



(RESEARCH ARTICLE)



Effect of *Azospirillum brasilense* on garlic (*Allium sativum* L.) cultivation

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Abstract

The aim of the study was to study how *Azospirillum brasilense* influences the quality and growth of garlic cv "Bianco Polesano". An experiment was carried out with 2 treatments: 1) soil inoculated with *Azospirillum brasilense* (ALO1 strain of the company AZ BEL) and fertilized; 2) soil without *Azospirillum*, irrigated with water and fertilizer (control). The test showed a significant increase in the agronomic parameters analysed in plants treated with *Azospirillum*. In fact, all garlic bulbs in which the alpha proteobacterium has been inoculated have shown a significant increase in the weight of the bulbs, the diameter of the bulbs and the weight of the roots. This alpha proteobacterium can also have a positive effect on the solubilization and absorption of minerals present in the soil, especially iron, promoting plant growth, improving product quality, stimulating the hormones GA and IAA and the synthesis of vitamins such as B1, B3, B5. Furthermore, plants treated with *Azospirillum brasilense* are more resistant to biotic and abiotic stresses of various kinds.

Keywords: Bulb quality; Rhizobacteria; Horticulture; Vegetable plants; Biofertilizer; PGPB; Alpha proteobacterium

1. Introduction

Azospirillum is a kind of bacteria that have the ability to colonize both the rhizosphere and the intercellular spaces of the root. Represents one of the bacteria plant growth promoters (PGPB) most studied, with its 19 described species. The main known species are *A. brasilense*, *A. lipoferum*, *A. halopraeferens* and *A. oryzae*, which are widely used as biostimulants and bio-fertilizers. *Azospirillum* is associated with several plant species, more than 113 and 35 botanical families and can be considered a genus that easily enters into symbiosis with plant species [1-2].

It has the ability to promote plant growth through different mechanisms: biosynthesis and release of amino acids, indoleacetic acid, cytokinins, gibberellins and other polyamines, promoting root growth and, consequently, increasing the absorption of water and nutrients by plants [3].

Azospirillum also has the ability to fix atmospheric nitrogen (N₂) through the process of biological nitrogen fixation and can therefore contribute directly to making N available to non-leguminous species. The use of plant growth promoting bacteria (PGPB) can be an alternative to reduce the use of chemical fertilizers [4].

The micro-organisms associated with PGPB are distinguished by their ability to provide nutrients and hormones directly and/or indirectly through biological processes and cycles [5]. In this context, the species *Azospirillum brasilense* has demonstrated agronomic relevance as it has a number of benefits in order to: significantly influence the biological capacity of nitrogen fixation [6]; increase the activity of nitrates reductase; promote the production of hormones such as auxins (promotes the development of roots), gibberellins; promote the solubility of phosphates; encourage beneficial mycorrhizal associations of plants and may indirectly act on the growth of fungi or phytopathogenic bacteria, protecting the plant [7-8]. Although the use of the *Azospirillum brasilense* is a promising

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alternative studied by different authors [9-12], the studies on its use are very limited on the cultivation of garlic. The aim of this work was to evaluate the effects of *Azospirillum brasilense* inoculation on bulb growth and garlic production (*Allium sativum* L.).

2. Material and methods

2.1. Greenhouse experiment and growing conditions

The experiments began in early October 2018 (mean temperature 18.1 °C), were carried out in experimental greenhouses of the CREA-OF of Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E) on bulbs of onion (cv "Bianco Polesano"). The bulbs were placed in pots ø 14 cm; 60 bulbs for thesis divided into replicas of 20 bulbs each, for all types of garlic.

All bulbs were fed with the same amount of nutrients supplied through controlled release fertilizer (5 kg m⁻³ of Osmocote® 6 months containing 190 g kg⁻¹ N, 39 g kg⁻¹ P, 83 g kg⁻¹ K) blended with the growing medium before transplant.

The 2 experimental groups in cultivation were:

Group without *Azospirillum brasilense* (CTRL), with only water.

Group with *Azospirillum brasilense* strain ALO1 (AZ BEL biostimulant) in dilution 5:100 (1 L of 5:100 dilution inoculum was used with *Azospirillum brasilense* for every 10L of peat, same proportion for the control thesis where only water was used);

The lighting of the greenhouse at the plant level was about 12,000 lux with high pressure sodium lamps. The plants were lit for 16 hours a day. On the 22th of June, bulbs weight, bulb diameter and roots weight were recorded.

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

3.1. Plant growth

The test showed a significant increase in the agronomic parameters analysed in plants treated with *Azospirillum brasilense*. In fact, garlic bulbs treated with this alpha-protobacterium, have shown a significant increase in bulb weight, bulb diameter and root weight.

In fact, the diameter of the bulb was 26.97 mm in the thesis with *Azospirillum*, against 20.75 mm of the control (Fig.1A), 22.51 g in the thesis treated, against 11.71 g of the control with respect to the weight of the bulb (Fig.1B and Fig.2). There is also an increase in root weight, 35.78 g in the thesis treated with *Azospirillum brasilense* compared to 22.36 g in the control (Fig. 1C and Fig. 3).

The test also showed that the garlic bulbs treated with AZ BEL were growing faster, that they were more resistant to water stress and that the secondary hairs of the roots grew more quickly.

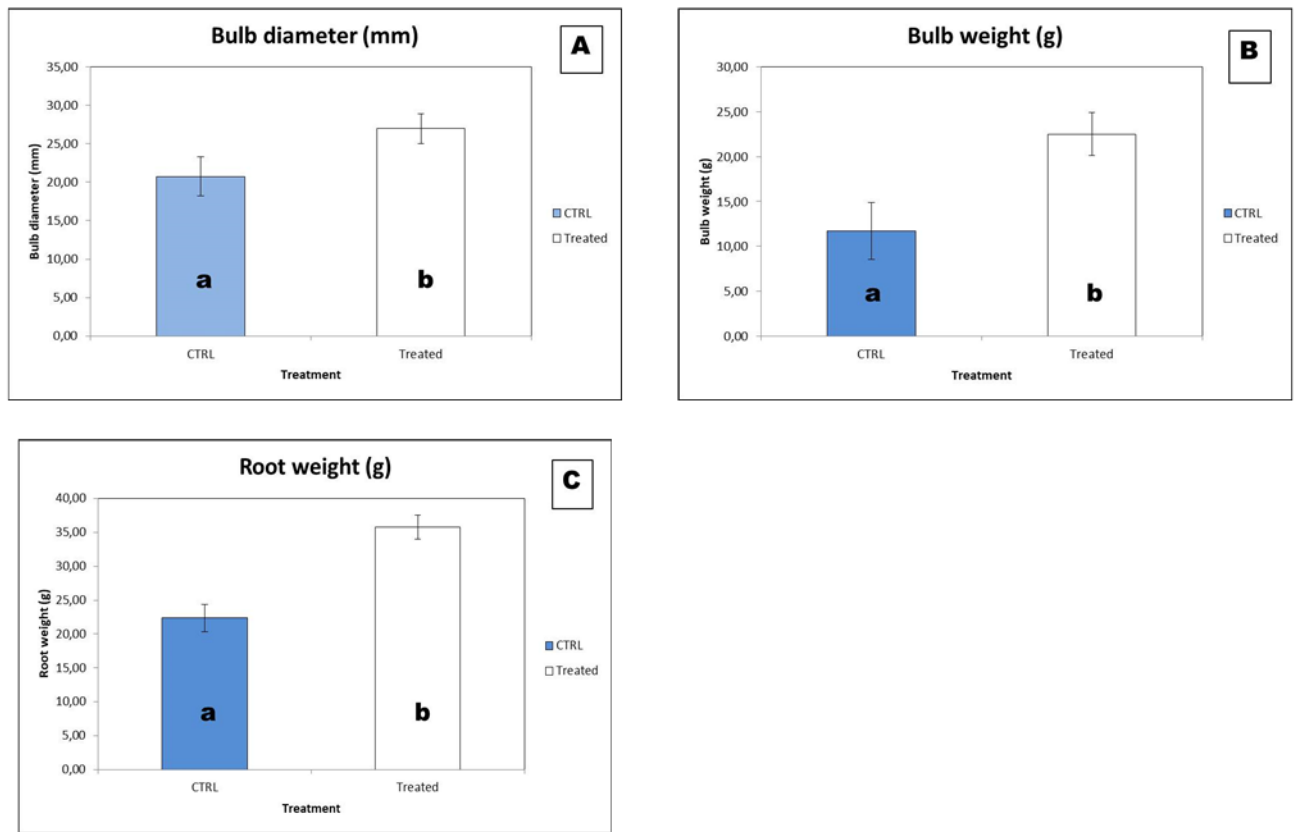


Figure 1 Effect of *Azospirillum brasilense* on the growth of garlic bulbs. Each value reported in the graph is the mean of three replicates \pm standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test ($P = 0.05$).

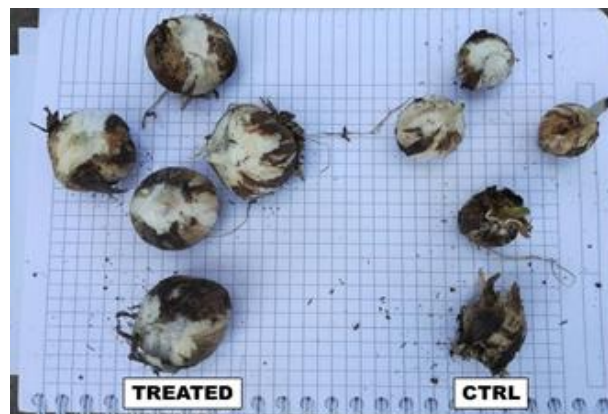


Figure 2 Effect of *Azospirillum brasilense* on growth of bulbs, treated (left), control (right)



Figure 3 Effect of *Azospirillum brasilense* on roots growth, treated (right), control (left)

4. Discussion

There are divergent studies on the efficacy of use of *A. brasilense*, some studies have positive results, others have no significance. The differences can probably be due to different growing conditions, as indicated by Novakowski et al. (2011) [13]. Another factor that can contribute to different inoculation responses is the hybrid used, because some hybrids are more sensitive to inoculation than others. Revolti (2014) [14] and Pereira et al. (2015) [15], studied the influence of genotypes on the inoculation of *A. brasilense* and concluded that the response to inoculation varies according to the genotypes used, that is, hybrids influence the response to inoculation. Researchers such as Bárbaro et al. (2008) [16] indicate the possibility of selecting strains suitable for local soil and climate conditions, as well as the crops and their cultivars used in each region [17-20]

In this test, garlic bulbs treated with *Azospirillum brasilense* showed a significant increase in diameter, bulb weight and root weight. Evidence has shown that this alpha-proteobacterium stimulates garlic bulbs already at the time of transplantation ensuring a higher water supply and an increase in the solubilization of minerals present in the substrate and in the soil [21-22], in particular iron/phosphorus and the synthesis of hormones such as GA and IAA, influencing many processes beneficial for the plant: in particular a greater resistance to biotic and abiotic stress (salt and water stress) and a better preservation of the product, with a higher content of vitamins B1, B3, B5. The results also show a faster growth of garlic bulbs treated with *Azospirillum* and an increase in the growth of secondary root hairs, which probably increase the efficiency of the absorption of water and nutrients.

5. Conclusion

The test has shown that the use of *Azospirillum brasilense* can improve the quality of garlic bulbs cv "Bianco Polesano", in particular by significantly increasing the diameter, weight of the bulbs and the weight of the roots. This alpha proteobacterium can also have a positive effect on the solubilization and absorption of minerals present in the soil, especially iron, promoting plant growth, improving product quality, stimulating the hormones GA and IAA and the synthesis of vitamins such as B1, B3, B5. Furthermore, plants treated with *Azospirillum brasilense* are more resistant to biotic and abiotic stresses of various kinds.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Disclosure of conflict of interest none to declare.

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