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BACTERIAL INOCULANTS FOR TOMATO SEED TREATMENT

Oana-Alina Boiu-Sicuia [1,2]*, Sorina Dinu [1], Lavinia-Diana-Nicoleta Barbu [1,2]

[1] Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., Bucharest, Romania
[2] University of Agronomic Sciences and Veterinary Medicine – Bucharest, Faculty of Biotechnology, 59 Mărăşti Blvd., Bucharest, Romania

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Abstract
Tomatoes are important vegetables in Romanian greenhouse and open field production. Improving their growth is a continuous concern that begins with seed germination and seedlings production and covers all culture types, conventional and organic. Microbial inoculants are valuable products that can trigger both growth promotion and plant protection. This study presents the beneficial traits of some bacterial inoculants applied as seed treatment on tomatoes. The tested inoculants contain single strains or a mixture of two beneficial bacteria, Bacillus amyloliquefaciens and Paenibacillus graminis. These bacterial treatments improve several growth parameters such as: plant fresh and dry weight, root length and branching. Although, the bacterial seed treatments delay plant emergence, the treatment stimulate later growth, revealing increased biomass weight. Compared to the untreated control, the mixt bacterial treatment improved plant fresh weight with 38.1% and the dry weight with 48.3%. Due to the biologic activity of these beneficial bacteria on plant growth, the mixed microbial inoculant offers similar benefits to a complex commercial fertilizer used for horticultural purposes.

Keywords: Bacillus amyloliquefaciens, Paenibacillus graminis, tomato seed treatment.

1. INTRODUCTION

Tomatoes are important vegetables in Romanian greenhouse and open field production. Due to the governmental support of tomato growers through the ”tomato program”, the total harvested area for tomatoes started to grow from 40 041 ha in 2017 to 40 741 ha in 2018 and 45 700 ha in 2019, respectively (https://gov.ro).

Improving tomato growth is a continuous concern for both farmers and agricultural researcher (Passari et al., 2019). Growth promotion and yield improvement start from seed germination and seedlings production, and reach the final harvest. These cover all culture types, conventional as well as organic (Wang et al., 2020; Ye et al., 2020).

Microbial inoculants are valuable products that can trigger both growth promotion, yield increases and plant protection attributes (Mayak et al., 2004; Haney et al., 2015; Răut et al., 2017; Kong et al., 2018; Ferreira et al., 2019). Therefore, the aim of this paper is to present the beneficial traits of some bacterial inoculants applied as tomato seed treatment. The tested inoculants contain *Bacillus amyloliquefaciens* OS17 and *Paenibacillus graminis* FL400 plant-beneficial bacteria, either as single strains or as bacterial mixture.

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

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2. MATERIALS AND METHODS

Bacterial strains

Bacillus amyloliquefaciens OS17 and *Paenibacillus graminis* FL400 are selected strains from the RDIPP microbial collection. These strains were also registered in international microbial collection NCAIM, under the numbers (P) B.001415 and (P) B.001365 respectively, and patented at the Romanian State Office for Inventions and Trademarks (OSIM).

Bacterial formulation

Bacteria were grown in LuriaBertani broth for 3 days, under continuous shaking at 28°C. Cells' biomass was harvested separately for each strain and pellets were resuspended in sterile phosphate buffer. The bio-products were maintained as single strain inoculants or as a consortium of mixed strains (1:1 v/v). Bacterial inoculants were then supplemented with carboxy-methyl-cellulose, at a final 2‰ concentration. Each inoculum was standardized at a final concentration of 10^8 cfu/ml (figure 1).

Figure 1. Important stages in bacterial growth and bio-products formulation

Plant growth conditions

The plant growth promotion tests were carried out on tomatoes (*Lycopersicon esculentum* Mill.) Buzău 1600 variety. The freshly prepared bacterial inoculants were applied as seed treatments. Five experimental variants were studied:

V1 = Untreated control
V2 = *Bacillus amyloliquefaciens* OS17
V3 = *Paenibacillus graminis* FL400
V4 = Mixed treatment (OS17 and FL400)
V5 = CropMax (comercial fertilizer)

Seeds were germinated in Cyg pouches (figure 2) incubated in Sanyo MLR351H growth chamber for 30 days under 16 hours photoperiod, 26ºC/light, 20ºC/dark, 14000 lx and 70% RH, using PNS (plant nutrient solution) for basic ferti-irrigation.

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Figure 2. In vitro grown tomatoes in Cyg pouches: emerged (a) and young (b) seedlings

One month after seeding, several biometric measurements were performed, such as tomato shoots length and fresh and dry weight of the plants.

3. RESULTS AND DISCUSSIONS

Tomato plants emerged five to seven days after sowing, depending on the seed treatment. Bacterial treatments delayed seeds germination by one to two days, compared to the untreated control. One month after seeding, shoots length was measured. No significant differences were noticed between untreated control, *B. amyloliquefaciens* OS17 seed treatment and CropMax fertilizer regarding shoots length. Shoots were, however, higher if the seed was treated with mixed bacterial inoculum of *B. amyloliquefaciens* OS17 and *P. graminis* FL400 strains (figure 3).

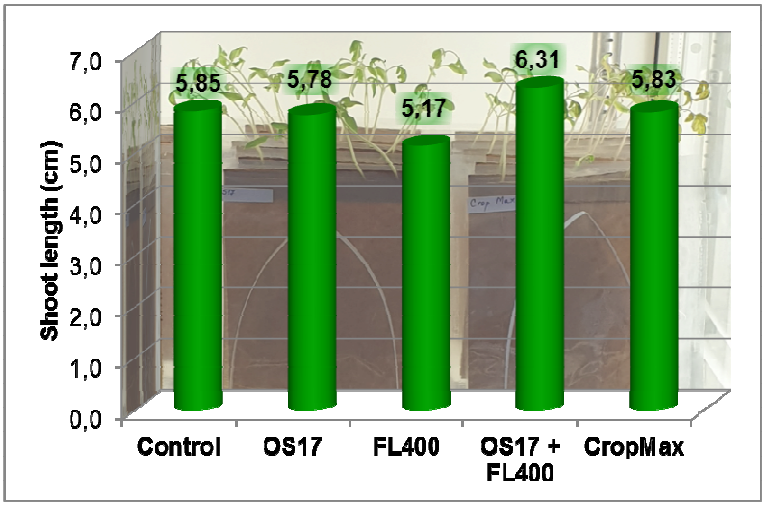

Figure 3. Tomato shoot length one month after seeding in Cyg pouches

Greener leaves effect and deeper roots were observed in CropMax fertilized tomatoes and mixed bacterial treatment (figure 4d), revealing a robust growth. Deeper roots could give some beneficial attributes to the plants in a classical growth system, were tomatoes are directly sown in the field (and the root tip is not chiselled as in seedlings production). Such root growth could help the plants to explore deeper soil layers, and possible soil water reserves, increasing plant adaptation in non-irrigated or drought conditions.

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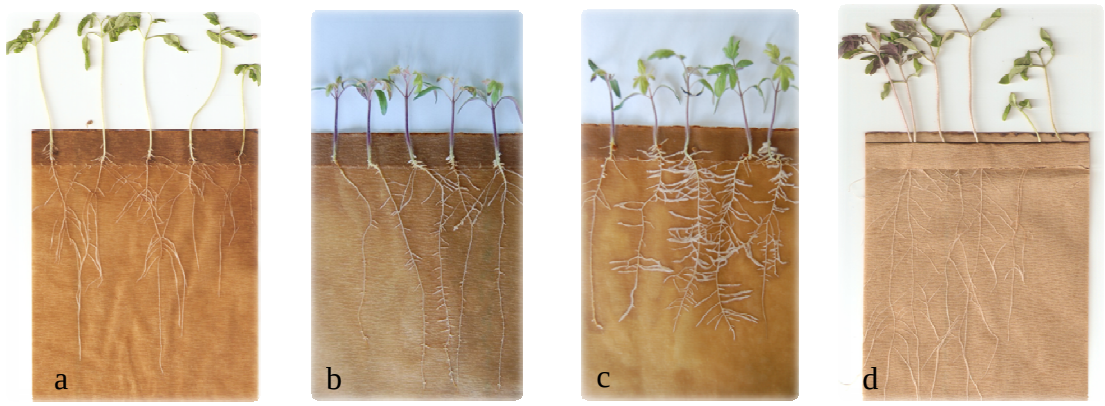

Figure 4. Comparative growth of tomato seedlings

The bacterial seed treatments substantially increased root branching (figures 4 b, c) compared to the untreated control (figure a). Such root growth improvement gives important advantages to the plants in any growth system, either soil or soil-less; directly sown or seedling planted cultures. Well-developed root systems increase plant sustainability and improve water and nutrient uptake.
Bacterial treatments also promoted plant vigour. Biomass fresh and dry weights were improved by bacterial seed treatments (figure 5 a, b). The mixed treatment revealed a synergic effect between the two plant beneficial strains.

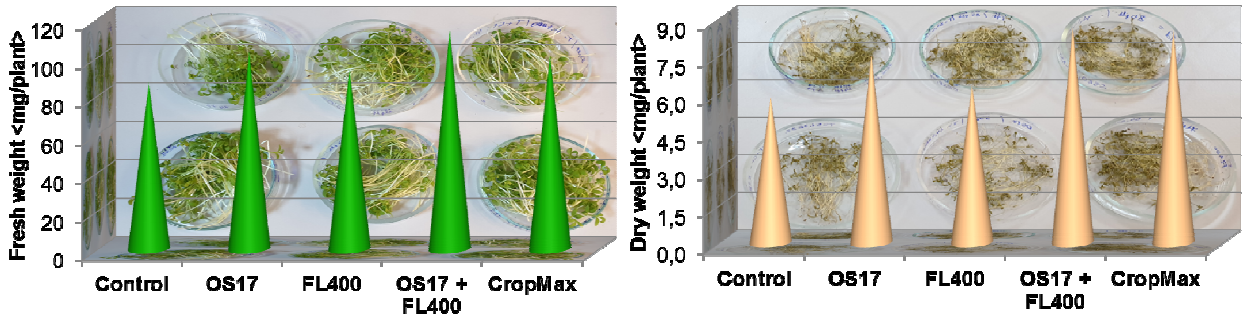

Figure 5. Tomato fresh (a) and dry (b) weight of one month plants

Best results were obtained the mixed bacterial seed treatment based on OS17 and FL400 strains for both fresh (114 mg/plant) and dry (8.6 mg/plant) plant weight.

4. CONCLUSIONS

- *Bacillus amyloliquefaciens* OS17 and *Paenibacillus gramins* FL400 are having synergic effects when applied together as tomato seed treatments.
- Bacterial seed treatments delay plant emergence but stimulate later growth, revealed as fresh and dry biomass weight.
- Mixed seed treatment with OS17 and FL400 beneficial strains improved plant growth similarly to CropMax commercial fertilizer.

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*Corresponding author, E-mail address: sicuia_oana@yahoo.com