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Effect of soil organic matter on chickpea inoculated with *Azospirillum brasilense* and *Rhizobium leguminosarum* bv. *ciceri*

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Abstract

Azosprillum inoculated with *Rhizobium* improved the nodulation of chickpea-*Cicer arietinum*. This interaction was further enhanced by organic matter present in the growth medium.

Introduction

Scientific literature has reported already the positive effect that *Azospirillum* can have on *Rhizobium*-legumes symbioses. Sarig et al. (1986) reported a positive effect, under field condition, of *Azospirillum* inoculation on *Vicia sativa*, *Pisum sativum*, *Hedysarum coronarum* and *Cicer arietinum* spontaneously nodulated. Yahalom et al. (1987) reported the results obtained by an experiment carried out in in vitro conditions and concluded that a possible reason for increased susceptibility of the plant to *Rhizobium* infections, following *Azospirillum* application, may be due to the fact that it stimulates a formation of a larger number of epidermal cells that differentiate in infectable root hairs.

In our research we have focused on the effect of *Azospirillum* on chickpea-*Rhizobium* symbiosis and we have explored more in detail how soil organic matter can affect the interaction.

We have chosen chickpea for several reasons, among others because this plant respond very well to bacterial inoculation and because this system can be a good model of interaction, at root level, of plant and plant-growth promoting rhizobacteria (PGPR). Blocking nodulation (either increasing soil organic matter, or placing plants in a suboptimal light exposition), for instance, it is possible to study competition between *Azospirillum* and *Rhizobium* in the rhizosphere. In a previous paper (Del Gallo and Fabbri, 1990) we described the fact that *Azospirillum* can have a remarkable positive influence on chickpea-*Rhizobium* symbiosis, but we have also found that a strong competition between the two bacteria can be present at the root level, and that this competition can be affected by soil organic matter.

From field experiments previously conducted (unpublished results) we have observed that plants bred or selected to grow in an artificial environment (like our intensively exploited soils, continuously enriched by chemical fertilizers) respond to PGPR inoculation less than old variety or wild-type plants. This may be due to the fact that such a plant is pushed to utilize chemical fertilizers and to divert its energetics pool – carbon resources accumulated through the photosynthesis – to seed production instead of to the rhizosphere, to support a useless associated microflora. Moreover, in these particular conditions – such as a soil exploited for intensive agriculture – having all nutrients it needs concentrated, the plant develops a reduced root apparatus and has, thus, a reduced rhizosphere.

Chickpea, instead, is a plant generally cultivated in poor environments and has no need for large chemical fertilizers applications. Even recently bred cultivars are selected to grow in such a poor environment and association with its one

Rhizobium and, possibly, with an associated microflora.

Material and methods

The bacterial strains utilized were: *Azospirillum brasilense* Cd and *Rhizobium leguminosarum* bv. *ciceri* USDA 3379 (Nitragin 27A8), kindly supplied by Peter Van Berkum. *Cicer arietinum* cv Califfo (an italian winter cultivar) seeds were kindly supplied by F. Calcagno. 3-days-old sterile chickpea seedlings were put aseptically in sterile pots (four plants per pot) and were inoculated with single or mixed cultures of bacteria (about 1×10^5 Rhizobia and 1×10^6 Azospirilla per plant) grown at the late exponential phase.

The soil utilized was a mixture of sand and agroperlite (2:1) enriched (or not) with peat in order to have 0, 8% or 22% of organic matter (o.m., Walkley–Black). In both 8% and 22% o.m. C/N ratio was 28. Nitrogen content was measured by Kjeldhal. Plants were grown in a Heraeus Heraphyt 2000 growth chamber with 16 hrs daylight; temperature was kept at 30°C during the light-period and at 25°C during the dark-period; humidity was kept constantly at 60%. Plants were watered twice a week alternatively with a Jensen N-free solution (Gibson, 1980) and tap water.

Plants grown at 22% o.m. were not nodulated – possibly because of the high N content of the substrate, 0.44%.

Controls not inoculated or inoculated with *Azospirillum* alone were carried out only at 22% o.m. level. At 0 and 8% o.m. plants without *Rhizobium*, or inoculated only with Azospirillum, did not survive up to seed supply (about 20 days). We inoculate Rhizobium either together with Azospirillum, when the seedling was planted in the pot, or 66 hours later. Twenty plants were considered for each treatment.

Data were elaborated statistically by Duncan's Multiple Range Test. In each group of data, different letter shows data statistically different from the control (a) for $p = 0.01^{**}$ or for $p = 0.05^{*}$.

Results and discussion

The results obtained from the three experiments showed a positive effect of *Azospirillum* inoculation on *Rhizobium*-chickpea symbiosis.

Azospirillum effect on roots and shoots

As described before (Del Gallo and Fabbri, 1990) the positive effect of *Azospirillum* inoculation was quite pronounced in the all experiments carried out, even more pronounced in plant inoculated with *Azospirillum* and later on with *Rhizobium* (Tables 1 and 2 and Figures 1 and 2).

Plants inoculated with both *Azospirillum* and *Rhizobium* had 2-fold dry weight with respect to plants inoculated with *Rhizobium* alone. This effect was more stressed at 22% o.m. level, when nodulation was inhibited: *Rhizobium* alone showed a slight positive effect on the plant, while *Azospirillum* increased 6-fold shoot dry weigh above controls. When the latter was inoculated together with *Rhizobium*, instead, its effect seemed to be inhibited, probably because of competition problems on root surface. However, the high level of organic matter by itself stimulated plant growth and precociousness. Plants grown at 22% o.m. were in a more advanced growth stage (at 52 days they were filling already

Table 1. Root dry weight. Root d.w. at 62 days, 22% o.m. was not determined because plants had already finished their life cycle.

				Organic matter						
	Sand			8%			22%			
	R	R + A	R + A(66)	R	R + A	R + A(66)	C	R	A	R + A
42 days	0.39 a	0.50 b*	0.53 b*	0.49 a	0.66 b*	0.80 c*	2.02 a	1.96 a	2.87 b*	2.17 ab
52 days	0.46 a	0.51 b*	0.61 c*	0.52 a	0.70 b*	0.80 c*	0.60 a	1.40 b*	4.75 c**	1.76 b*
62 days	0.52 a	0.63 b*	0.88 c*	0.68 a	0.99 b**	1.12 b**	n.d.	n.d.	n.d.	n.d.

In each group of data, different letter shows data statistically different from the control (a) for $p = 0.01^{**}$ or for $p = 0.05^{*}$.

Table 2. Shoot dry weight. Shoot d.w. at 62 days, 22% o.m. was not determined because plants had already finished their life cycle.

	Organic matter									
	Sand			8%			22%			
	R	R + A	R + A(66)	R	R + A	R + A(66)	C	R	A	R + A
42 days	0.44 a	0.60ab**	0.70 b**	0.65 a	0.76 b*	0.85 c*	2.54 a	2.82 a	3.55 b*	3.06 ab
52 days	0.60 a	0.70 b*	0.82 c*	0.83 a	0.89 ab	1.01 b*	1.00 a	1.50 a	5.85 c**	2.11 b*
62 days	0.81 a	1.00 b*	1.01 b*	1.39 a	2.13 b*	1.85 b*	n.d.	n.d.	n.d.	n.d.

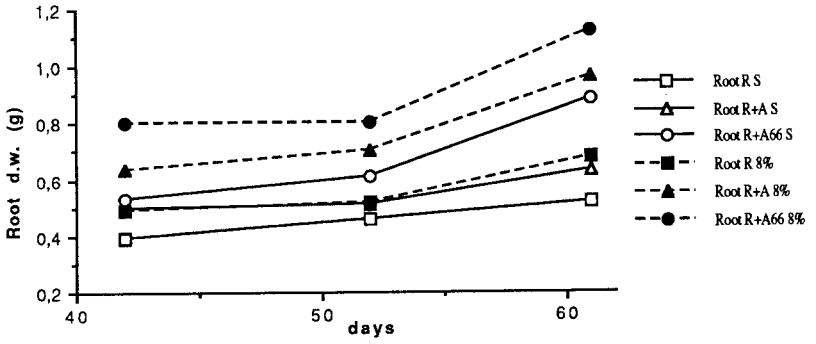

Fig. 1 Root dry weight of plant grown with 0 and with 8% organic matter. Statistic analysis of data is reported in table 1. R = rhizobium, A = azospirillum, R + A 66 = rhizobium was inoculated 66 h later than azospirillum, S = sand.

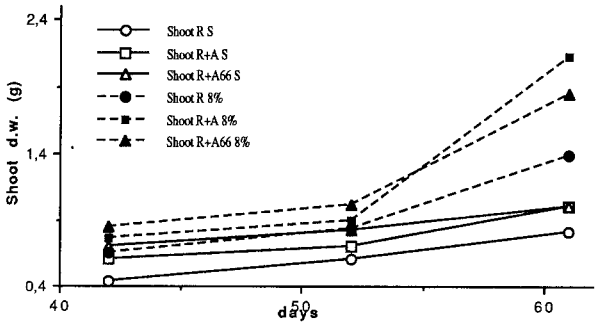

Fig. 2. Shoot dry weight of plant grown with 0 and with 8% of organic matter. Statistic analysis of differences are in Table 2.

pods, while plants grown at 0 or 8% o.m. did not start flowering yet) than plants grown at 0 and 8% o.m., so that a comparison between the two groups is impossible.

Effect on nodule formation

As already described by Sarig et al. (1986) and Yahalom et al. (1987) *Azospirillum* affected chickpea nodulation both by anticipating nodule formation and increasing nodule number, size and dry weight (Tables 3 and 4, Fig. 3).

Table 3. Number of nodules per plant

	Sand			8% Organic matter		
	Rhiz.	Rhi + Azo	R + A (66)	Rhiz.	Rhi + Azo	R + A (66)
42 days	6.00 a	16.25 b*	10.00 b*	16.75 a	27.66 b**	27.08 b**
52 days	11.01 a	15.37 a	14.00 a	19.81 a	17.94 a	20.19 a
62 days	15.00 a	21.00 a	18.00 a	15.44 a	26.37 b*	23.13 b*

Table 4. Nodules dry weight, g per plant

	Sand			8% Organic matter		
	Rhiz.	Rhi + Azo	R + A (66)	Rhiz.	Rhi + Azo	R + A (66)
42 days	0.010 a	0.030 b**	0.049 c**	0.031 a	0.042 b*	0.076 c*b**
52 days	0.028 a	0.050 b*	0.070 c*b**	0.032 a	0.051b*	0.089 c*b**
62 days	0.030 a	0.060 b**	0.088 c**	0.057 a	0.168 b**	0.189 b**

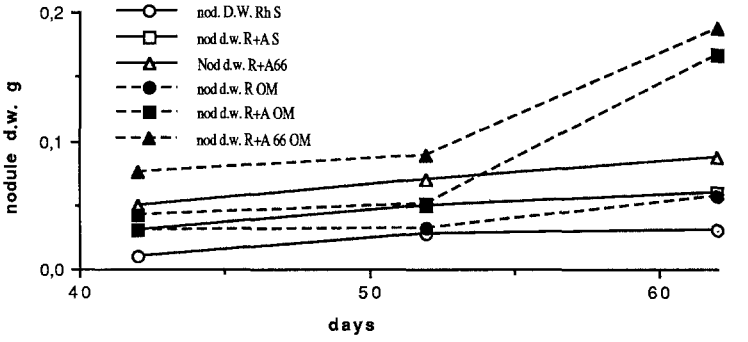

Fig. 3. Nodules dry weight.

Effect on acetylene reduction activity

Results are shown in Figure 4. *Azospirillum* effect on acetylene reduction activity per plant was more accentuated in sand-grown plant, at 42 and 52 days. At the end of the experiment, instead, the activity decreased and reached the same value of plants inoculated with *Rhizobium* alone. At 0 and 8% o.m., *Azospirillum* effect was more pronounced, particularly when it was inoculated before *Rhizobium*. These values, however were similar, at the end of the experiment.

Nitrogenase specific activity

The nitrogenase specific activity values are shown in Figure 5. Soil organic matter seemed to affect only *Azospirillum* inoculation, *Rhizobium*, instead, seemed not to be influenced at all: both groups of plant grown at 0 and at 8% o.m. showed the same pattern.

Rhizobium-inoculation 66 hours later than *Azospirillum* seemed not to affect nitrogenase activity in a different way than both bacteria inoculated together, but this was only on sand. When o.m. was present in the substrate, instead,

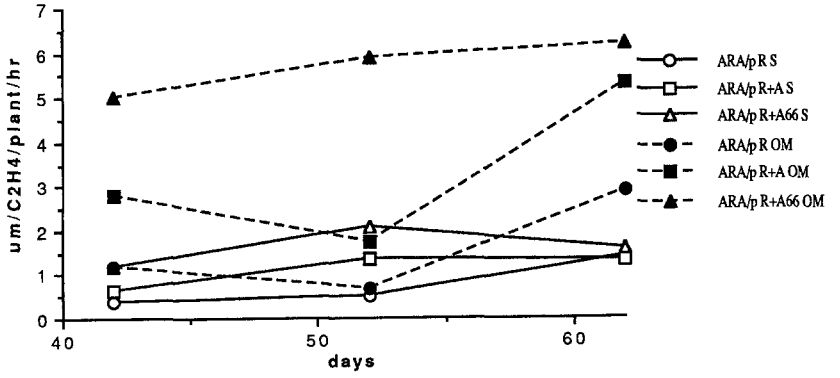

Fig. 4. Acetylene reduction activity/plant/h. All data were significantly different from the control (*Rhizobium* alone) except for plant inoculated with *Azospirillum* and *Rhizobium* together, without o.m. in sand, at 52 and at 62 days.

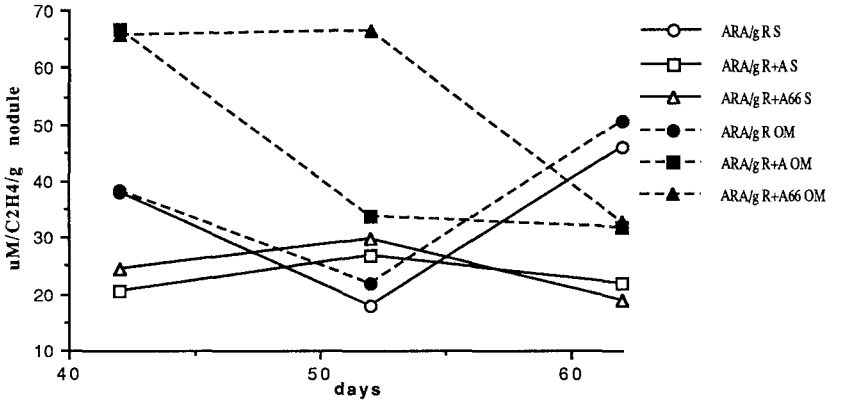

Fig. 5. Nitrogenase specific activity. Data were statistically different from controls (*Rhizobium* alone) only at o.m. 8%, in 42 and 52 days-old plants.

a pronounced effect was present until 52 days; 10 days later this difference disappeared. When both bacteria were inoculated together results showed a similar peak of nitrogenase specific activity, but for a shorter period. This may be due to the fact that the former group of plants was in a more advanced growth stage, and many senescent inactive nodules were present.

Conclusions

In conclusion, *Azospirillum* inoculation, and PGPR in general (Burns et al., 1981; Grimes and Mount, 1984), can have a remarkable influence on Rhizobium-legumes symbiosis. Burns et al. (1981) reported that nodulation enhancement by *Azotobacter vinelandii* was probably caused by a non-excretable protein produced by this bacterium. Sarig et al. (1986) hypothesized that, following Azospirillum inoculation, more root hairs become susceptible to rhizobial infection.

However, it is difficult to explain, according to these hypotheses, the effect of Azospirillum on nitrogenase specific activity. An indirect effect of hydrogen recycling by Azospirillum hydrogenase, for instance, or a nitrogen fixation direct contribution inside nodules by this bacterium, can be present.

Further studies, however, are necessary to clarify the interaction.

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