Agronomie 21 (2001) 553–560

Original article

Effects of inoculation with *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) under different growth conditions

Bianca HAMAOUI[a], Jihad M. ABBADI[b], Saul BURDMAN[a], Adnan RASHID[b], Shlomo SARIG[a], Yaacov OKON[a]*

[a] Department of Plant Pathology and Microbiology and the Otto Warburg Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel
[b] College of Science and Technology, Al-Quds University, Abu-Dis, Palestinian Authority

(Received 27 July 2000; revised 15 April 2001; accepted 28 May 2001)

Abstract – The effects of the inoculation of chickpeas (*Cicer arietinum* L.) and faba beans (*Vicia faba* L.) with *Azospirillum brasilense* strain Cd were studied under different growth conditions. In greenhouse experiments with both legumes, inoculation with *A. brasilense* significantly enhanced nodulation by native rhizobia and improved root and shoot development, when compared with non-inoculated controls. Moreover, the bacterial treatment was shown to significantly reduce the negative effects on plant growth caused by irrigation with saline water. In field experiments, inoculation of chickpeas with *A. brasilense* peat-based inoculants also resulted in a significant increase in nodulation, root and shoot growth, and crop yield as compared with non-inoculated controls.

***Azospirillum brasilense* / *Cicer arietinum* / *Vicia faba* / *Rhizobium* sp.**

Résumé – Effets de l'inoculation avec *Azospirillum brasilense* sur le pois chiche (*Cicer arietinum* L.) et les fèves (*Vicia faba* L.) dans différentes conditions de croissance. Les effets de l'inoculation des pois chiches et des fèves avec *Azospirillum brasilense* souche Cd ont été étudiés dans différentes conditions de croissance. Dans les expériences en serre avec les deux légumineuses, l'inoculation avec *A. brasilense* a augmenté de façon significative la nodulation par les rhizobia indigènes et amélioré le développement des racines et des tiges comparativement à des témoins non inoculés. De plus, il a été montré que le traitement bactérien réduit de façon significative les effets négatifs sur la croissance des plantes dus à l'irrigation avec de l'eau salée. Dans les expériences au champ, l'inoculation des pois chiches avec *A. brasilense* incorporé à de la tourbe a permis également un accroissement significatif de la nodulation, de la croissance des racines et des tiges et du rendement de la culture comparativement à un témoin non inoculé.

***Azospirillum brasilense* / *Cicer arietinum* / *Vicia faba* / *Rhizobium* sp.**

1. INTRODUCTION

The free-living N_2-fixing rhizobacteria of the genus *Azospirillum* live in close association with plants. These bacteria are capable of increasing the yield of important crops grown in various soil and climatic regions [13]. Positive effects of *Azospirillum* are mainly attributed to improved root development and to the subsequent

Communicated by Jean-Jacques Drevon (Montpellier, France)

* Correspondence and reprints
okon@agri.huji.ac.il

increase in the rate of water uptake and mineral utilization [14, 19].

Positive effects following inoculation with *Azospirillum* and other plant growth promoting rhizobacteria have been reported for several legumes naturally nodulated or coinoculated with *Rhizobium* under greenhouse and field conditions [5, 9, 16, 17, 22]. The increase in dry matter production and in nitrogen content of *Azospirillum* inoculated-legumes may be attributed to an early nodulation, an increased number of nodules, higher N_2-fixation rates and a general improvement in root development [5, 24]. The increase in nodulation may be explained, at least partially, by the promoting effects of *Azospirillum* on root hair formation and by an increased secretion of *nod*-gene inducing signals by the roots [3, 23].

The picture emerging from experimental data indicates that the combined inoculation with *Azospirillum* and *Rhizobium*, or inoculation with *Azospirillum* of naturally nodulated legumes, may potentially increase legume yields under limited water and nitrogen availability. It may also lead to a decrease in the need for nitrogen fertilization, lowering production costs and reducing the negative impacts of excessive fertilization on the environment [5, 6].

Cicer arietinum L. (chickpeas, "houmous") and *Vicia faba* L. (faba bean, broad beans, "ful") are consumed in large quantities by both the Israeli and Palestinian populations. They are winter-springcrops grown in semi-arid regions requiring frequent supplemental irrigation at the critical reproductive stages. Severe shortage of fresh water in those regions imposes the necessity of exploring alternative sources of irrigation water. The possibility of using saline water stored in existing large aquifers in the Middle East for crop production needs to be supported by basic and applied research regarding plant-water and soil-water relationships.

Based on this background, attempts were made to assess the effect of inoculation with *Azospirillum brasilense* on greenhouse-grown chickpeas and faba beans and on chickpeas grown in the field, on soils containing natural *Rhizobium*. The plants were grown under different water regimes and in one experiment with chickpeas, a treatment of irrigation with saline water was included.

2. MATERIALS AND METHODS

2.1. Bacteria and inoculum preparation

Azospirillum brasilense strain Cd (ATCC 29729) was grown for 24 h on liquid malate minimal medium [12] at 30 °C. For the greenhouse experiments, cells were harvested by centrifugation (1 000 *g*, 10 min, twice) and resuspended for inoculation in 0.1 M phosphate buffer, pH 7.0 (10.6 $g \cdot l^{-1}$ K_2HPO_4 and 5.3 $g \cdot l^{-1}$ KH_2PO_4). For the field experiments, *A. brasilense* strain Cd was grown as described above and peat-based inoculants of the bacterium were prepared as described previously [18]. The bacterial concentration of the inoculum was determined by dilution counts taken directly from the applied inoculant in all experiments.

2.2. Greenhouse experiments

Plants were grown in 2-L pots filled with different types of soil according to each experiment. Three to 5 seeds were sown in each pot. At this time, 1-ml inoculum suspensions containing about 5×10^7 colony forming units (CFU)$\cdot ml^{-1}$ were added to the soil. Alternatively, 1-ml phosphate buffer was added to the non-inoculated controls. Seedlings were thinned out to 1 per pot a week after emergence. All experiments were conducted using a completely randomized design, with the number of replicates varying within the different experiments. Dry weight parameters were determined after drying the plant material at 70 °C for 72 h, in an air-forced oven.

2.2.1. Experiment 1 (Al-Quds University, November 1998–April 1999)

Cicer arietinum cv. Bulgarian (Hazera Co., Haifa, IL) seeds were sown in pots filled with a mixture (1:1) of washed sand and Rhodoxerafs, Terra Rossa soil, typical of the region. The pots were watered twice a week with 200-ml water. The temperature inside the greenhouse ranged between 15 and 25 °C. The plants were naturally nodulated by native rhizobia from the local soil. Dry weight parameters were measured at 6 stages of growth: 41, 49, 56, 63, 71 and 82 days after sowing (d.a.s.). Each harvest time included 10 randomized replicates (plants) per treatment.

2.2.2. Experiment 2 (Kibbutz Kfar Menachem, December 1999–May 2000)

C. arietinum cv. Yarden (Hazera Co., Haifa, IL) seeds were sown in pots filled with local Chromoxererts, Brown Alluvial soil. In this experiment, the inoculation of *A. brasilense* was also assayed in combination with saline water irrigation. Treatments were irrigation with fresh (tap) or with underground saline water (EC, 4.8 $ds \cdot m^{-1}$; 714 $mg \cdot L^{-1}$ NaCl), with or without inoculation with *A. brasilense*. Plants received 200-ml water twice a week, until 60 d.a.s. Afterwards, the pots were irrigated with 500-ml water every two days. The temperature inside the greenhouse varied between 25 and 29 °C. The plants were nodulated with native rhizobia from the local soil. Five plants per treatment were harvested at each sampling (84 and 105 d.a.s.).

2.2.3. Experiment 3 (Al-Quds University, October–December 1999)

The experimental conditions were as in Experiment 1 but with *Vicia faba* L. (local cultivar). Eight plants per treatment were harvested at each sampling for root and shoot dry weight determination (45, 59 and 66 d.a.s.). In addition, shoot length measurements were taken during plant growth (8 replicates per treatment).

2.2.4. Experiment 4 (Kibbutz Kfar Menachem, February–May 2000)

The experimental conditions were the same as in Experiment 2 but with *V. faba* cv. Cyprus (M. Ben Shachar Ltd., Petach Tikva, IL) irrigated with tap water only. Six plants per treatment were sampled at each sampling (45 and 75 d.a.s.) for root and shoot dry weight determination.

2.3. Field experiments

Two field experiments were carried out with *C. arietinum* cv. Yarden. In both, *A. brasilense* was applied using a peat-based inoculant containing 1×10^9 CFU·g^{-1} peat. The peat carrier was resuspended with tap water previous to inoculation and added over the furrow, each plant receiving approximately 5×10^7 CFU. Non-inoculated controls were treated with the same amount of sterilized peat inoculant. The experiments consisted of a completely randomized design with 5 and 4 replicates (plots) per treatment in experiments 1 and 2, respectively. Each plot consisted of six 12-m long rows, with a sowing density of 20 seeds·m^{-1}. The two innermost rows of each plot were used to assess the yield parameters at the final harvest. Plant material for sampling during the experiments was taken from the two adjacent rows. Dry weight values were obtained in the same manner as for the greenhouse experiments.

2.3.1. Experiment 1 (Havat Misholim, November 1998–May 1999)

The Havat Misholim experimental site is located in the Lachish area (Northern Negev), and is characterized by a Chromoxererts, Brown Alluvial soil. Seeds were sown on November 17, 1998. At this time, the soil contained 15 and 17.5 mg·kg^{-1} soil N and P, respectively. The inoculation was carried out 3 d.a.s. The experimental field was irrigated periodically, with a total of 200-mm water. Precipitation during the growth period was less than 200 mm. The experiment was terminated 190 d.a.s.

2.3.2. Experiment 2 (Bane-Naem, January–June 1999)

Bane-Naem is located in the Hebron area on a Terra Rossa (Rhodoxeralfs) - Rendzina of the Mountains complex soil. The seeds were sown on January 12, 1999 and inoculated with *A. brasilense* over the furrow, immediately after sowing. The field was not irrigated during the growth period. Total precipitation during the experiment was about 120-mm. The experiment was terminated 150 d.a.s.

3. RESULTS

3.1. Greenhouse experiments

3.1.1. Experiment 1

In Experiment 1, a tendency of improved root and shoot development of chickpeas was observed following inoculation with *Azospirillum brasilense* (Fig. 1a, b). These positive effects were mainly observed at the later stages of plant development when statistically significant differences ($p = 0.05$) were also found between inoculated and non-inoculated plants. At the end of the experiment (82 d.a.s.), the inoculated plants showed an increase above controls in the order of 54 and 51% in shoot and root dry weight values, respectively.

3.1.2. Experiment 2

In a preliminary greenhouse experiment carried out in Kibbutz Kfar Menachem, it was found that chickpea plants inoculated with *A. brasilense* and irrigated with saline water did not differ in plant dry weight parameters from plants inoculated with the bacterium and irrigated with tap water. This suggested that inoculation with *A. brasilense* has the potential to prevent, or at least diminish, the damaging effects of salinity on growth of this legume.

An additional experiment (Experiment 2) was carried out in the same greenhouse, in order to evaluate the above hypothesis. In this experiment, the inoculated plants showed an enhanced nodule formation in comparison with non-inoculated controls (Tab. I). This increased nodulation by 39% was significant for plants

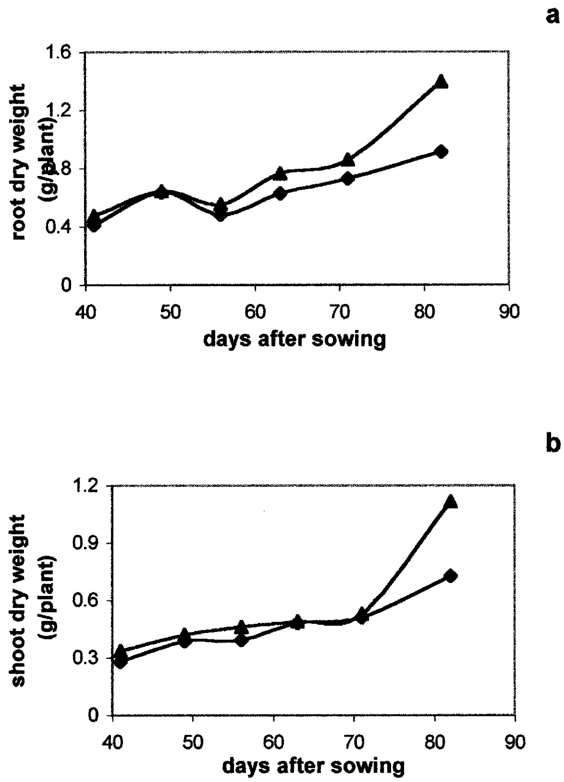

Figure 1. Effects of *Azospirillum brasilense* strain Cd on root (a) and shoot (b) dry weight of chickpeas (*Cicer arietinum* L. cv. Bulgarian) as measured at different stages of growth, in the greenhouse experiment 1 (Al-Quds University). Results represent averages from 8 plants per treatment at each harvest time. At the final harvest (82 days after sowing, d.a.s.), the differences between the treatments were significant ($p = 0.05$) for both parameters, according to Student t-Test. ▲, *A. brasilense*; ◆, control.

irrigated with normal (tap) water. This increased nodulation in inoculated plants could be partly explained by the fact that inoculation promoted earlier nodulation, as could be observed by the relatively higher nodulation in the upper part of the roots in comparison with the controls (Fig. 2).

Inoculation with *A. brasilense* also promoted chickpea plant growth as can be deduced from the differences in root and shoot dry weight obtained between inoculated and non-inoculated plants (Tab. I). At 84 d.a.s., most of the differences with fresh water irrigation were found to be statistically significant ($p = 0.05$) in favor of the inoculation treatment (Tab. I). Moreover, similarly to the preliminary experiment, *A. brasilense* was shown to prevent or diminish the negative effects caused by the salinity. The clear improvement in plant root development following inoculation with *A. brasilense*, under the two irrigation treatments, is illustrated in Figure 3.

One hundred and five d.a.s., a significant increase ($p = 0.05$) in shoot dry weight was observed in inoculated plants as compared with controls. No significant differences were observed between inoculated and non-inoculated roots (Tab. I), probably because at this stage, the roots had already reached their maximum development as allowed by the pot capacity.

3.1.3. Experiment 3

This experiment with faba beans showed the same trend of enhanced nodulation and plant growth as observed for chickpeas in the experiments described above. Inoculation with *A. brasilense* significantly ($p = 0.05$) increased nodule number and nodule dry weight as observed in the different sampling dates (Fig. 4a, b). For most samplings, the percentage of increase in nodule dry weight following inoculation with *A. brasilense* was higher than that observed in nodule

Table I. Effects of *Azospirillum brasilense* strain Cd on nodule, root and shoot dry weight (dw, g/plant) of chickpeas (*Cicer arietinum* L. cv. Yarden) irrigated with tap or saline water, as measured 84 and 105 days after sowing (d.a.s.) in the greenhouse experiment 2 (Kfar Menachem). Values represent averages of 5 replicates per treatment at each sampling. Different letters indicate significant differences ($p = 0.05$) between treatments in each irrigation category, according to Student t-Test.

Days after sowing		84		105	
Treatment	Nodule dw	Root dw	Shoot dw	Root dw	Shoot dw
Tap water					
A. brasilense	0.37 a	2.64 a	5.33 a	5.06 a	11.28 a
Control	0.26 b	1.49 b	4.98 a	5.57 a	8.67 b
Saline water					
A. brasilense	0.27 a	2.72 a	5.10 a	4.35 a	7.25 a
Control	0.24 a	2.29 b	4.15 a	4.31 a	6.32 b

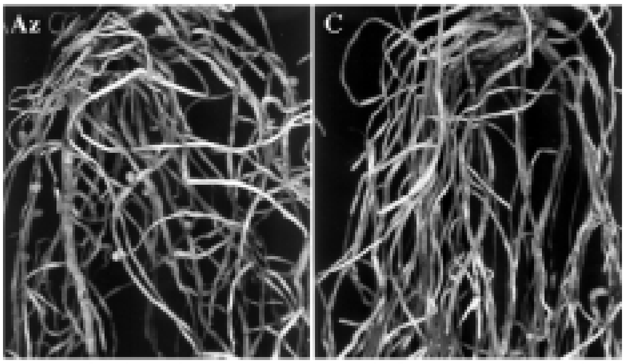

Figure 2. Effect of *A. brasilense* strain Cd on nodulation of chickpeas (*C. arietinum* L. cv. Yarden) irrigated with tap water, as observed 84 d.a.s., in the greenhouse experiment 2 (Kfar Menachem). Az, root inoculated with *Azospirillum*; C, non-inoculated control root.

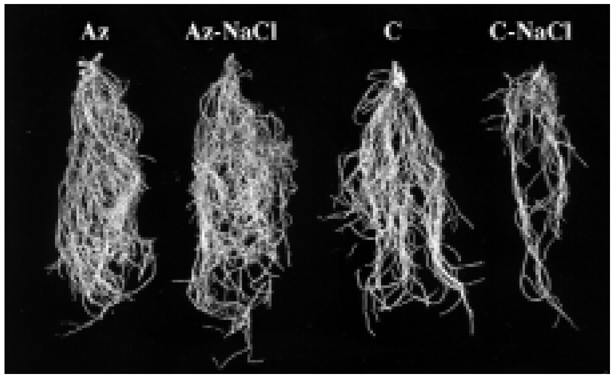

Figure 3. Effect of *A. brasilense* strain Cd on root morphology and development of chickpeas (*Cicer arietinum* L. cv. Yarden) 84 d.a.s., in the greenhouse experiment 2 (Kfar Menachem). Az, root inoculated with *Azospirillum* and irrigated with tap water; Az-NaCl, *Azospirillum*, saline water; C, non-inoculated, tap water; C-NaCl, non-inoculated, saline water.

number (for example, approximately 125% for the first, in contrast to 100% for the latter at the third sampling). This indicates that in addition to the enhanced nodulation, *A. brasilense* also increased the average weight of the individual nodules. It was also observed that the amount and the dry weight of upper nodules (those nodules formed in the upper 5 cm of the root crown) were significantly ($p = 0.05$) higher than in controls (not shown).

The positive effects of the inoculation on plant growth of faba beans were expressed in significant ($p = 0.05$) higher values of shoot length and root and shoot dry weight of this treatment as compared with non-inoculated controls in most of the sampling dates (Fig. 4c, d, e).

3.1.4. Experiment 4

In this additional experiment with faba beans, positive effects on plant growth were also observed following inoculation with *A. brasilense*. Forty five d.a.s., the average plant dry weight (roots plus shoots) of inoculated plants was 11.9 g, in contrast to 9.9 g for the non-inoculated controls. Seventy five d.a.s., the average shoot dry weights were 11.4 and 8.7 g per plant for inoculated and non-inoculated plants, respectively (significant difference at $p = 0.05$).

3.2. Field experiments

3.2.1. Experiment 1

In this field experiment carried out at the Havat Misholim experimental site, chickpea roots were very well nodulated by native rhizobia, with active pink well developed nodules. In *A. brasilense*-inoculated plots, nodules were mainly formed on the principal roots, whereas in non-inoculated controls, few smaller nodules were observed, mainly on the lateral roots.

The inoculated plants showed significantly ($p = 0.05$) higher root dry weight compared to controls (in the order of 47%) as observed from an initial sampling, 50 d.a.s. Similar results were obtained 150 d.a.s. No significant differences in shoot dry weight were observed at these stages between treatments (not shown).

The experiment was terminated 190 d.a.s., at which time a clear beneficial trend deriving from the inoculation treatment was observed for the assessed parameters (Tab. II). Although significant differences ($p = 0.05$) between treatments were obtained only for shoot dry weight, p-values close to 0.05 were found for the other parameters (for example, $p = 0.06$ for seed dry weight).

3.2.2. Experiment 2

In the field experiment carried out in Bane-Naem the benefits obtained from the inoculation treatment were demonstrated by following the development of the roots and the shoots along the season (not shown). The significant ($p = 0.05$) beneficial effects of *A. brasilense* on yield parameters as observed at the final harvest time are shown in Table III.

As previously indicated, the field was not irrigated during this experiment. As a consequence, and due to a very dry winter season during the experiment (total precipitation of about 120 mm), the chickpea yield was very

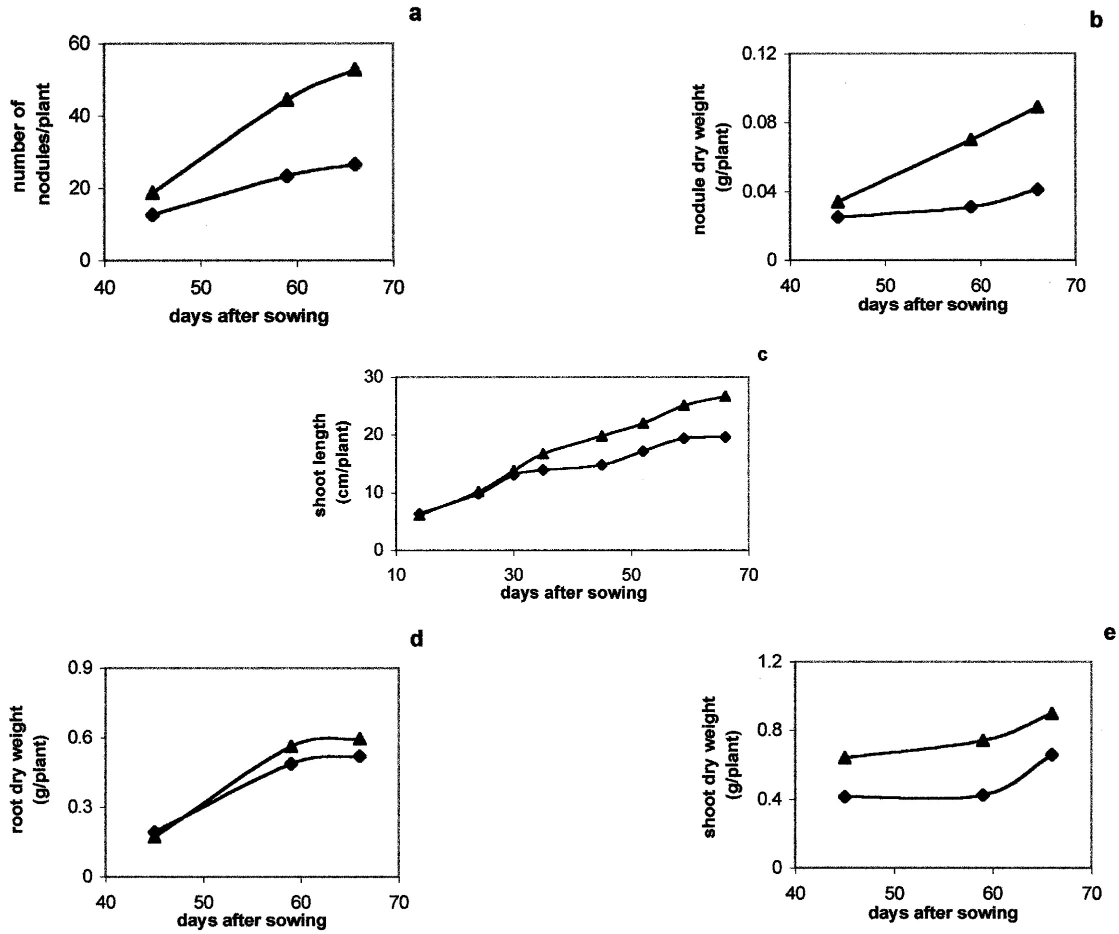

Figure 4. Effects of *A. brasilense* strain Cd on nodulation (a, b) and on plant growth parameters (c, d, e) of faba beans (*Vicia faba* L., local cultivar) at different sampling dates in the greenhouse experiment 3 (Al-Quds University). Significant differences ($p = 0.05$) between treatments according to Student t-Test were found in all sampling dates (45, 59 and 66 d.a.s.) for a, b and e; 35, 45, 52, 59 and 66 d.a.s. for c; and 59 and 66 d.a.s. for d. ▲, *A. brasilense*; ◆, control.

low. Nevertheless, plants inoculated with *A. brasilense* yielded about 4 times more than the non-inoculated controls.

4. DISCUSSION

Results of greenhouse and field experiments carried out in different soils indicate that inoculation with *Azospirillum brasilense* at an optimal cell concentration (about 5×10^7 CFU/plant) consistently improves root and shoot growth of chickpeas and faba beans often resulting in an increase in grain yield. These results confirm previous observations on the positive effects on plant growth and yield caused by azospirilla on legumes, naturally nodulated or coinoculated with *Rhizobium*. These include many species of cultivated legumes under a variety of soil and environmental conditions [5].

The positive effects of the inoculation treatment on chickpeas and faba beans could be explained by an enhancement of root branching and root growth as observed in most of the greenhouse- and field-grown plants at the earlier stages of plant development. These favorable effects on root growth are known to improve the efficiency of mineral and water uptake in inoculated plants [14, 19]. In addition to these, the consistent enhancement of nodulation observed in this work of the *A. brasilense*-inoculated plants may also play an important role in improving plant growth. Earlier nodulation,

Table II. Effect of inoculation with *A. brasilense* strain Cd on yield parameters of chickpeas (*C. arietinum* L. cv. Yarden) 190 d.a.s. (field experiment 1, Havat Misholim). Values represent averages of 5 replicates per treatment, each consisting of two 1 m-samples from the two innermost rows of each plot. Different letters indicate significant differences (p = 0.05) between treatments according to Student t-Test. dw, dry weight.

Treatment	Shoot dw ($g \cdot m^2$)	Pod dw ($g \cdot m^2$)	Pod number per m^2	Seed dw ($g \cdot m^2$)
A. brasilense	817 a	784 a	842 a	567 a
Control	615 b	669 a	752 a	462 a

increase in total nodule number and earlier onset of N_2-fixation have been reported for several legumes inoculated with *Azospirillum* [4, 5, 10, 15, 18, 20, 24, 25]. Similar effects to those caused by *Azospirillum* on the improvement of the legume-*Rhizobium* symbiosis were reported for other plant growth promoting rhizobacteria [6].

Results from a hydroponic system showed that inoculation with *A. brasilense* increased secretion of *nod*-gene inducing flavonoids by common bean roots [3] and alfalfa [23]. Moreover, in different legumes, *A. brasilense* was shown to promote the formation of higher amounts of root hairs, which are the sites for rhizobial infection [3, 11, 25]. These may explain, at least in part, the consistent enhancement of nodulation observed in legumes inoculated with *A. brasilense*.

Promotion of nodulation and N_2-fixation in legumes is of economic importance, particularly for chickpeas and faba beans. The greater number of active nodules following inoculation with *A. brasilense* is expected to contribute higher amounts of fixed nitrogen for higher yields under field conditions [2, 13 14]. It is well established that the potential of *A. brasilense* in enhancing plant growth and yields is markedly higher in sustainable agriculture when the positive effects of this bacterium are more evident than under optimal growth conditions. In the present work, the field experiment in Havat Misholim was carried out under almost optimal growth conditions, with a relatively high crop yield potential (about 5 ton seeds/ha). Under those conditions the benefits obtained from the inoculation treatment were less evident than in the experiment carried out in Bane-Naem, where the soil and the water conditions were substantially poorer.

The negative effects of salinity on plant growth, nodulation and nitrogenase activity of chickpeas are well established [21, 26]. In this study, inoculation of chickpeas with *A. brasilense* increased nodulation, root and shoot development under both tap and saline water irrigation. Although the dry weight of plants irrigated with saline water was similar to or smaller than those grown under normal conditions, the positive effects of *A. brasilense* were generally obtained in comparison to saline-treated non-inoculated plants. It is possible that the enhanced root branching and the improved root metabolism caused by the bacterium [14] are important factors in relieving the stress caused by salinity. This possibility is strengthened by recent observations in wheat, where inoculation with *Azospirillum* improved coleoptile growth in seedlings grown in darkness under osmotic and salt stresses [1, 7]. Inoculated seedlings were shown to have higher turgor than non-inoculated controls at low water potential under osmotic stress. The observed stimulated growth of the inoculated seedlings was accompanied by significant decreases in osmotic potential and relative water content at zero turgor, in volumetric cell wall modulus of elasticity, and in absolute symplastic water volume, and by a significant rise in apoplastic water fraction parameters. These results are consistent with a better water status in *Azospirillum*-inoculated wheat seedlings under water, osmotic and salt stress, where both effects on cell wall elasticity and/or apoplastic water were evident [8].

The observation on relief of salinity stress in chick peas, is potentially important for the Mediterranean region since, in many cases, high salinity water is the only available source for supplementing the water needs of winter legumes by irrigation, after the winter rains have stopped.

Table III. Effect of inoculation with *A. brasilense* strain Cd on yield parameters of chickpeas (*C. arietinum* L. cv. Bulgarian) 150 d.a.s. (field experiment 2, Bane-Naem). Values represent averages of 4 replicates per treatment, each replicate consisting of 0.5 m-samples harvested from the two innermost rows of each plot. Different letters indicate significant differences (p = 0.05) between treatments according to Student t-Test.

Treatment	Pod number per m^2	Pod dry weight ($g \cdot m^2$)	Seed number per m^2	Seed dry weight ($g \cdot m^2$)
A. brasilense	186 a	242 a	220 a	46 a
Control	48 b	60 b	46 b	9 b

Acknowledgements: We thank Prof. Jos Vanderleyden from the F.A. Janssens Laboratory of Genetics (Catholic University of Leuven, Belgium) for helpful discussions. This investigation was carried out in the framework of the "Belgian-Israeli-Palestinian Cooperation in Applied Research" project, supported by the Charles R. Bronfman (CRB) Foundation, and the "Palestine Consultancy Group" (PCG).

REFERENCES

[1] Alvarez M.I., Sueldo R.J., Barassi C.A., Effect of *Azospirillum* on coleoptille growth in wheat seedlings under water stress, Cereal Res. Comm. 24 (1996) 101–107.

[2] Bashan Y., Inoculants of plant growth-promoting bacteria for use in agriculture, Biotechnol. Adv. 16 (1998) 729–770.

[3] Burdman S., Volpin H., Kigel J., Kapulnik Y., Okon Y., Promotion of *nod*-gene inducers and nodulation in common bean (*Phaseolus vulgaris*) roots inoculated with *Azospirillum brasilense* Cd, Appl. Environ. Microbiol. 62 (1996) 3030–3033.

[4] Burdman S., Kigel J., Okon Y., Effects of *Azospirillum brasilense* on nodulation and growth of common bean (*Phaseolus vulgaris* L.), Soil Biol. Biochem. 29 (1997) 923–929.

[5] Burdman S., Vedder D., German M., Itzigsohn R., Kigel J., Jurkevitch E., Okon Y., Legume crop yield promotion by inoculation with *Azospirillum*, in: Elmerich C., Kondorosi A., Newton W.E. (Eds.), Biological Nitrogen Fixation for the 21st Century, Kluwer Academic Publishers, Dordrecht, 1998, pp. 609–612.

[6] Burdman S., Jurkevitch E., Okon Y., Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture, in: Subba Rao N.S., Dommergues Y.R. (Eds.), Microbial Interactions in Agriculture and Forestry, Vol. 2, Science Publishers, Enfield, 2000, pp. 227–248.

[7] Creus C.M., Sueldo R.J., Barassi C.A., Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses, Plant Physiol. Biochem. 35 (1996) 939–944.

[8] Creus C.M., Sueldo R.J., Barassi C.A., Water relations in *Azospirillum*-inoculated seedlings under osmotic stress, Can. J. Bot. 76 (1998) 238–244.

[9] Groppa M.D., Zawoznik M.S., Tomaro M.L., Effect of co-inoculation with *Bradyrhizobium japonicum* and *Azospirillum brasilense* on soybean plants, Eur. J. Soil Biol. 34 (1998) 75–80.

[10] Iruthayathas E.E., Gunasekaran S., Vlassak K., Effect of combined inoculation of *Azospirillum* and *Rhizobium* on nodulation and N_2-fixation of winged bean and soybean, Sci. Hortic. 20 (1983) 231–240.

[11] Itzigsohn R., Kapulnik Y., Okon Y., Dovrat A., Physiological and morphological aspects of interactions between *Rhizobium meliloti* and alfalfa (*Medicago sativa*) in association with *Azospirillum brasilense*, Can. J. Microbiol. 39 (1993) 610–615.

[12] Okon Y., Albrecht S.L., Burris R.H., Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants, Appl. Environ. Microbiol. 33 (1997) 85–88.

[13] Okon Y., Labandera-Gonzales C.A., Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation, Soil Biol. Biochem. 26 (1994) 1591–1601.

[14] Okon Y., Vanderleyden J., Root-associated *Azospirillum* species can stimulate plants, ASM News 63 (1997) 366–370.

[15] Plazinski J., Rolfe B.G., Influence of *Azospirillum* strains on the nodulation of clovers by *Rhizobium* strains, Appl. Environ. Microbiol. 49 (1985) 984–989.

[16] Rodelas B., Gonzales Lopez J., Martinez Toledo M.V., Pozo C., Salmeron V., Influence of *Rhizobium*/*Azotobacter* and *Rhizobium*/*Azospirillum* combined inoculation on mineral composition of faba bean (*Vicia faba* L.), Biol. Fertil. Soils 29 (1999) 165–169.

[17] Rodelas B., Gonzales Lopez J., Pozo C., Salmeron V., Martinez Toledo M.V., Response of faba bean (*Vicia faba* L.) to combined inoculation with *Azotobacter* and *Rhizobium leguminosarum* bv. viceae, Appl. Soil. Ecol. 12 (1999) 51–59.

[18] Sarig S., Kapulnik Y., Okon Y., Effect of *Azospirillum* inoculation on nitrogen fixation and growth of several winter legumes, Plant and Soil 90 (1986) 335–342.

[19] Sarig S., Blum A., Okon Y., Improvement of the water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*, J. Agric. Sci. 110 (1988) 271–277.

[20] Singh C.S., Subba Rao N.S., Associative effects of *Azospirillum brasilense* with *Rhizobium japonicum* on nodulation and yield of soybean (*Glycine max*), Plant and Soil 90 (1979) 335–342.

[21] Soussi M., Lluch C., Ocana A., Comparative study of nitrogen fixation and carbon metabolism in two chick-pea (*Cicer arietinum* L.) cultivars under salt stress, J. Exp. Bot. 50 (1999) 1701–1708.

[22] Tchebotar V.K., Kang U.G., Asis C.A., Akao S., The use of GUS-reporter gene to study the effect of *Azospirillum*-*Rhizobium* coinoculation on nodulation of white clover, Biol. Fertil. Soils 27 (1998) 349–352.

[23] Volpin H., Burdman S., Castro-Sowinski S., Kapulnik Y., Okon Y., Inoculation with *Azospirillum* increased exudation of rhizobial *nod*-gene inducers by alfalfa roots, Mol. Plant-Microbe Interact. 9 (1996) 388–394.

[24] Yahalom E., Okon Y., Dovrat A., Early nodulation in legumes inoculated with *Azospirillum* and *Rhizobium*, Symbiosis 6 (1988) 69–80.

[25] Yahalom E., Dovrat A., Okon Y., Czosnek H., Effect of inoculation with *Azospirillum brasilense* strain Cd and *Rhizobium* on the morphology of burr medic (*Medicago polymorpha* L.), Isr. J. Bot. 40 (1991) 155–164.

[26] Zurayk R., Adlan M., Baalbaki R., Saxena M.C., Interactive effects of salinity and biological nitrogen fixation on chickpea (*Cicer arietinum* L.) growth, J. Agron. Crop Sci. 180 (1998) 249–258.