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EVALUATION OF NATIVE BACTERIAL STRAINS AS PLANT GROWTH PROMOTERS FOR GREENHOUSE TOMATOES

Daniel-Nicolae COJANU[1, 2], Maria-Cristina LUMÎNARE[1, 2], Oana Alina BOIU-SICUIA[1, 2], Călina Petruța CORNEA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research - Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, 013813, District 1, Bucharest, Romania

Corresponding author email: daniel.cojanu13@yahoo.com

Abstract

Various bacterial strains have been described, over time, as plant growth promoters. The aim of this study was to evaluate the effect of some native bacterial strains applied as soil treatment on tomato seedlings, grown in greenhouse conditions, Giurgiu County. Seven bacterial strains were used in this study, three Bacillus cereus/thuringiensis strains, and one strain of each Acinetobacter guillouiae, Bacillus safensis, Paenibacillus castaneae, and P. lautus species. Certain plant growth parameters were evaluated to compare bacterial effects on the plant growth, such as leaves' chlorophyll content, plant height and stem diameter. Compared to the untreated tomato seedlings, most of the tested bacterial strains improved the plants' growth. Among all the analyzed strains, the highest chlorophyll content was registered in tomato leaves when treated with Acinetobacter guillouiae (2.07 mg/g fresh weight). The obtained results confirm the hypothesis of using selected beneficial bacteria as plant growth promoters in tomato crop. Further evaluations are needed, in order to establish the positive effects of these bacterial inoculants on tomato fruits quality and quantity.

***Key words**: beneficial bacteria, native strains, plant growth promoters, seedlings, tomato.*

INTRODUCTION

Tomatoes are a widely cultivated and consumed plants across the world, with significant economic, nutritional, or cultural importance. In December 2022, the FAO reported that global tomato production in 2021, for both processing and fresh consumption, reached slightly over 189.1 million metric tons (mt). This marked a 2% increase from the 2020 production of 184.8 million mt and a 4% rise from the average production of the previous three years (2018-2020) at 182.7 million mt. Romania is one of the notable tomato producers in European Union (EU), with 9.2% of the total cultivated area (EUROSTAT, 2019). The evolution of tomato area and production cultivated in 2022, evaluated at 17170 ha and 298920 tons, was slightly decreasing compared to previous years, e.g. 2021, when surface cultivated marked 18130 ha, with a production of 500200 tons (FAO, accessed February 2024). While facing new problems in terms of agricultural technology, such as climate change, higher impact of new pests, diseases or weeds, farmers tend to use higher quantities of pesticides year by year, to respond to it. Chemical fertilizers and pesticides, while effective in boosting crop yields and controlling pests, can have detrimental effects on the environment and human health.

In the context of the increasing use of chemicals in agriculture, both worldwide and Romania, the need for new sustainable solutions has become more evident. Bacteria plant growth promoters offer a sustainable and eco-friendly alternative to synthetic chemicals. These beneficial bacteria can improve soil health and fertility (Aloo et al., 2022), enhance nutrient solubilization (Varma et al., 2020) by plants, protect crops against diseases (Aghisa et al., 2019), nitrogen fixation (N_2) (Souza et al., 2015), solubilizing potassium (Varma et al., 2019) or as phytohormones. By promoting plant growth naturally, bacteria help reduce the reliance on harmful chemicals in agriculture.

Among the most studied bacteria for their plant biostimulant proprieties, belong to genera such as genera such as *Azotobacter*, *Bacillus*, *Pseudomonas*, *Azospirillium*, and *Rhizobium* (Hurek and Reinhold Hurek, 2003; Oosten et al., 2017). Even though PGPMs (plant growth promoting microorganisms) have several advantages, their adoption in worldwide agriculture has been hindered primarily because the majority of PGPM products have low field lifetimes, mainly to their sensitive thermo-, photo- or hydro-reaction. Therefore, testing and identifying the best microorganisms as plant growth promoters, becomes an increasingly important direction in the world of research, new molecular techniques shortening the discovery process of the proprieties of these microorganisms.

MATERIALS AND METHODS

Bacterial strains

To observe growth promoting effects in tomato seedlings, 7 native bacterial strains (noted as follows, 53.3, 61.4, 59.3, 59.2S, 65.3, 50.4, LvD1) were used (Table 1).

Table 1. Native bacterial strains used in the experiment

Strain	Source isolation	Stored collection
B. cereus/thuringiensis - 53.3	Soil	RDIPP Bucharest
Paenibacillus lautus – 61.4	Soil	RDIPP Bucharest
B. safensis – 59.3	Soil	RDIPP Bucharest
Paenibacillus castanae – 59.2S	Soil	RDIPP Bucharest
B. cereus/thuringiensis – 65.3	Soil	RDIPP Bucharest
B. cereus/thuringiensis – 50.4	Soil	RDIPP Bucharest
Acinetobacter guillouaie – LvD1	Insect	RDIPP Bucharest

Bacterial strains used in the study were identified using Biolog GEN III, and some of them (50.4, 65.3) were also identified by 16S rDNA partial sequencing (Cojanu et al., 2024, Sicuia et al., 2017). Previously, the strains were growned on Luria Bertani Agar (LBA) medium (ROTH), then transferred into Erlenmeyer flask containing 50 ml of liquid Luria Bertani (LB). After inoculation the flasks were transferred to Thermoshaker, for 5 days, 120 rpm, at 30ºC.

Plastic containers and substrate

Containers used in the experiment were specifically chosen (dimensions L x l x h cm, 80 x 40 x 40 cm), in order to allow the growth of 5 tomatoes seedelings per container, during the experiment observations. The soil used was universal substrate “Agro Cs”, with modified reaction, who ensures optimal aeration and accelarates root growth.

Tomato seedlings (*Lycopersicon esculentum*)

The native variety Moldoveanu F1 is known for the firm texture of fruits, resistance to diseases and for organoleptic characteristics, such as exceptional taste, vibrant red colour or sweetness.

The seedlings were obtained in controlled growth conditions (greenhouse), in special sowing pots. Randomly, for the experiment, 5 seedlings were chosen (per bacterial strain), uniform in growth, colour and with no specific disease symptoms.

In this bioassay experiment, tomato seedlings were exposed to a liquid bacteria culture to observe the effects of bacterial treatment on plant growth and development. The experiment involved three rounds of bacterial administration to the tomato seedlings.

1. ***Initial Bacterial Treatment***:

- Tomato seedlings were treated with 80 ml of liquid bacteria culture per plant. The seedlings were allowed to soak in the bacterial suspension for 2 hours to ensure thorough exposure. After the 2-hour baiting period, the seedlings were planted in soil, and the remaining liquid from the initial treatment was used to wet the seedlings equally (Figure 1a).

Figure 1. Bacterial inoculation:
a. root immersion; b. soil application

2. ***Subsequent Bacterial Treatments***:

- Following the initial bacterial administration, two more rounds of bacterial treatment were carried out. Each round involved administering 40 ml of liquid bacteria culture per plant to ensure consistent and continued exposure to the bacterial culture (Figure 1b).

Observations and Data Collection

Throughout the experiment, observations were made on the growth and development of the tomato seedlings, including leaves chlorophyll content, plant height and stem diameter.

Analysis of chlorophyll

For acquiring total chlorophyll compounds, both a and b, a systematic protocol was followed. Initially, a quantity of 1 g of tomato leaves (4 repetitions per bacterial strain) was carefully gathered and subsequently transferred to a mortar and pestle (Figure 2).

Figure 2. Tomato leaves samples for chlorophyll extraction

In conjunction, 1 g of quartz sand and 0.1 g of $CaCO_3$ were added to the mixture. The leaves were ground, and 20 ml of 100% acetone (ADRA CHIM SRL, % min 99) was poured over the resulting blend, which was then transferred to Falcon tubes.

The tubes were left undisturbed overnight at a temperature of 4°C. The following day, filtration was performed using filter paper with a weight of 389.84 g/m^2. Decimal dilutions were prepared by combining 200 microliters of the leaf mixture with 1800 microliters of acetone. Subsequently, measurements were taken using a spectrophotometer at wavelengths of 470 nm, 645 nm, and 662 nm.

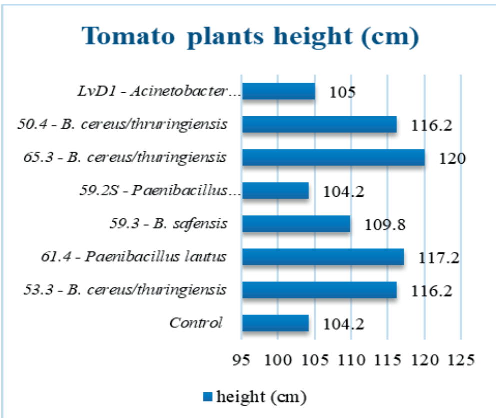
RESULTS AND DISCUSSIONS

All tested bacterial strains exhibited a positive influence on the growth of tomato plants (Figure 3). The parameters that were monitored, including plant height, stem diameter, number of leaves and chlorophyll levels, showed improvements in response to the application of the bacterial strains.

Figure 3. Tomato plants after 25 days from bacterial immersion

The **height** of the plants can serve as an indicator of their health state and overall growth. Taller tomato plants indicate vigour and a healthy growth rate. The results showed that most of the bacterial strains improved plant height, with positive values over control test (104.2 cm), from 0.2% - 104.2 cm (59.2S – *Paenibacillius castanae*) up to 15.38% - 120 cm (65.3 - *B. cereus/thuringiensis*) (Table 2).

Table. 2 Tomato seedlings height improvement with bacterial strains

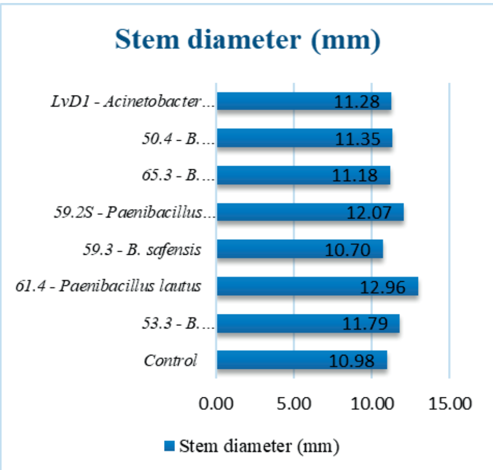


A thicker stem indicates a stronger and more robust plant. Monitoring **stem diameter**, along with other growth indicators, can help growers assess the well-being of their tomato plants and make informed decisions regarding care and maintenance. The observations were made with instrument for precise measuring (Shubler, mm), 1 cm above substrate for all plants included in test (Figure 4).

Figure 4. Stem diameter measurements

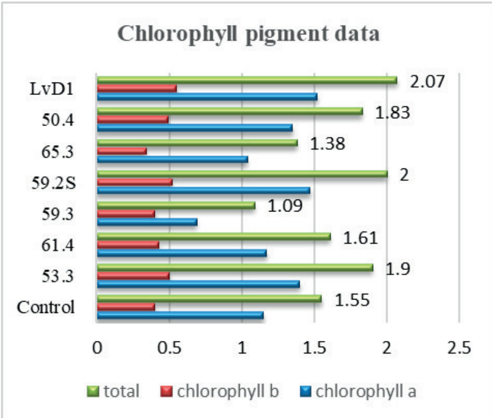
Bacterial strains used in test showed positive influence improving stem diameter values, over control (10.98 mm), from 1.8% - 11.18 mm (65.3 - *B. cereus/thuringienis*) to 18% - 12.96 mm (61.4 - *Paenibacillus lautus*) (Table 3).

Table 3. Stem diameter improvement with bacterial strains



Chlorophyll is a vital pigment found in plants that plays a vital role in photosynthesis, a or b type being the primary pigments responsible for capturing light. In tomato plants, chlorophyll and b are essential for the plant's ability to absorb and use light energy efficiently. The total amount of chlorophyll in tomato plants is an important indicator of their overall health and photosynthetic capacity. Higher levels of chlorophyll generally indicate that the plant is receiving an adequate amount of light and nutrients for optimal growth. Total chlorophyll levels are listed in Table 4.

Table 4. Total chlorophyll content analysis in tomato plants



The results suggest that different bacterial strains have varying effects on the total chlorophyll content in tomato plants. Some bacterial strains may enhance chlorophyll production, while others may have neutral impact. *Acinetobacter guillouaie* strain (LvD1), had highest total chlorophyll content compared to the control. Strains 53.3, 592S, and 50.4 also showed higher chlorophyll content than control group.

The bacterial strains involved in the test showed, for most of the followed parameters, a positive impact. Influence of plant promoting bacteria in tomato growth was analysed, research results being already reported (Adedayo et al., 2022; Gashash et al., 2022; Poria et al., 2022; Katsenios et al., 2022; Kalozoumis et al., 2021; Moustaine et al., 2017; Zaamer et al., 2016). Bacterial strains used as plant growth promoting agents improved tomato plants growth both quantitative and qualitative. The total chlorophyll content was significantly stimulated by *Acinetobacter guillouaie, Penibacillus castanae* or *B. cereus/ thruingiensis strains* comparison with other treatments and control; these results are similar to those demonstrated by Gashash et al., 2022. Comparing other test results, plant height improvement by promoting bacteria is also confirmed by other researchers (Siahaan et al., 2022).

CONCLUSIONS

Use of bacterial strains as promoting growth agents, led to improved health plant indicators, proving that the strategy of inoculation of bacteria, can represent a new tool in tomato crop technology. Better establishment of a new microorganism in the rhizosphere of a plant is directly influenced by inoculation methods. Moreover, factors like soil nutrient, pH, different soil temperatures or water should be taken into consideration, directly influencing the microorganism proprieties in plant promoting growth. Screening the influence of this factors can lead to a proper and better use of bacterial strains into an integrated management of tomato crop and more.

Further analyses are required in order to observe differences in qualitative tomato factors.

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