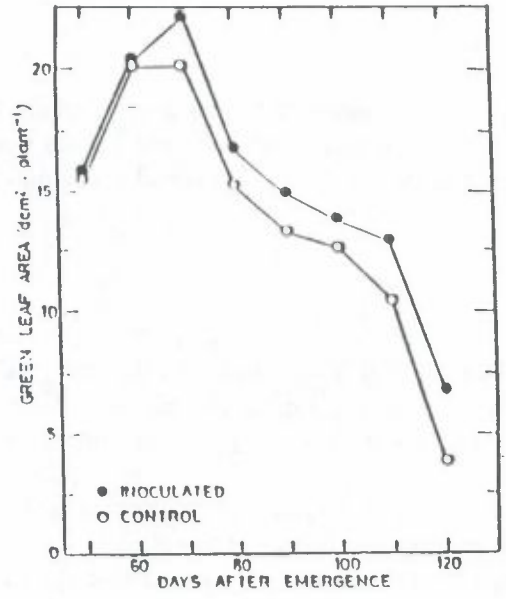

Figure 5. Effect of *Azospirillum brasilense* inoculation on green leaf area of *Sorghum bicolor* in 5L hydroponic system.

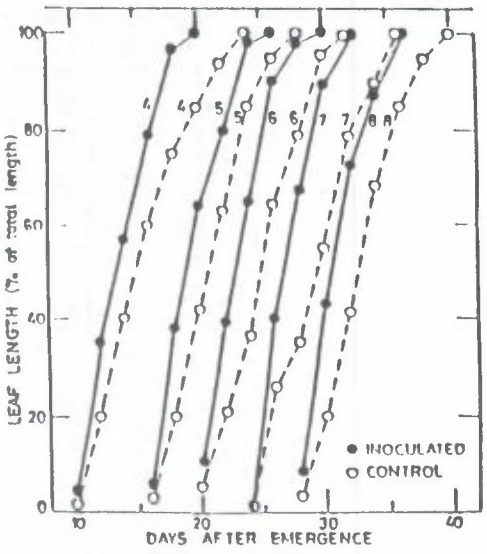

Figure 6. Effect of *Azospirillum brasilense* inoculation on leaf appearance and leaf elongation rate in *Sorghum bicolor* grown in pots. The values of leaf length are expressed as % of the total length of each leaf separately. The numbers near the graphs express the position of the leaf from the basis of the stem.

Grain yield

In 5L hydroponic systems inoculation increased grain yield by 18% over that of controls (Table 3). The increase was due mainly to the greater weight of kernels (a 14% increase was observed in the weight of 1000 kernels). The number of kernels per panicle did not vary between treatments.

4. Discussion

Several studies have shown yield increases in cereal plants inoculated with *Azospirillum* (Sarig et al., 1984; 1988; Wani, 1988). They focused on the biological nitrogen fixation ability of these bacteria as the main reason for this increase (Wani, 1988). The use of ^{15}N natural abundance method in this study(pot experiment), however, provided evidence for the absence of biological N fixation in sorghum inoculated with *Azospirillum*. It is probable that the increase in N content in leaves of inoculated plants may be derived from enhanced uptake of this element from the soil (Okon and Kapulnik, 1986). The hydroponic experiments showed that the effect of inoculation on dry matter accumulation begins at early stages of plant development (24–28 DAE). At this early stage of growth, it is less likely that the effect is related to improvement

Table 3. Effect of inoculation with *Azospirillum brasilense* on grain yield in *Sorghum bicolor* in 5 liter hydroponic systems (120 DAE, full maturity)

	Grain yield (g/plant)	H.I.	Weight of 1000 seeds (g)	Number of seeds/panicle
Inoculated	21.4a	0.33	23.0a	930
Control	18.1b	0.34	20.2b	896

Numbers followed by the same letter in each column do not differ at $P = 0.05$.

of the water status of the plant, especially in hydroponics. It is more probable that the increased growth is due to the production of phytohormones by the bacterium which promote growth and branching of roots (Tien et al., 1979; Okon and Kapulnik, 1986). It is also possible that the plant produces phytohormones as a reaction to *Azospirillum* colonization of the roots (Fallik et al., 1989). A detailed analysis of root development and function in the hydroponic system has shown that inoculation increased the total number of length of adventitious roots (Sarig, Ph.D. Thesis, unpublished results).

The positive effect of inoculation on later stages of plant growth in hydroponics was expressed by delayed leaf senescence; this would bring greater assimilation at later growth stages.

Most likely, the delayed leaf senescence under inoculation (with PEG) allowed for better kernel growth, as seen in final total kernel weight. Final total kernel weight was the only yield component affected by inoculation. The delayed leaf senescence under inoculation most likely results from a better plant water status, as compared with the controls (Eck and Musick, 1979).

The effect of *Azospirillum* inoculation in moderating the damage caused by osmotic (drought) stress illustrates the potential importance of *Azospirillum* inoculants for improving growth and yield of summer cereal and grass crops in areas subjected to drought stress (Okon et al., 1988).

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