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# Inoculation of *Azospirillum brasilense* Cd on Chick Pea (*Cicer arietinum*)*

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**Abstract**

The response to *Azospirillum brasilense* Cd inoculation, in greenhouse conditions, of an italian chick pea cultivar "Calia" has been studied. The results show a statistically significative positive effect of the inoculation on plant root and shoot compared to the uninoculated controls or to the *Rhizobium*-inoculated (but not nodulated) plants.

## Introduction

Nitrogen-fixing bacteria of the genus *Azospirillum* live associated with roots of *Gramineae* and other plants utilized in agriculture. Field experiments conducted in many countries by different research groups (Smith et al., 1976; Reynders and Vlassak, 1982, Fallik et al., 1988, Del Gallo et al., 1989) demonstrated inequivocally that *Azospirillum* inoculation increases crop yield. Okon and Kapulnik (1986) have shown that inoculation of wheat, sorghum and corn with an inoculum size of $10^6$ to $10^7$ *Azospirillum*-cells per plant has a marked effect on root tip morphology, proliferation of root hairs, root surface area, root branching and on the general development of root system.

Even though Azospirilla can express high nitrogenase activity in laboratory conditions, nitrogen contribution to plant is still controversial – or it seems to be very low – and the increase repeatedly observed in growth and yield of inoculated plant seems to be induced by plant growth promoting substances produced by the bacterium, rather than by a nitrogen contribution (Okon and Kapulnik, 1986). Actually, since 1979 (Tien, T.M. et al.), it has been observed that *Azospirillum brasilense* produces in pure cultures large amount of auxines, and, in a lower extent, gibberellins and some cytokinin-like substances.

*Reviewed

According to several data reported on literature *Azospirillum* seems to interact positively with the symbiosis *Rhizobium*-Legumes (Sarig et al., 1986; Yalom et al., 1987; Plazinski and Rolfe, 1985).

We have studied the response to *Azospirillum brasilense* Cd inoculation, in greenhouse conditions, on an italian cultivar of chick pea (*Cicer arietinum*) "Calia" not inoculated and inoculated with *Rhizobium leguminosarum* by *ciceri* Na 936.

## Materials and Methods

In a first experiment, 3-day-old sterile chick-pea seedlings were put aseptically in sterile pots (four plants per pot) and were inoculated with single and mixed cultures of bacteria grown at the late exponential phase; the soil utilized was a mixture of peat and sand (2:1 weight) containing 0.44% of nitrogen (Kjeldhal) and 12.34% carbon (Walkley-Black); C/N 28. Plants were grown in a greenhouse.

In a second experiment, plants were aseptically grown in Leonard jar (two plants per jar), without nitrogen, in an agroperlite support imbibed with Jensen solution (Bergersen, 1980). *Rhizobium* inoculum was added either at the same time with *Azospirillum*, or 66 hours later.

Plants were collected after 33 days and 42 in the first experiment and after 33 days in the second; shoot and root dry weight and root length were measured.

For each treatment – uninoculated control, *A. brasilense* alone, *R. leguminosarum* alone, *A. brasilense* and *R. leguminosarum* mixed inoculum – 20 plants were considered for the final analyses.

Data were analyzed using the Duncan's Multiple Range Test in both experiments.

## Results and Discussion

The results obtained from both experiments show a statistically significative positive effect of *Azospirillum* inoculation on root and shoot of chick pea, compared to the uninoculated controls (Fig. 1 and 2). The differences are even more accentuated in a stress situation like a Leonard jar cultivation.

In both experiments, actually, inoculated plants did not nodulate – except for a few inactive nodules- and acethylene-reduction test revealed very low or inexistent activity. This may be due to combined nitrogen present in the soil in the first experiment, or, in the second experiment, to the difficulty, already observed, of the chick pea cultivar utilized, to nodulate in in vitro conditions (Rita Di Bonito, personal communication).

In the first experiment *Azospirillum* effect was quite marked, particularly after 42 days from sowing, when control plants are more stressed. However, plants did not seem to miss nodulation – except for controls. This is probably due to the presence of assimilable nitrogen mentioned before.

In the second experiment the effect of *Azospirillum* is even more evident, in

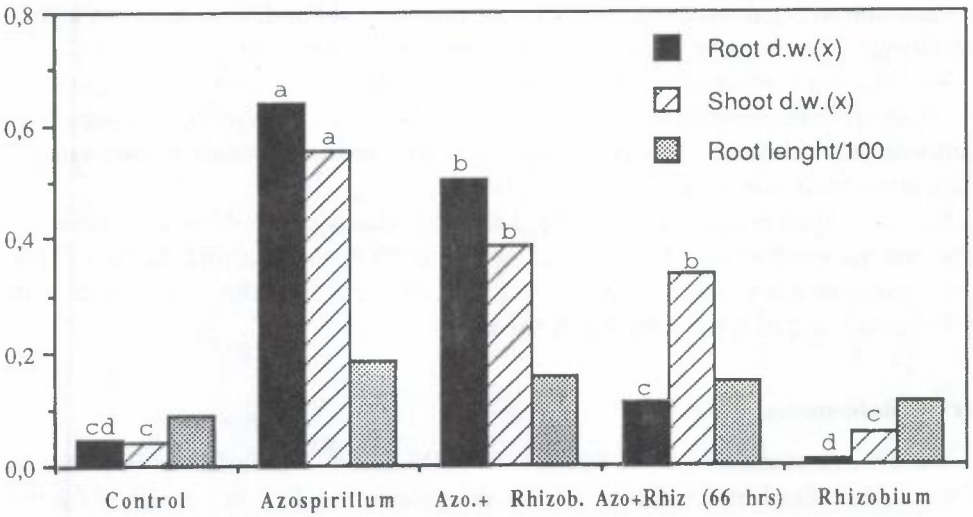


Figure 1. Root and shoot dry weight of inoculated and non-inoculated plants grown in a sand-peat soil (nitrogen content by Kjeldhal, 0.44%, organic matter by Walkley-Black 21.22) after 33 and 42 days from germination. Columns of the same color marked by the same letter (in this and in the following figure) do not differ significantly at $P = 0.05$ using Duncan's multiple range test.

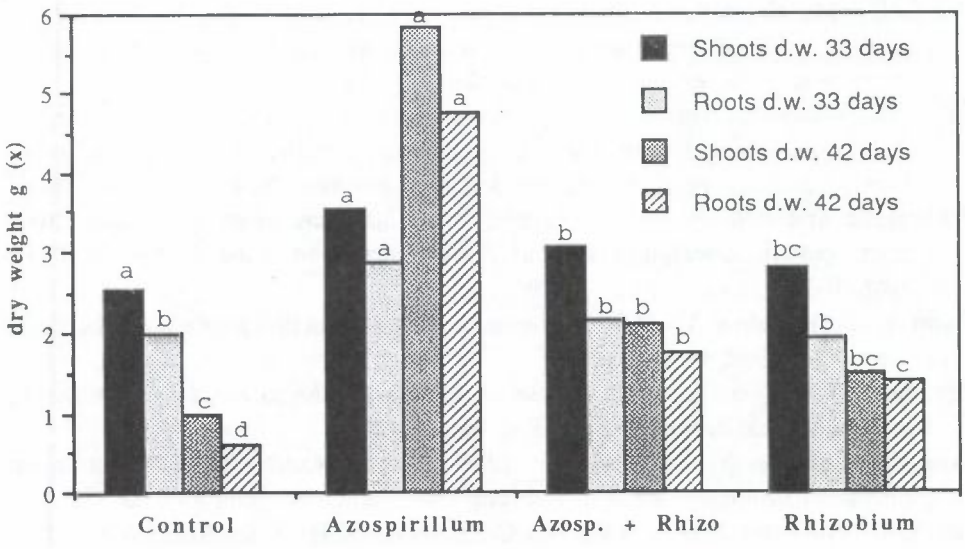


Figure 2. Root length ($cm \times 10^{-2}$) and dry weight, shoot fresh and dry weight (g) of inoculated plants and controls harvested after 3 days. Plant were grown in Leonard jar, in mineral Jensen solution without nitrogen.

comparison with uninoculated controls, particularly on shoot development, while root development seems to be inhibited by the presence of *Rhizobium*.

The inhibition effect of *Rhizobium* (non-nodulating, in our case) on *Azospirillum*, observed in both experiments, can be due to a *Rhizobium*-saturation of *Azospirillum* adhesion-sites. However, this effect is present also when *Rhizobium* is inoculated 3 days after *Azospirillum*.

Further experiments are necessary to better clarify the interactions occuring between *Azospirillum* – and, in general, plant growth promoting rhizobacteria – and the symbiosis *Rhizobium*-legumes concerning, in particular, the relationship with different amount of organic matter in the soil.

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