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Inoculation of *Arthrobacter* sp. improves the growth of *Acanthocalycium* sp. and the fruits nutraceutical quality and the flowers longevity

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Abstract

Research objective: The aim of this work is on the inoculation in the growing medium of *Arthrobacter* sp. and its effects on the growth, flower quality and longevity of cactus plants. To this end, the study of biometric parameters, i.e. plants and flowers (height, weight, number and duration), as well as root growth (weight), and the presence of disease were quantified as measures of plant productivity and compared with those obtained from non-inoculated plants. The use of alginate microspheres as a means of bacterial inoculation was also considered.

Materials and Methods: Seedlings of *Acanthocalycium* (2 years old) *Ferrarii*, *A. Glaucum* and *A. Violaceum* were immediately planted in pots after purchase, in substrate containing (sand 20%, pumice 20% and peat 60%; pH 7; electrical conductivity 0.7 dS/m; total porosity 80% (v/v). A randomised complete block design was applied for the experiments: the pots were randomly divided into two series, one series was inoculated with the bacteria and the other non-inoculated was used as a control. Each month, plant growth was assessed according to (1) plant height, (2) vegetative and root weight, (3) number of flowers per plant (4) flower duration. In addition, the occurrence of possible diseases, in particular *Fusarium* sp. and *Verticillium* sp., was assessed. Total flavonoids, total phenols and antioxidant activity were also analysed.

Results and Discussion: The experiment showed that the use of *Arthrobacter* sp. can indeed significantly improve the vegetative and root growth of *Acanthocalycium* sp. cacti. In addition, there was a significant improvement in the number and floral longevity as well as in the phenol, flavonoid and antioxidant content of the fruits of the inoculated plants compared to the untreated control. The experiment also showed that mortality due to the pathogens *Fusarium* sp. and *Verticillium* sp. was significantly reduced in the treated plant. In agreement with these authors, we found that PGPB treatments increased agronomic parameters and improved *Fusarium* and *Verticillium* resistance. In general, the application of biostimulants can increase the synthesis of bioactive compounds in plants by increasing resistance to phytopathologies. Inoculation with *Arthrobacter* sp. led to a significant reduction in the number of dead plants with respect to the diseases analysed. As a result of the stimulating action demonstrated in *Acanthocalycium*, bioproducts suitable for nutraceutical purposes may be developed in the future. Therefore, new microbe-assisted technologies can help plants to resist stress conditions, improving their tolerance and productivity.

Conclusions: Due to its 'multifunctionality', the genus *Acanthocalycium* is considered as one of the species of the future, for ornamental use and medicinal aspects, and new results may help reveal its potential in the context of the bio-economy and circular economy. As natural resources and cultural practices are crucial in defining the quality of flowers when destined for food/nutraceutical applications, the inoculation of *Acanthocalycium* with *Arthrobacter* sp. can be envisaged to provide better plant growth under conditions of environmental stress, or as a soil fertiliser, but also to improve the synthesis of natural products used for therapeutic applications.

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Keywords: Microorganism; Sustainable applications; Plants; Rhizosphere; Plant-microbial interaction

1. Introduction

According to EU strategy, current agriculture is implemented through more sustainable cultivation practices, according to the Farm to Fork Strategy, which is at the heart of the European Green Deal. Many crop species have been shown to benefit from the use of Plant Growth Promoting Bacteria (PGPB) due to their positive effects on plant growth, yield quantity and quality, and adaptability to biotic and abiotic stresses [1]. Microorganisms applied to the soil can affect plants directly by producing (i.e., synthesizing plant growth regulators hormones, volatile organic compounds, trehalose, exopolysaccharides) or by regulating the metabolism of different molecules (e.g., by ACC deaminase activity or indirectly by pathogen antagonism or induced systemic resistance [2]. The positive effects of PGPB on different crops have been shown in several studies, prompting interest in such promising bacterial strains and their possible applications in agriculture [3].

Arthrobacter sp. is one of the most adaptable species for promoting plant growth. It was originally isolated from the leaf cavities of the fern *Azolla fliculoides* Lam., where the bacteria are also found in the sporocarps [4]. Among the other characterizations of the bacteria were the ability to synthesize polysaccharides and IAA, halotolerance and lack of ACC deaminase activity, and the ability to counteract salt stress in plants [5]. Additionally, *Actinobacteria* have been isolated from *Opuntia ficus indica*, revealing the components of distinct *Streptomyces* clades based on their molecular phylogeny [6]. These bacteria are quite distinct from other actinobacterial genera; they are thought to be important for drought-tolerant plants with potential improvement in plant growth under abiotic stress. In this study, we explored the potential compatibility of the use of *Arthrobacter* sp. in drought-tolerant crops, based on the underexploited plant-bacteria interactions in cactus species and promising results in other crops [7]. Although *Acanthocalycium* sp. is considered a hardy species, the implementation of modern cultivation practices improves productivity, quality and crop sustainability; especially in the organic farming system, the demand for plant biostimulants, biological fertilisers or biofertilisers has been increasing in recent years [8]. Therefore, this study aims to contribute to the discussion on the effectiveness of PGPB use in agriculture. More specifically, the aim of this work is on the inoculation in the growing medium of *Arthrobacter* sp. and its effects on the growth, flower quality and longevity of cactus plants. To this end, the study of biometric parameters, i.e. plants and flowers (height, weight, number and duration), as well as root growth (weight), and the presence of disease were quantified as measures of plant productivity and compared with those obtained from non-inoculated plants. The use of alginate microspheres as a means of bacterial inoculation was also considered.

Figure 1 Details of the plants used in the trial

2. Material and methods

2.1. Bacteria Inocula Preparation

An *Arthrobacter* sp. strain from the CREA Horticulture and Floriculture Center, Pescia (PT), was used in this experiment. A standard procedure was used to identify and compare *Arthrobacter* globiformis strain ATCC 8010 with the strain found in Azolla leaf cavities [26]. It was stored at -80 °C in glycerol solution. Bacteria were grown on tryptic soybean medium (TSB) (Sigma-Aldrich) at 30°C and refreshed once a week. The bacterial cultures (OD600=1.3) were encapsulated, formulated and applied as bioinoculants in alginate beads. A sterile Na+-alginate solution (6% w/v) was used to dilute the culture (1:2 v/v) to form microspheres. A sterile CaCl2 solution (0.2 M) was added to form microspheres. These microspheres were washed three times with milli-Q water and stored at room temperature. A resuspended microspheres in medium were used to prepare the inoculum. Thirty-six microspheres were divided into seven tubes each of 50 mL. Twenty mL of minimal culture medium (saline (0.9% NaCl) added with 2.5 g/L glucose) was added to the tubes. After 48 hours, the cultures (OD 600 nm =7× 10^6 CFU) were ready to be inoculated near the roots of the acclimatised plants.

2.2. Plant Materials and Phenological Evaluations

Seedlings of *Acanthocalycium* (2 years old) *Ferrarii*, *A. Glaucum* and *A. Violaceum* were immediately planted in pots after purchase, in substrate containing (sand 20%, pumice 20% and peat 60%; pH 7; electrical conductivity 0.7 dS/m; total porosity 80% (v/v).

The temperature at the time of transplanting was 20 °C and the RH 55%. The plants were acclimatised for 5 months and then inoculated as reported above. A randomised complete block design was applied for the experiments: the pots were randomly divided into two series, one series was inoculated with the bacteria and the other non-inoculated was used as a control. Each month, plant growth was assessed according to (1) plant height, (2) vegetative and root weight, (3) number of flowers per plant (4) flower duration. In addition, the occurrence of possible diseases, in particular *Fusarium* sp. and *Verticillium* sp., was assessed. Total flavonoids, total phenols and antioxidant activity were also analysed.

Phenological observations were conducted for 2 years. Plant samples (in summer and autumn) and flower samples (in summer) were collected from inoculated and non-inoculated plants. All samples were oven-dried at 60 °C for 72 hours and kept in dry conditions until analysis.

2.3. 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 and discussion

The experiment showed that the use of *Arthrobacter* sp. can indeed significantly improve the vegetative and root growth of *Acanthocalycium* sp. cacti (**Table 1** and **Figure 2**). In addition, there was a significant improvement in the number and floral longevity as well as in the phenol, flavonoid and antioxidant content of the fruits of the inoculated plants compared to the untreated control (**Table 1**). The experiment also showed that mortality due to the pathogens *Fusarium* sp. and *Verticillium* sp. was significantly reduced in the treated plant (**Table 2**).

PGPBs may be useful to improve plant tolerance to abiotic stresses as a result of climate change, soil salinization, and aridity. In addition to living in the symbiotic association of *Azolla-Anabaena*, this species is widely found in the soil [9,10]. This bacterium has proven to be an excellent microbial inoculum for *Acanthocalycium* sp. A further advantage of bacterial inoculums is the ability to use bioencapsulated microorganisms as inoculants [11-13]. Inoculating cactus growth with *Arthrobacter* sp. improved flower quality and flowering duration characteristics, and improved yield [14-16]. In fact, inoculated plants produced more flowers that lasted longer. According to these results, the extension of flowering periods has a positive effect on plant supply and marketing, which is especially appealing to domestic and international markets [17]. Recently, Morais et al. [5] reported that PGPB inoculation significantly accelerated strawberry crop maturity. *Pedobacter* strain inoculation led to an increase in fruit size, particularly length and shape, and total soluble solids content (°Brix) among the same authors [18-20]. In addition to strawberry, similar results have been reported for other crops, including raspberry, tomato, sugar, beet, and barley [21,22]. In agreement with these

authors, we found that PGPB treatments increased agronomic parameters and improved *Fusarium* and *Verticillium* resistance. In general, the application of biostimulants can increase the synthesis of bioactive compounds in plants by increasing resistance to phytopathologies [23-26]. Inoculation with *Arthrobacter* sp. led to a significant reduction in the number of dead plants with respect to the diseases analysed. As a result of the stimulating action demonstrated in *Acanthocalycium*, bioproducts suitable for nutraceutical purposes may be developed in the future. Therefore, new microbe-assisted technologies can help plants to resist stress conditions, improving their tolerance and productivity [27]. In addition to affecting plants' biochemical, physiological, molecular, and morphological characteristics, increased temperatures also decrease crop productivity [28]. The inoculation of heat-stressed soybean plants with thermotolerant *Bacillus cereus* improved biomass, chlorophyll content, fluorescence, and antioxidant capacity. By increasing nutrient availability, the application of PGP microbes in wheat, rice, maize and oilseed rape improved the stress response from this point of view [29]. During heat stress, photosynthesis rates in heat-tolerant potato genotypes were unaffected or increased slightly, whereas leaf sucrose accumulation was increased and starch accumulation was decreased. The application of PGPB offers an ecological approach to improve plant production and counteract the negative effects of abiotic stresses [30-32]. Soil application of *Arthrobacter* sp. on *Acanthocalycium* plants improved plant growth and flower production with statistically significant differences compared to the control. From these results, it can be stated that the application of *Arthrobacter* sp. on *Acanthocalycium* sp. is able to mitigate the negative effect on growth due to seasonal conditions, such as high temperatures and dryness in summer. The results of this experiment confirmed the possibility of a wide application of *Arthrobacter* species as inoculum in different crops. So far, this is the first report on the positive effects of this bacterial species on the flowering and quality of cactus plants of the genus *Acanthocalycium*.

Table 1 Evaluation of the effect of *Arthrobacter* sp. on the agronomic parameters and fruits quality of *Acanthocalycium* sp

Acanthocalycium Ferrarii	**PH (cm)**	**VW (g)**	**RW (g)**	**FN (n°)**	**FD (days)**	**Fruit Total phenolic compounds (mg gallic acid eq./g d.w.)**	**Fruit Total flavonoids (µg quercetin eq./g d.w.)**	**Fruit Antioxidant activity (µmol Trolox eq./g d.w.)**
Not inoculated	16.22 b	56.24 b	44.55 b	4.11 b	3.22 b	12.24 b	5.36 b	13.26 b
Inoculated	19.33 a	61.33 a	47.33 a	6.22 a	5.11 a	15.66 a	7.24 a	15.94 a
Anova	***	***	***	***	***	***	***	***
Acanthocalycium Glaucum	**PH (cm)**	**VW (g)**	**RW (g)**	**FN (n°)**	**FD (days)**	**Fruit Total phenolic compounds (mg gallic acid eq./g d.w.)**	**Fruit Total flavonoids (µg quercetin eq./g d.w.)**	**Fruit Antioxidant activity (µmol Trolox eq./g d.w.)**
Not inoculated	15.23 b	45.33 b	34.33 b	2.33 b	3.11 b	11.33 b	4.88 b	11.68 b
Inoculated	17.66 a	48.66 a	36.78 a	4.55 a	4.33 a	16.24 a	6.67 a	16.33 a
Anova	***	***	***	***	***	***	***	***
Acanthocalycium Violaceum	**PH (cm)**	**VW (g)**	**RW (g)**	**FN (n°)**	**FD (days)**	**Fruit Total phenolic compounds (mg gallic acid eq./g d.w.)**	**Fruit Total flavonoids (µg quercetin eq./g d.w.)**	**Fruit Antioxidant activity (µmol Trolox eq./g d.w.)**
Not inoculated	19.88 b	57.88 b	46.55 b	4.44 b	1.66 b	10.44 b	3.33 b	9.96 b
Inoculated	22.11 a	63.66 a	48.99 a	6.33 a	3.22 a	13.56 a	6.77 a	12.12 a
Anova	***	***	***	***	***	***	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$); Parameters: PH = plant height (cm); VW = vegetative weight (g); RW = roots weight (g); FN = flowers number; FD: flowers duration (days) Treatments: Not inoculated: control; Inoculated: *Arthrobacter* sp.

Table 2 Number of plants attacked by *Fusarium* sp. and *Verticillium* sp

Acanthocalycium Ferrarii	**Fusarium sp. (n°)**	**Verticillium sp. (n°)**
Not inoculated	3.22 a	2.24 a
Inoculated	1.11 b	0.00 b
Anova	***	***
Acanthocalycium Glaucum	Fusarium sp. (n°)	Verticillium sp. (n°)
Not inoculated	4.66 a	3.88 a
Inoculated	1.22 b	0.66 b
Anova	***	***
Acanthocalycium Violaceum	Fusarium sp. (n°)	Verticillium sp. (n°)
Not inoculated	5.32 a	3.22 a
Inoculated	0.00 b	0.00 b
Anova	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05); Treatments: Not inoculated: control; Inoculated: *Arthrobacter* sp.

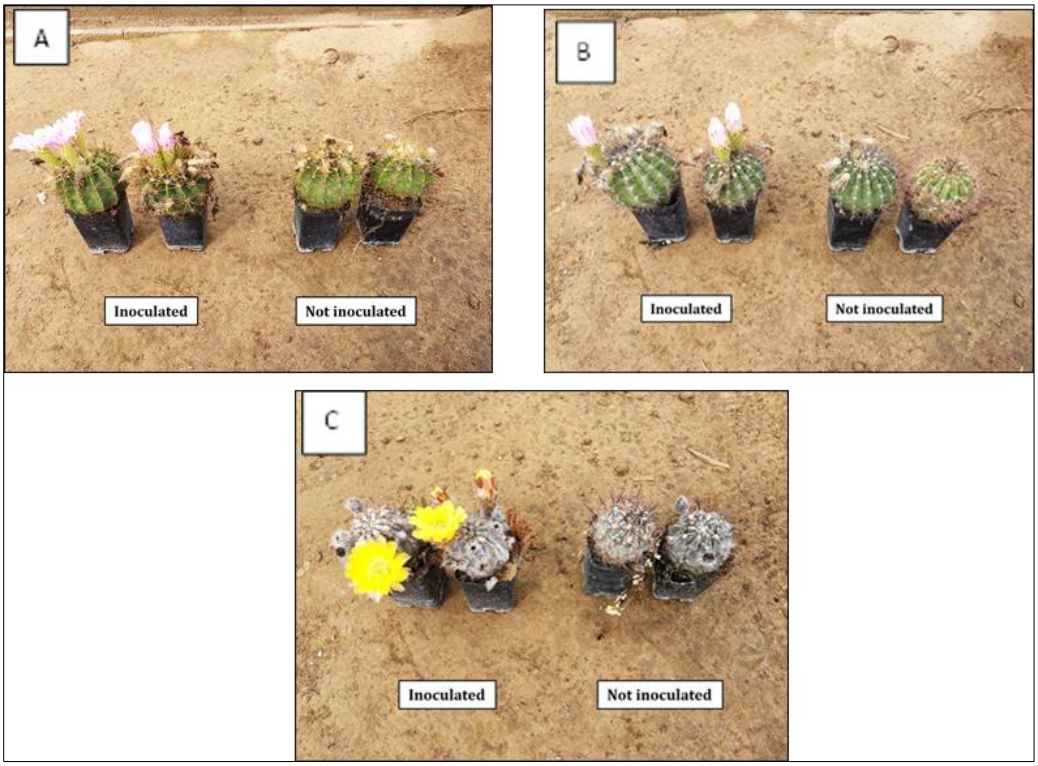

Figure 2 Effect of *Arthrobacter* sp. on plant and flower development in *Acanthocalycium Violaceum* (A), *A. Ferrarii* (B), *A. glaucum* (C)

4. Conclusion

Due to its 'multifunctionality', the genus *Acanthocalycium* is considered as one of the species of the future, for ornamental use and medicinal aspects, and new results may help reveal its potential in the context of the bio-economy and circular economy. As natural resources and cultural practices are crucial in defining the quality of flowers when

destined for food/nutraceutical applications, the inoculation of *Acanthocalycium* with *Arthrobacter* sp. can be envisaged to provide better plant growth under conditions of environmental stress, or as a soil fertiliser, but also to improve the synthesis of natural products used for therapeutic applications. Further studies aim to better understand the connection pathways involved in the *Acanthocalycium-Arthrobacter* interaction in order to assess the contribution of bacteria to cactus metabolism and to bridge the gap in the use of *Arthrobacter* sp. from the laboratory to the field scale.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

Statement of informed consent

The present research work does not contain any studies performed on animal/humans subjects.

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