ORIGINAL PAPER

Optimization of the production of organic cherry tomatoes through an innovative bioinoculation technology

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ABSTRACT

Biological products are sustainable alternatives to reduce the overuse of chemical fertilizers in agriculture. However, little is known about the effects of such products on cherry tomatoes. This study evaluated the performance of *Azospirillum argentinense Az39 (AZ)* and an extract of *Macrocystis pyrifera* (AE) on this variety of tomato. The products were assayed on their own and in combination, and two methods of application were tested: seed inoculation and seedling immersion. The treatments were assessed in terms of their efficacy in promoting crop growth and flowering. The combination of AE and AZ led to an increase in germination (10%), the fresh and dry weight (74% and 80%) of plants, and the chlorophyll content (50%) in the leaves of cherry tomato compared with the controls (application method with distilled water). In addition, the plants treated with AE+AZ had a larger number of floral petioles and flowers (300%) than the controls. No significant differences were found between inoculation and immersion, and a small volume of solution was enough for inoculation to be successful, i.e., for bacteria to effectively colonize the seeds. These findings demonstrate that seed inoculation with AE+AZ is a promising biotechnological tool to improve the production of cherry tomatoes.

Keywords: algae extract, PGPR, cherry tomato, flowering, germination, inoculation, production.

RESUMEN

Los productos biológicos basados en rizobacterias promotoras del crecimiento vegetal (PGPR) y extractos de algas son alternativas sostenibles para reducir el uso excesivo de fertilizantes químicos en la agricultura. Sin embargo, se sabe poco acerca de los efectos de estos productos en los tomates cherry (Solanum lycopersicum L. var. cerasiforme). Este estudio evaluó el rendimiento de Azospirillum argentinense (AZ, antes conocido como A. brasilense Az39) y un extracto de Macrocystis pyrifera (AE) en esta variedad de tomate. Los productos se evaluaron por separado y en combinación, y se probaron dos métodos de aplicación: inoculación de semillas e inmersión de plántulas. Los tratamientos se evaluaron en términos de su eficacia para promover el crecimiento y la floración de los cultivos. La combinación de AE y AZ condujo a un aumento en la germinación (10%), en el peso fresco y seco (74% y 80%) de las plantas y en el contenido de clorofila (50%) en las hojas de tomate cherry en comparación con el tratamiento con agua destilada (testigos). Además, las plantas tratadas con AE+AZ presentaron un mayor número de pecíolos florales y flores (300%) que los testigos. No se encontraron diferencias significativas entre la inoculación y la inmersión, por lo que un pequeño volumen de solución fue suficiente para que la inoculación fuera exitosa, es decir, para que las bacterias colonizaran eficazmente las semillas. Estos hallazgos demuestran que la inoculación de semillas con AE+AZ (posiblemente seguida de inmersión de plántulas en la misma solución) es una herramienta biotecnológica prometedora para mejorar la producción de tomates cherry.

***Palabras clave**: extracto de algas, PGPR, tomate cherry, floración, germinación, inoculación, producción.*

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most consumed fruit worldwide and one of the most important horticultural crops in Argentina (Argerich and Troilo, 2011; Massantini *et al*., 2021). Given that the global food demand is expected to keep increasing, a major challenge for agriculture is to boost productivity while optimizing the use of land (Ollivier and Bellon, 2013; De Schutter, 2014). One of the main factors that determine tomato yields is floral differentiation, a process which is triggered whenever a minimum amount of carbohydrates is available. While it lasts, changes in this availability have an impact on the outcome (Kumar *et al*., 2022). If the carbohydrate balance is low, the plant may abort the differentiation of several floral primordia. If it is high, the number of flowers can end up being larger than in each inflorescence under normal circumstances. Growth conditions, therefore, can strongly affect this stage and subsequent fruit production (Contreras-Magaña *et al*., 2013; Falla *et al*., 2020).

The species *S. lycopersicum* includes varieties shapes and colors of fruits. The variety known commercially as cherry tomato (*S. lycopersicum* var. cerasiforme), whose fruits are less than 35 mm in size, is believed to be the ancestor of the tomato which is now widely distributed in tropical and subtropical regions around the world (Kumar *et al*., 2022). One of the reasons for the expansion of cherry tomato is that it is considered a "protective food" due to its nutritional value and its high levels of antioxidant molecules, e.g., carotenoids like lycopene, ascorbic acid, vitamin E, and phenolic compounds such as flavonoids (Rune and Michelle, 2011; Sepat *et al*., 2013; Ramya *et al*., 2016). Although its yields under greenhouse conditions are generally lower than those of other tomato varieties (Zangoueinejad *et al*., 2019), its cultivation in Argentina is mostly carried out in this way, since it allows for a year-round fresh supply. Cultivation in the field occurs to a much lesser extent.

As is the case for many other crops, the application of chemicals to grow cherry tomatoes has serious environmental and health consequences. However, the organic agriculture segment has rapidly developed in the last decades (Willer *et al*., 2010; Van der Werf, 2020). A growing number of consumers (sometimes called "ecological consumers") are willing to pay higher prices for food that is certified organic, i.e., which has been produced without agrochemicals, including inorganic fertilizers (De la Cruz-Lazaro *et al*., 2010; Rihn *et al*., 2019). The principles governing organic agriculture comprise a return to closed cycles of energy and materials, maximized recycling, and the use of rotation systems, fertilizers of biological origin, and renewable energy (Muhie, 2022).

The inoculation of bacteria on seeds is an example of a biotechnological tool that can be implemented to improve crop productivity. The terrestrial ecosystem is highly dependent on microbial activity. Soil quality and plant yields are influenced by multiple metabolic reactions carried out by microorganisms in the rhizosphere, and the incorporation of plant growth-promoting rhizobacteria (PGPR) can be positive for crops (Mehmood *et al*., 2018; Basu *et al*., 2021). Some of the mechanisms through which these microorganisms exert their beneficial activity include biological nitrogen fixation, the production of phytohormone-type compounds that increase energy, germination and plant growth, and the secretion of compounds that improve the root structure, nutrient uptake, and the protection against pathogens (Raj *et al*., 2020). For instance, the incorporation of PGPR with organic biochar has resulted in significant improvements in cauliflower yield and quality (Širić *et al*., 2022). Such increased production can be linked to the role of soil microbial populations in the degradation and provision of nutrients needed for plant growth and development (Chaudhary *et al*., 2023).

Nevertheless, one of the ecological disadvantages of inoculation is that synthetic materials are used as a support for the bacteria. Extracts of algae or seaweed, whose exploitation only began towards the middle of the last century in Argentina, could serve as a non-toxic and environmentally friendly alternative. They contain natural nutrients and bioactive substances that can improve the yield, quality, and vigor of plants (Buschmann *et al*., 2017). The nutritional and hormonal composition of a *Macrocystis pyrifera* extract, as well as its metabolomic profile, has proved to be suitable for the preparation of bacteria-based formulations to promote plant growth (Iparraguirre *et al*., 2023). This extract could thus ensure the maintenance of cell growth, the viability, and the survival of *A. argentinense* Az39 (formerly *A. brasilense* Az39; Dos Santos *et al*., 2022), a PGPR that is commonly used in commercial inoculants. Replacing currently applied synthetic fertilizers with this biological alternative has the potential to guarantee appropriate cherry tomato yields while decreasing the risks of groundwater and soil contamination.

Taking all of this into account, this study aimed to determine whether the application of a formulation made up of *A. argentinense* Az39 and *Macrocystis pyrifera* extracts would have a positive synergistic effect on the germination, the establishment of seedlings, and the number of flowers in cherry tomato. Solutions of the algae extract and the bacterium were assessed individually and in combination, and they were applied using two different methods: the inoculation of seeds and immersion of seedlings prior to being transplanted.

MATERIALS AND METHODS

Biological materials

INTA-41 cherry tomato seeds were provided by INTA Río Cuarto (National Agricultural Technology Institute located at Río Cuarto- Argentina). This variety has a semi-determinate growth, and each plant produces more than 6 kg of yellow, grape-like fruits, with good firmness and sweet flavor, with an average of 15 g each. The seeds have a 115-day anthesis cycle, and floral differentiation lasts 150 days. They are usually transplanted into the greenhouse 34 days after being sown (Contreras-Magaña *et al*., 2013).

The alga extract (AE) was prepared with the brown alga Macrocystis pyrifera, which was harvested in the province of Santa Cruz (Argentina). BIOTEC S.A Laboratories (Argentina) provided the seaweed extracts. The algae were collected from the sea bottom/collected from the seabed; those that reached the coast naturally were discarded, since many of their active principles were then lost, and it was not possible to establish how long they had been out of the water. The harvested specimens were assessed for their state of development, because, as with other plants, their chemical constitution depends on their developmental stage. They were dried naturally in the shade until they reached 18% moisture and then washed intensely with demineralized water to remove excess sodium. The extract was made with a hydroalcoholic solution (80% demineralized water and 20% propylene glycol). The process for obtaining the AE was detailed in Iparraguirre *et al*. (2023).

The PGPR used was *A. argentinense* Az39. A fresh bacterial inoculum provided by the IMyZA INTA Institute (Argentina) was grown in a minimal culture medium (NFb or nitrogen-free biotin) for 24 hours at 28°C with shaking at 180 rpm and a pH of 6.8-7.0 until reaching a final concentration of 1x10^8 colony-forming units per milliliter (CFU/ml).

Inoculation and immersion treatments

Inoculation on tomato seeds

Seeds were used for inoculation, selected according to their size, choosing seeds of similar size to achieve sample homogeneity, and their impurities were removed. The seeds were superficially disinfected with 2% sodium hypochlorite. A hundred of them were weighed and the bacterial dose per gram of seeds was established. A 150µl aliquot of inoculant was used for every 30g of seeds, corresponding to a dose of 1x10^4 CFU/ml. For each treatment, 3 g of seeds were placed in a sterile polypropylene bag, leaving an air volume equivalent to that occupied by the seeds. The treatments were as follows: C IC, control with distilled water (300 µl); AZ IC, seeds inoculated with *A. argentinense* Az39 (15 µl); AE IC; inoculated seeds with pure AE (15 µl); and AE+AZ IC, seeds inoculated with *A. argentinense* Az39 (15 µl) + AE (15 µl). Each bag was closed and shaken for no less than 1 min to appropriately distribute the inoculant (manual homogenization). In order to ensure that all the seeds had the same wetted volume, all treatments were brought to the same final volume (300 µl in 30 g of seeds) by adding sterile distilled water.

Twenty minutes after inoculation, the seeds were placed in germination trays (one seed per well) and then in growth chambers (Conviron PR48) under the following conditions: 80% relative humidity, a photoperiod of 16 h light/8 h darkness, and a temperature of 24/20ºC.

Immersion of tomato seedlings before transplant

Five seedlings were randomly taken from each tray (per treatment) 34 days after sowing (DAS), and their physiological growth parameters were determined (see section 2.3). None of these plants had been previously inoculated. The remaining seedlings were immersed for 5 min in different solutions as follows: C IM, control with distilled water; AZ IM, seedlings immersed in AZ; AE IM, seedlings immersed in pure AE; and AE+AZ IM; seedlings immersed in AE+AZ. After that, they were transplanted into pots with sterile soil and kept in growth chambers (Conviron PR48), where the treatments were sown randomly. Four rows of eight seedlings were arranged, with a planting distance of 25 cm between each plant and 1.54 m between the rows, resulting in a density of 2.60 plants/m^2 (according to Perez and Coto, 2019). To evaluate growth parameters, the plants were collected 115 DAS (days after anthesis), and 150, 157, 164, and 190 DAS (reproductive stage).

Evaluation of plant growth parameters

The plant growth parameters were measured in both control and treated plants at specified times. Germination percentage was evaluated by counting germinated seeds with a length greater than 0.5 cm, using a manual magnifying glass and a ruler graduated in centimeters, at 5 DAS. Total length of plants at 155 DAS (when anthesis began) was determined using a tape measure graduated in centimeters. Fresh and dry weight of roots and shoots were evaluated at 115 and 190 DAS. The dry weight was determined by allowing the plants to dry in an oven at 60°C for 72 hours, and then the samples were weighed using an analytical balance (DENVER Instrument PK-202, USA). Chlorophyll content in leaves was measured using a non-destructive portable chlorophyll meter at 115 and 190 DAS (CL-O1, Hansatech Instruments Ltd., United Kingdom). The chlorophyll content was quantified in chlorophyll units (CU), and the measurements of at least 4 independent leaves from each plant were taken. The number of petioles and flowers was determined visually at 150, 157, 164, and 190 DAS (reproductive stage).

Statistical analysis

The experimental design was completely randomized with a factorial arrangement. The germination and growth assays were repeated twice, and the treatments were performed in quadruplicate. The intervening factors were as follows: 1) 2 levels of method of application (inoculation and immersion) and 2) 4 levels of treatment (Control, AZ, AE and AE+AZ). A two-factor ANOVA (considering application and treatment) was performed for each variable. Significant differences between treatments ($p<0.05$) were determined with Fisher's Least Significant Difference (LSD) test.

RESULTS

These two factors (method of application and treatments), as well as the interactions between them, had no significant effects ($p\geq0.05$). Therefore, only the results of the inoculation processes and seed treatments are described. As shown in figure 1, all the inoculation treatments resulted in an increase in the germination percentage ($p<0.05$) with respect to control plants. Although no significant differences were detected between treatments, the highest percentage (93%) corresponded to those seeds that received the combined inoculation (AE+AZ).

Figure 2 shows plant length, measured 115 days after sowing (DAS). Once again, the longest plants were those that grew from seeds or seedlings that had been inoculated with or immersed in AE+AZ (51.2 and 50.5 cm, respectively), with no significant differences between methods.

The fresh weight of shoots was determined 115 and 190 DAS (figure 3). On both days, the shoots of treated plants weighed more ($p<0.05$) than those of control plants, and those of plants exposed to AE+AZ increased the shoot weight (74%) ($p<0.05$) compared with controls. No significant differences were found between methods of treatment application. Similarly, the dry weight of the shoots of treated plants was significantly higher ($p<0.05$) after the different treatments (figure 4). The shoots of plants grown from seeds inoculated with either AZ or AE weighed more than those grown from seedlings immersed in the solutions separately. However, the highest weight (15.6 g) was recorded in plants treated with the combination of both (AE+AZ).

When it came to the fresh and dry weight of roots, which was measured 115 DAS (figure 5), there was a significant increase in treated plants by approximately 80% ($p<0.05$) compared to the controls. No significant differences were found between AE and AZ on their own or between inoculation and immersion. The highest values (8.5 g) were obtained from plants treated with AE+AZ.

On days 115 and 190 after sowing, the leaves of treated plants had more chlorophyll than those of the controls (figure 6). The chlorophyll content was 66% ($p<0.05$) higher in plants exposed to AE+AZ than in controls, regardless of the method of application.

Figure 7 shows the number of floral petioles 150, 157, 164, and 190 DAS. As with the other parameters, plants treated with AE+AZ had the largest number (6.4 floral petioles). They were followed by plants treated with AE or AZ on their own. No significant differences were found between the application methods.

Finally, the treated plantshad a significantly greater number of flowers than control plants (figure 8). The largest number of flowers (30) was observed in plants that grew from seedlings immersed in AE+AZ, though this value was not significantly different than for those plants grown from seeds inoculated with the same solution (28 flowers). More specifically, flower production rose by 300% ($p<0.05$) in plants treated with AE+AZ with respect to the controls (8-9 flowers per plant). This should represent an improvement in subsequent fruit formation as well.

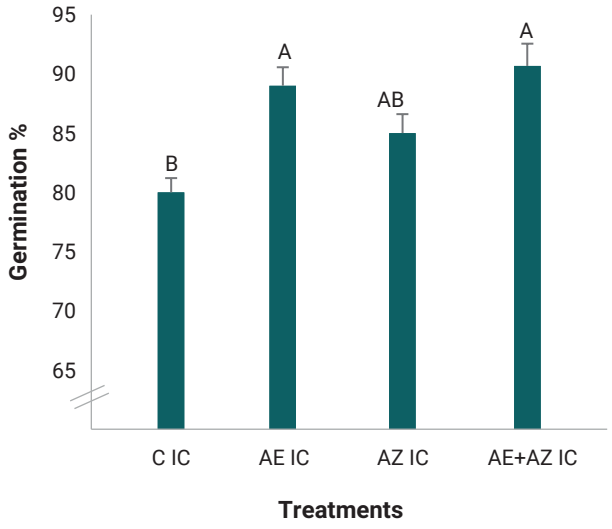

Figure 1. Germination percentage of tomato seeds at 115 DAS under different treatments (C IC: control; AZ IC: seeds inoculated with AZ39; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) ($n = 24$).

DISCUSSION

Even though moderately implemented at present, the inoculation of seeds with beneficial bacteria is being adopted as it can increase crop productivity through biological means (Van der Werf, 2020; Basu *et al*., 2021) and thus contribute to reducing the use of potentially toxic compounds as fertilizers. Furthermore, biological products play a key role in soil management and nutrient mobilization by safeguarding against diseases and strengthening plant defenses, managing stress tolerance, contributing to post-harvest fruit care, and governing the broader ecosystem. (Padmaperuma *et al*., 2020).

The inoculated bacteria should become intimately associated with the germinating seed and be predisposed to future colonization (Mangmang *et al*., 2015). This means that the success of the strategy depends largely on the interactions established between the plant and the bacteria, which are usually crop- or even cultivar-specific. In turn, these interactions may be favored by the characteristics of the site of inoculation, the modes of inoculation, and the culture conditions. Another crucial factor for efficacy is the inoculant formulation. Ideally, it should ensure the survival of bacteria for a period long enough for them to settle in the soil, their natural carrier, and begin exerting their beneficial activity on the plant (Sahu *et al*., 2016; Kaminsky *et al*., 2019; Lopes *et al*., 2021).

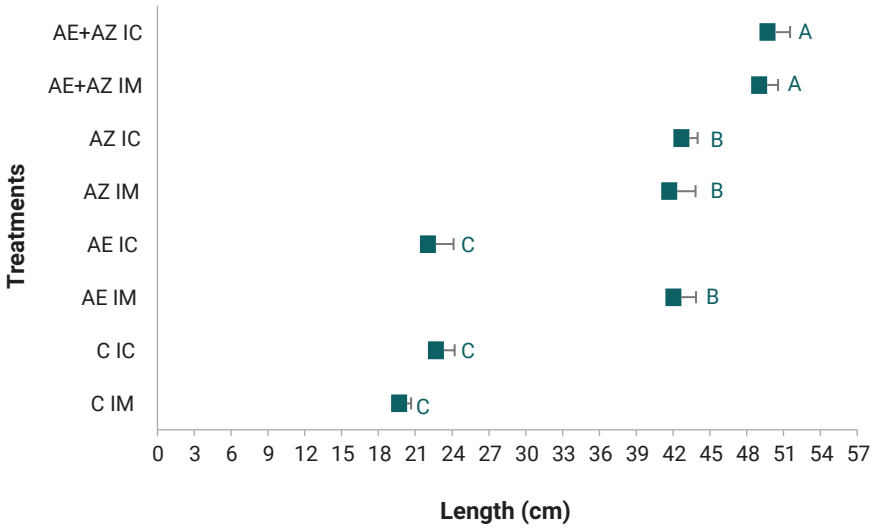

Figure 2. Total length of tomato plants at 115 days after sowing (DAS) (start of flowering) under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ + AE; C IM: immersion control; AZ IM: seedlings immersed in an AZ culture; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in an AZ+AE solution). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) ($n = 32$).

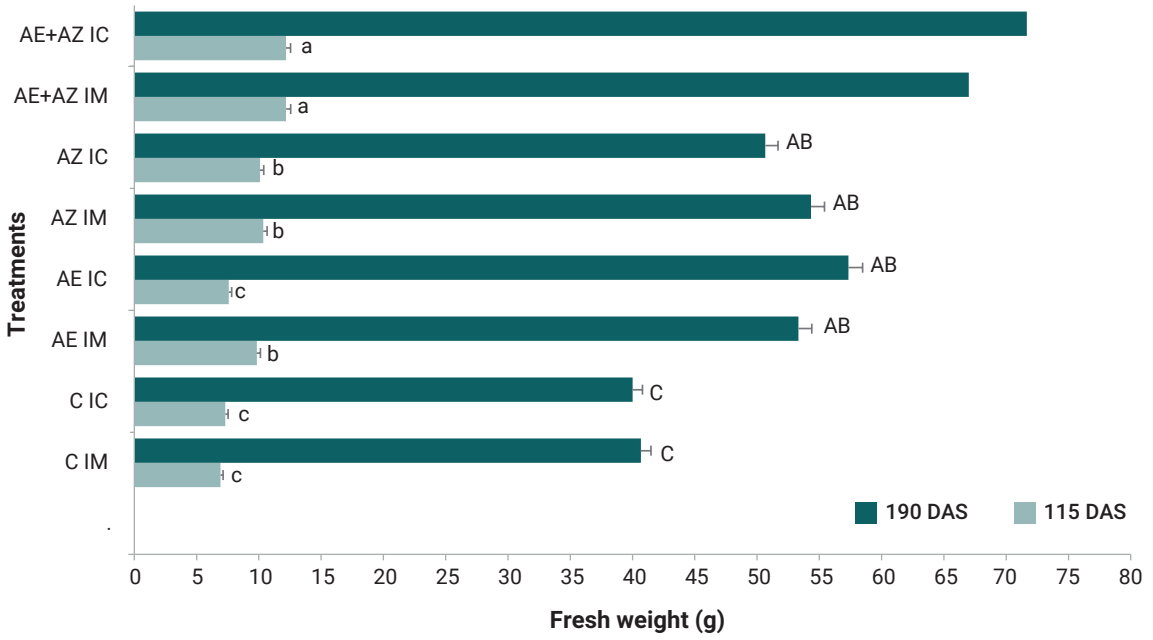

Figure 3. Fresh weight of tomato plant shoots at 115 and 190 DAS under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE; C IM: immersion control; AZ IM: seedlings immersed in an AZ culture; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in a solution of AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) (n = 32). Capital letters compare weight between treatments 115 DAS, while lowercase letters compare weight between treatments 190 DAS.

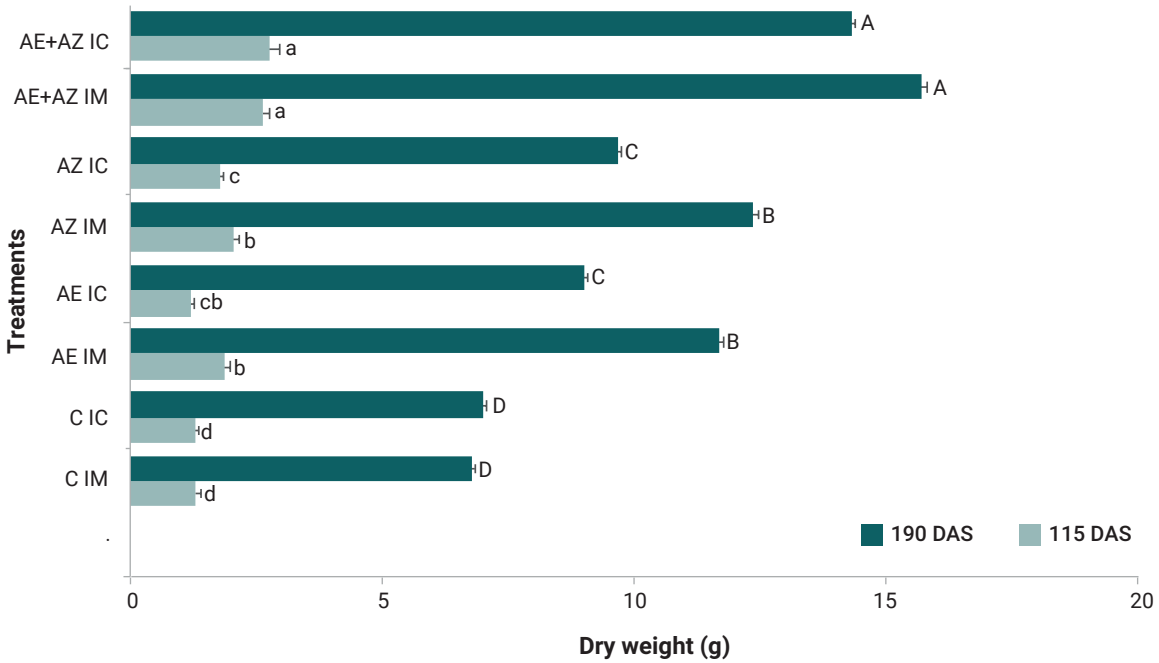

Figure 4. Dry weight of tomato plant shoots at 115 and 190 DAS under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE; C IM: immersion control; AZ IM: seedlings immersed in a solution of AZ; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in a solution of AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) (n = 32). Capital letters compare weight between treatments 115 DAS, while lowercase letters compare weight between treatments 190 DAS.

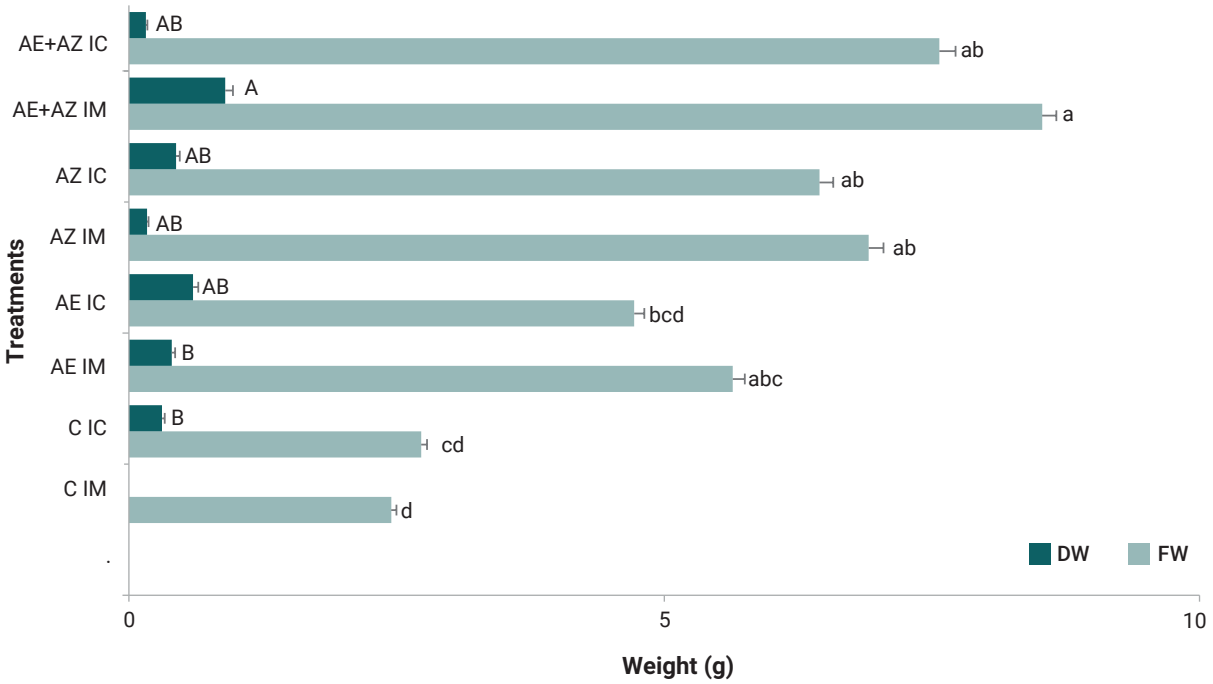

Figure 5. Fresh and dry weight of tomato plant roots at 115 DAS under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE; C IM: immersion control; AZ IM: seedlings immersed in a solution of AZ; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in a solution of AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) (n = 32). Capital letters compare fresh weight between treatments, while lowercase letters compare dry weight between treatments.

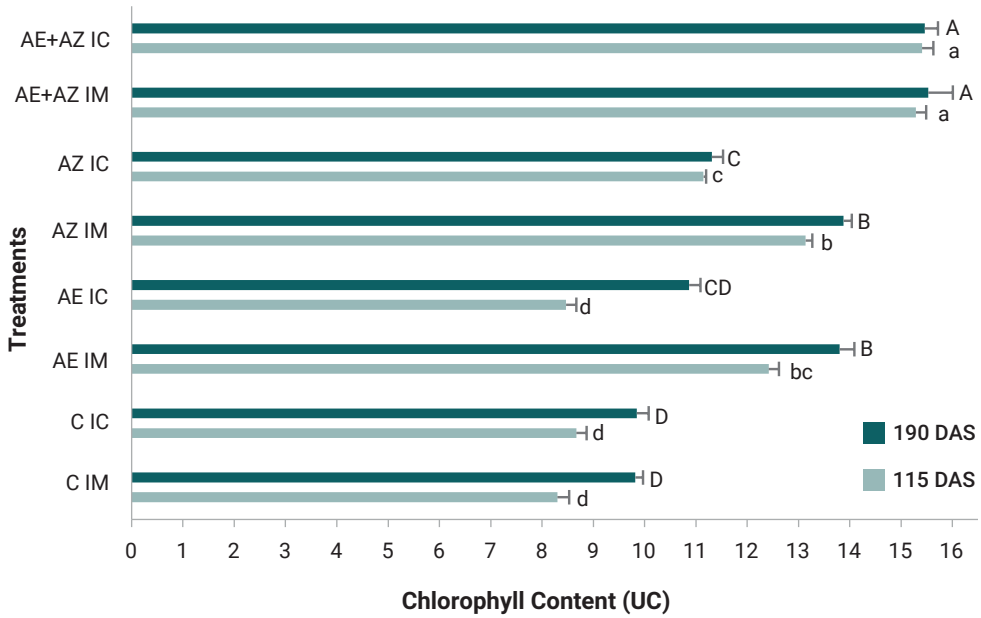

Figure 6. Chlorophyll content (CU) in tomato plants at 115 and 190 DAS under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE; C IM: immersion control; AZ IM: seedlings immersed in a solution of AZ; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in a solution of AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) (n = 32). Capital letters compare weight between different treatments 190 DAS, while lowercase letters compare weight between different treatments 115 DAS.

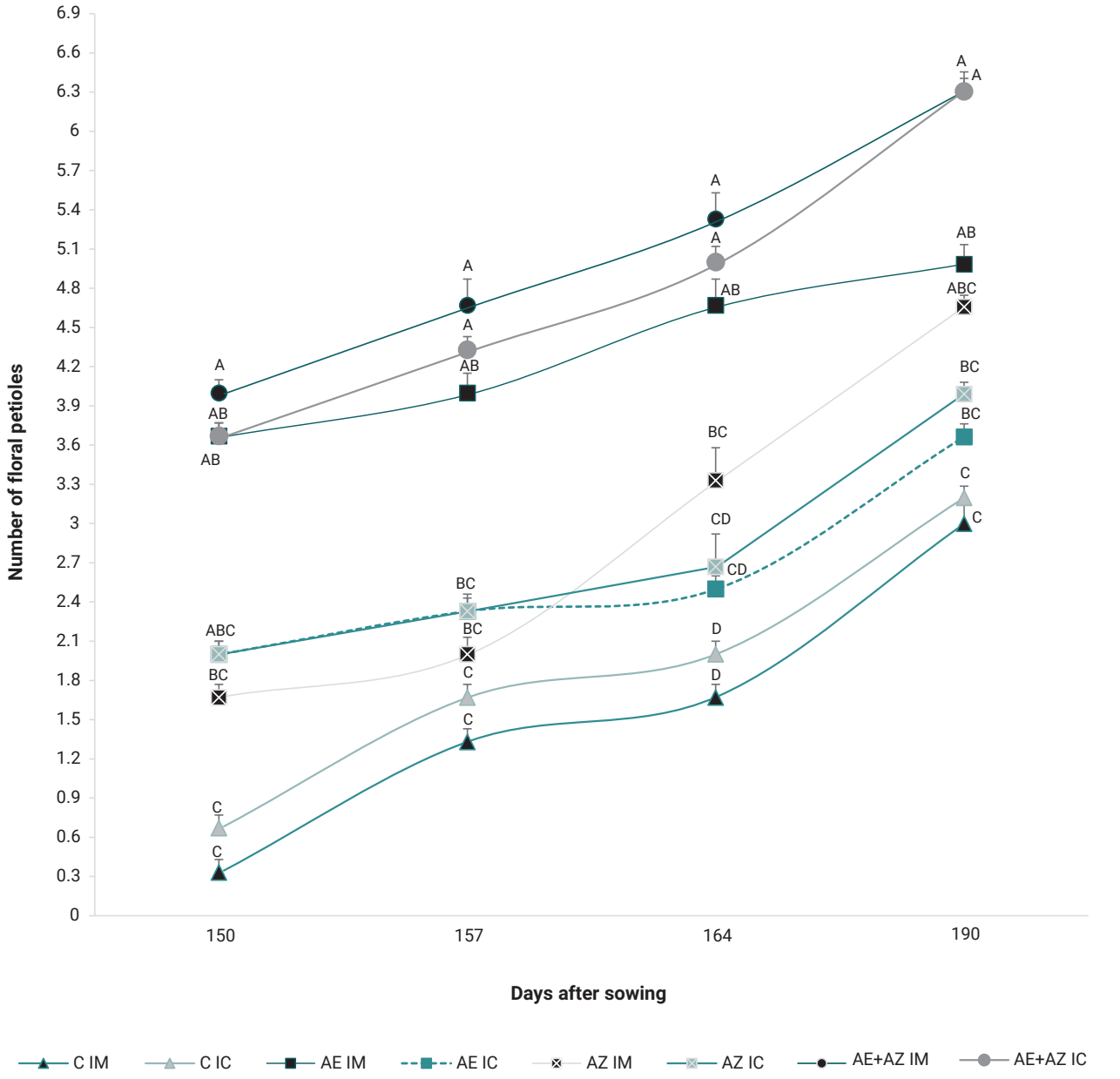

Figure 7. Number of floral petioles in tomato plants at 150, 157, 164, and 190 DAS under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE; C IM: immersion control; AZ IM: seedlings immersed in a solution of AZ; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in a solution of AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) (n = 32).

A. argentinense Az39 is a bacterium commonly used as an inoculant, recognized for its well-established abilities to promote plant growth. It is an efficient phosphate solubilizer and nitrogen fixer (Suhameena *et al*., 2020), and it synthesizes hormones such as auxins and gibberellic acid (Iparraguirre *et al*., 2023). These bioactive compounds can improve germination and modify the architecture of the root in a way that increases water absorption and the transport of assimilates (Salomon *et al*., 2014; Cordero *et al*., 2018; Dos Santos *et al*., 2022). The potential of combining the bacterium *A. argentinense* Az39 with an extract of the algae *Macrocystis pyrifera* (AE) as a natural matrix or support for inoculation was explored (Iparraguirre *et al*., 2020, 2023). The biochemical analysis of this combination showed a variety of mineral nutrients, hormonal compounds and alginic acid derivatives, which promoted bacterial growth (Iparraguirre *et al*., 2023). In the present study, the effects of this formulation (AE+AZ) and of each product separately on cherry tomatoes, a widely cultivated crop throughout the world, were evaluated. According to the results, treatment with either AZ, AE, or AE+AZ was always associated with an enhancement

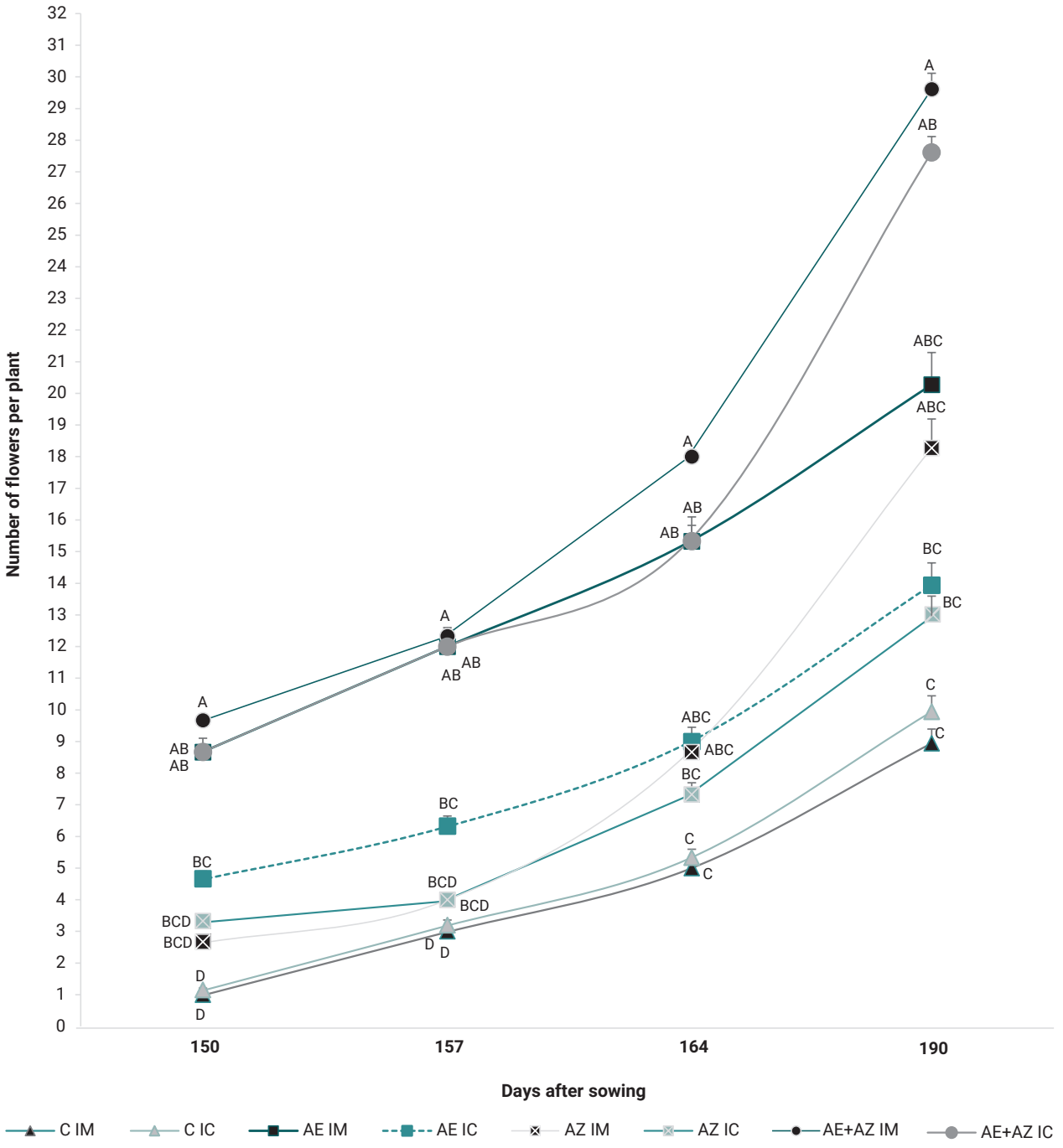

Figure 8. Number of flowers in tomato plant at 150, 157, 164, and 190 DAS under different treatments (C IC: inoculation control; AZ IC: seeds inoculated with AZ; AE IC: pelleted seeds with pure AE; AE+AZ IC: seeds inoculated with AZ+AE; C IM: immersion control; AZ IM: seedlings immersed in a solution of AZ; AE IM: seedlings immersed in pure AE; AE+AZ IM: seedlings immersed in a solution of AZ+AE). The bars represent the mean ± SE; different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p<0.05$) ($n = 32$).

of all the growth parameters assessed, namely germination percentage, fresh and dry weight of shoots and roots, plant length, chlorophyll content, and number of petioles and flowers. In other words, the seeds exposed to the treatments had a higher chance of germinating than the controls, and the plants that grew from those seeds were longer, had a more complex root system, more chlorophyll in their leaves, and more petioles and flowers. It makes sense that all the parameters were improved, since they are highly dependent on one another a higher percentage of germinated seeds means more implanted seedlings; a larger number of roots enables a more efficient absorption of water and nutrients, and a higher content of chlo-

rophyll leads to better photosynthesis. These factors usually result in more growth, i.e., longer plants with more flowers and, eventually, more fruit. The highest values for all these variables were consistently obtained when the two products (alga extract and bacterium, AE+AZ) were combined.

The AE appears to have played a double role. On the one hand, much like the bacterium, *M. pyrifera* is able to produce high levels of auxins and gibberellic acid (GA_3) (Iparraguirre *et al*., 2023). As mentioned before, these hormonal compounds present in the combination not only promote bacterial growth in the extract by functioning as a matrix (Iparraguirre *et al*., 2023), but also have multiple benefits for plant growth. These benefits include promoting germination and stimulating seedling implantation. The algae extracts also contain photoprotective compounds like carotenoids, which have been linked to protection against oxidative stress (Cohen *et al*., 2015). These findings could explain the better effect observed in plants treated with AE immersion compared to plants whose seeds were inoculated with AE. The high percentage of gibberellins, along with other phytohormones such as auxins and cytokinins, detected in the extract would improve seedling establishment and subsequent development when applied before transplantation via the immersion method, making the AE readily available for root absorption (Iparraguirre *et al*., 2023). It is known that there is cross-communication between gibberellins (GA) and other hormones, such as positive interactions with auxin, which promote cellular expansion, differentiation, and root elongation (Stirk *et al*., 2014). Additionally, it is known that our AE has a high content of IAA, a hormone that plays an important role in stimulating root growth in plants (Brumos *et al*., 2018). In fact, auxin, particularly indole-3-acetic acid (IAA), is an essential plant hormone that performs numerous functions in plant growth and development, including phototropic and gravitropic responses, apical dominance, the formation of lateral and adventitious roots, cell elongation and plant height control (Bartoli *et al*., 2013; Iparraguirre *et al*., 2023). In conclusion, the exogenous application of AE via immersion would act as a fertilizer/phyto-stimulant, while when applied to seeds, it would primarily have a beneficial effect on germination.

On the other hand, the extract seems to be effective as a support for *A. argentinense* Az39; it might adequately encapsulate and protect the living bacterial cells, thus contributing to their likelihood of surviving long enough to multiply and exert their favorable activity on the plant. In the soil, the polymers that make up the extract are broken down by native microorganisms, and the encapsulated bacteria are gradually released (probably when the seed germinates and the seedling sprouts) (Hernández-Carmona *et al*., 2012). The bacterial population can thus continue growing in greater numbers over time, so their bioactive effects on the plant are also longer-lasting (Nabti *et al*, 2010; Iparraguirre *et al*., 2023). In short, the findings show that *M. pyrifera* and *A. argentinense* can act synergistically to promote growth and flowering in cherry tomatoes, and could therefore enhance its fruit production. When an algal extract, which contains a variety of phytohormones, is combined with *A. argentinense* Az39 (a bacterium that can also produce phytohormones), the concentration of zeatin can increase in the treated plants. Zeatin, being present in higher amounts, stimulates the activity of chloroplasts, promoting chlorophyll biosynthesis. In turn, a higher chlorophyll content enhances the plant's ability to perform photosynthesis, improving its overall growth and development (Li *et al*., 2018).

Interestingly, no significant differences were found between seed inoculation and seedling immersion, the two application methods tested. This indicates that inoculation with AE+AZ could be used to ensure seed germination and the initial colonization by bacteria. The seedlings could then be immersed in the same solution to reinforce the positive effects before they are finally transplanted.

CONCLUSION

Based on the findings of the present study, the combination of *Azospirillum argentinense* Az39 and an extract of *Macrocystis pyrifera* (AE) resulted in a significant increase in the germination rate, as well as in the fresh and dry weights of roots and shoots, and in the leaf chlorophyll content. Moreover, the cherry tomato plants treated with AE+AZ exhibited a larger number of floral petioles and flowers than the controls (treated with distilled water). Notably, a small volume (150µl of inoculant for every 30g of seeds) of solution was sufficient for successful seed inoculation and subsequent bacterial colonization. These results demonstrated that seed inoculation with AE+AZ, potentially followed by seedling immersion in the same solution, represents a promising biotechnological tool for improving cherry tomato production. Although these results are encouraging, and the use of bioinoculants for tomato production is proposed as an alternative to synthetic agrochemicals, further studies are needed to address some of the assumptions outlined in this study.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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