

Allium cepa L. Inoculation with a Consortium of Plant Growth-Promoting Bacteria: Effects on Plant Growth and Development and Soil Fertility Status and Microbial Community [†]

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Abstract: The present work was aimed at investigating the effects of a four strains consortium—*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, and *Burkholderia ambifaria*—on crops of *Allium cepa* L. and its soil health. The bacterial consortium was inoculated on seeds of two different onion varieties; inoculated seeds and control ones (treated with autoclaved inoculum) were sown in open-field and followed until harvest. Plant growth development parameters, as well as soil physico-chemical and molecular profiles (DNA extraction and 16S community sequencing on the Mi-Seq Illumina platform), were investigated. The results showed a positive influence of bacterial application on plant growth, with increased plant height (+18%), total chlorophylls (+42%), crop yields (+13%), and bulbs dry matter (+3%) than the control. The differences between control and treated experimental conditions were also underlined in the bulb extracts in terms of total phenolic contents (+25%) and antioxidant activities (+20%). Soil fertility and microbial community structure and diversity were also positively affected by the bacterial inoculum. At harvest, the soil with the presence of the bacterial consortium showed an increase of total organic carbon, organic matter, and available P and higher concentrations of nutrients than control. The ecological indexes calculated on the molecular profiles showed that community diversity was positively affected by the bacterial treatment. The present work allowed to remark the effective use of plant growth-promoting bacteria as a valid fertilization strategy to improve yield in productive landscapes, whilst safeguarding soil biodiversity.

Keywords: biostimulants; PGPB; seed inoculation; sustainable agriculture; Illumina sequencing

1. Introduction

One of the current concerns in agriculture is improving the sustainability of productive landscapes and at the same time, obtaining high production rates. To achieve this goal, one of the promising and sustainable innovations could be the use of natural plant biostimulants [1]. Among them, plant growth-promoting bacteria (PGPB) are considered sustainable biostimulant agents, effective also in the presence of abiotic stresses factors [1]. Key traits for biostimulation are nitrogen fixation, nutrients solubilizing capabilities, and production of phytohormones [2]. During the past decades, PGPB formulations gained increasing attention, being highly regulated by European Union by Regulation EU 2019/1009. Seed inoculation is the most common method of application. In this

work, we investigated the biostimulant effectiveness of seed inoculation with a bacterial consortium formed by *Azospirillum brasilense* Cd, *Burkholderia ambifaria* PHP7, *Gluconacetobacter diazotrophicus* Pa15, and *Herbaspirillum seropedicae* Z67. The consortium of these strains already demonstrated good biostimulant effects on *Lycopersicon esculentum* L. [3], *Cannabis sativa* L. [4], *Artemisia eriantha* Ten [5], and ancient *Triticum* genotypes [6].

In this work, we hypothesized that this PGPB consortium could positively affect growth, development, yield, and quality traits of *Allium cepa* crops. To demonstrate the validity of this hypothesis, two onion varieties were chosen (“Meranto” and “Moondance”). Firstly, the bacterial adhesion on seeds was assessed by Scanning Electron Microscope (SEM). Then, two parallel one-year field experiments were carried out to evaluate the effectiveness of seed PGPB treatment on plants (i.e., height and chlorophylls amount) and bulbs (i.e., dry weight, total phenolic content, antioxidant activity). To evaluate the influence of this treatment on soil structure and fertility status, crop field samples were investigated for their physico-chemical and molecular profiles (DNA extraction and Next Generation Sequencing—NGS).

2. Material and Methods

The bacterial consortium was prepared as previously described by Botta et al. [3]. The bacterial adhesion on onion seeds (var. Moondance and Meranto) was assessed by Gemini 500 SEM (Zeiss, Oberkochen, Germany) following the procedure described by Pagnani et al. [6]. For field experiments, onion seeds were treated with the bacterial consortium and dried overnight at room temperature. Controls were treated in the same way, utilizing autoclaved inoculum. Sowings were performed by automatic seeder following a split-plot arranged experimental design. During the cultivations, plant height and chlorophylls [7] were monitored. At harvest, yields were quantified and bulbs were extracted [8] and investigated for their total phenolic content (by Folin-Ciocalteu reagent) and antioxidant activity (by 2,2-difenil-1-picrylhydrazyl - DPPH, 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) - ABTS, and ferric reducing antioxidant power - FRAP assays) [9].

Soil physico-chemical analyses were carried out according to the official methods described in “DM 13/09/1999 GU N°248 21/10/1999”. Genomic DNA was extracted utilizing the NucleoSpin® Soil kit (Macherey Nagel, Düren, Germany), following the manufacturer’s protocol. DNA samples were amplified by a specific 16S protocol for the amplification of bacteria and archaea, using paired-end 16S community sequencing on the Mi-Seq Illumina platform (Bio-Fab Research, Roma, Italy). The 16S V3 and V4 region were targeted by gene-specific sequences [10]. Classifications were obtained through a metagenomics workflow on Greengenes database (<http://greengenes.lbl.gov>). Data were processed by QIIME2 and Diversity indices were calculated using the R (R Foundation for Statistical Computing, Vienna, Austria) statistical package vegan v2.5-665.

3. Results and Discussion

Figure 1 shows the micrographs obtained by SEM, in which it is clear that bacterial strains were able to adhere to seed surfaces. The bacterial adhesion occurs thanks to biofilm formation, a basic characteristic generally attributed to strains belonging to the PGPB and key element for their association with plants [11].

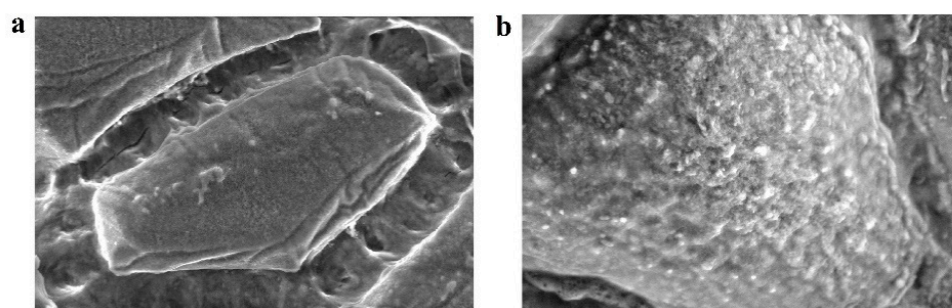


Figure 1. SEM micrographs at 5000× of (a) control and (b) inoculated *Allium cepa* seed.

The presence of PGPB promoted best growth and development of both plants and bulbs than the control (Figure 2). On average, increases were recorded in terms of: (i) plant height, 18% ($p < 0.001$); (ii) total chlorophylls, 42% ($p < 0.001$); (iii) crop yield, 13% ($p < 0.01$); (iv) bulbs dry matter, 3% ($p < 0.05$). Total phenolic contents and antioxidant activities of bulbs extracts also recorded positive increases—25% ($p < 0.01$) and 20% ($p < 0.05$), respectively. PGPB biostimulants properties are well-known and can be ascribed to several direct and indirect mechanisms [12]. The capability of synthesizing hormones, stimulates plant development and has positive influence on pigments synthesis [13]. The capability of solubilizing nutrients (e.g., P and K) allows plants to receive nourishment and helps them to thrive. Crop yield and bioactive compounds increases are other positive consequences of biostimulants effects.

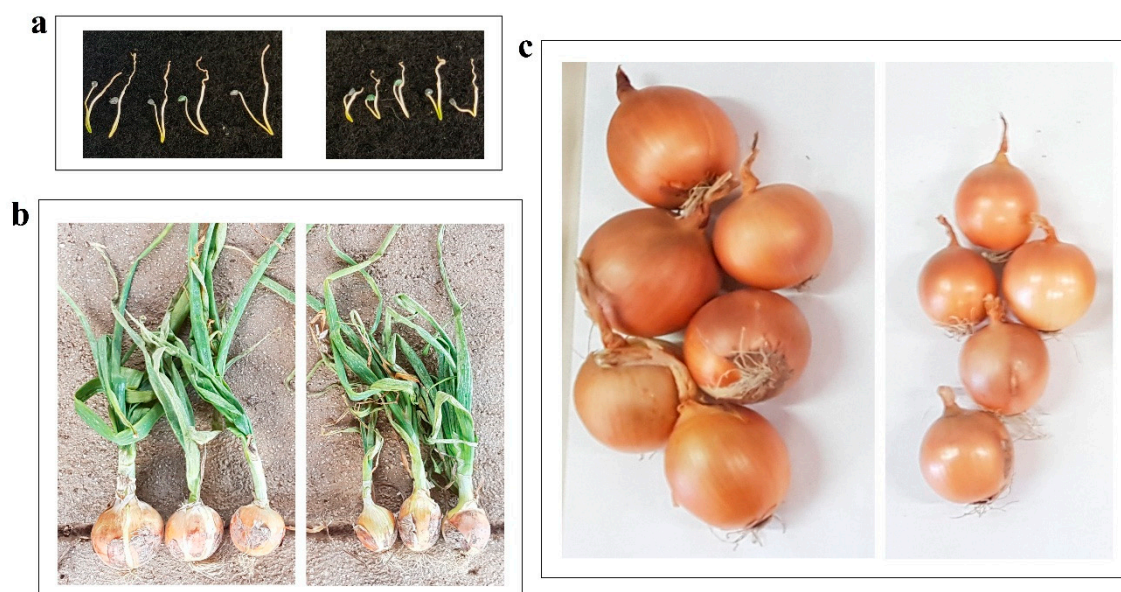


Figure 2. (a) Seedling, (b) plants, and (c) bulbs of *Allium cepa*. In the panels, inoculated plants are on the left, while controls are on the right.

PGPB positive influence was shown also in terms of soil fertility status. In both experimental fields, the application of PGPB consortium increased total organic carbon, organic matter, and available P and allowed to keep higher concentrations of the other nutrients. These findings have been already reported by other authors [14,15] and can be ascribed to the PGPB capability of nutrient mobilization and atmospheric nitrogen fixation [2]. The ecological indexes calculated on NGS results showed that seed bacterization was able to positively influence soil microbial community composition of both varieties, by increasing microbial biodiversity. The PGPB influence on the soil microbial community has been underlined also by other authors [16], however, the mechanisms behind it have not been yet completely clarified [17].

4. Conclusions

Our results underlined that the onion seed treatment with a selected PGPB consortium is an effective methodology that can significantly improve yield and quality of onion crops. Further studies should be undertaken to assess the biostimulant ability on different soils and environmental conditions. Anyhow, the results so far obtained suggest that increasing bacterial biodiversity could be a valid eco-friendly technique to increase food quality whilst safeguarding soil biodiversity.

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