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Combined bacterial and mycorrhizal inocula improve tomato quality at reduced fertilization

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ABSTRACT

Plant Growth Promoting Bacteria (PGPB) and Arbuscular Mycorrhizal Fungi (AMF) can positively affect plant nutrition and growth. Recent studies have also shown that rhizospheric microorganisms can result in improved fruit features. Aim of this work was to evaluate, in an industrial farming, the effects of three selected biostimulants (consisting of a mix of Plant Growth Promoting Bacteria and Arbuscular Mycorrhizal Fungi), employed in conditions of reduced fertilization on yield, fruit quality and nutritional value.

Tomato plants were inoculated with AM fungi and *Pseudomonas* sp. 19Fv1T or *P. fluorescens* C7, transplanted and grown in open field under conditions of reduced fertilization. The impact of the microorganisms on the fruit yield and nutritional value was assessed by measuring the production, fruit size and concentration of soluble sugars, organic acids, carotenoids and ascorbate.

The size and biomass of tomato fruits were affected by the inocula. Sugar concentration was increased by the selected microorganisms. All the mixtures induced an enhancement of malic acid, while double colonization with AMF and PGPB increased β-carotene concentration in fruits if compared to controls.

The results of the present study show that inoculation with soil microorganisms can help to drastically reduce the use of chemical fertilization, maintaining and, in some cases, even improving the tomato fruit yield and quality. This can lead to economical, environmental and human health benefits in relation to the increased sustainability.

1. Introduction

Plant growth-promoting bacteria (PGPB) represent a wide range of soil bacteria that can interact with plant roots, resulting in growth stimulation of their host. PGPB act as biostimulants, either directly by helping to provide nutrient to the host plant or indirectly by positively influencing root growth and morphology or by aiding other beneficial symbiotic relationships (Vessey 2003; Ramasamy et al., 2011; Gamalero et al., 2014). Rhizospheric fungi such as arbuscular mycorrhizae (soil fungi belonging to Glomeromycotina subphylum – Spatafora et al., 2016) are known to have plant growth-promoting effects, improving phosphorus and nitrogen absorption. This symbiosis directly influences plant responses (as growth and protein expression – Berta et al., 2014; Lingua et al., 2012; Bona et al., 2011, 2010) and plant physiology not only in the target organ (root), but also in shoot and in fruits and seeds (Bona et al., 2016). In particular, the AM symbiosis enhances yield and fruit quality in terms of taste, quality and vitamin concentration in strawberry fruits (Bona et al., 2015; Lingua et al., 2013; Castellanos-Morales et al., 2012, 2010), modulates sugar and carotenoid concentrations in tomato fruits (Bona et al., 2017; Copetta et al., 2011), induces the accumulation of carotenoids, chlorophylls and tocopherol in green and red leaf lettuces (Baslam et al., 2013), improves yield and quality of saffron (*Crocus sativus* L.) (Aimo et al., 2010), increases growth, flavour content and yield in *Allium sativum* L. in field conditions (Borde et al., 2009), impacts on phenolic content and antioxidant properties of artichoke leaves (Ceccarelli et al., 2010), modulates essential oil production in a number of plants, including *Artemisia annua* L. (Chaudhary et al., 2008) and in *Ocimum basilicum* L. (Copetta et al., 2006; Copetta et al., 2007).

This work is part of a project focused on the isolation and

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Table 1
Total fertilization inputs in the different treatments.

		CFD	CRD	Myc + 19Fv1T	Myc + C7	Myc + 19Fv1T + C7
Basal fertilization[a]	N (kg/ha)	150	150	150	150	150
	$(NH_4)HPO_4$ (kg/ha)	300	300	300	300	300
	KSO_4 (kg/ha)	330	330	330	330	330
Fertigation[b]	N (kg/ha)	109.78	84.44	84.44	84.44	84.44
	K_2O (kg/ha)	65.70	50.54	50.54	50.54	50.54
	CaO (kg/ha)	13.65	10.50	10.50	10.50	10.50
	SO_3 (kg/ha)	121.84	93.72	93.72	93.72	93.72

Plant treatments: CFD: Control 100, uninoculated plants with conventional fertilization; CRD: Control 70, uninoculated plants with 70% of the conventional fertilization; Myc + 19Fv1T: plants inoculated with AMF and with *Pseudomonas* sp. 19 Fv1T with 70% of the traditional fertilization; Myc + C7: plants inoculated with AMF and with *P. fluorescens* C7 with 70% of the traditional fertilization; Myc + 19Fv1T + C7: plants inoculated with AMF and with *Pseudomonas* sp. 19 Fv1T and *P. fluorescens* C7 with 70% of the traditional fertilization.

[a] Basal fertilization was homogeneously distributed in the field one week before transplanting.

[b] Fertigation was provided once a week by drip irrigation reaching the total amount reported in this table. The fertigation was provided according to a fertilization plan modulated in the different phenological phases of the tomato plants.

characterization of soil microorganisms to improve agronomic practices in the production of tomato with particular reference to the optimization of plant growth, yield and fruit nutritional value.

Previous results regarding the use of microorganisms alone and in combination in another variety of tomato were published by Bona et al. (2017). Aim of the present work was to evaluate, in an industrial tomato farming, the effects of three different selected biostimulants (consisting of PGPB and AM fungi mixed), in condition of reduced fertilization, on yield, fruit dimension, tomato parameters important for industrial transformation and fruit nutritional quality (sugar concentration, organic acid concentration, vitamin and antioxidant concentration) in order to check the effective potential use to reduce chemical fertilizer.

2. Materials and methods

2.1. Experimental design and plant growth

The experiment was carried out in a rectangular field with an area of about 2 ha. Plants were arranged in rows. Three rows (not on the borders) were used for the plants inoculated with the four different inocula. These three rows were separated by two rows of uninoculated plants. The distance between the rows was 1.2 m. Along each row, a set of 33 plants of each of the four inoculation treatments was separated by a set of ten uninoculated plants, used as spacers. The distance between adjacent plants within a row was 0.4 m.

The experiment included the growth of tomato plants at two different levels of fertilization i. e. 100% (traditional fertilization) and 70% of macronutrients, for a total of five treatments (99 plants each): CFD – uninoculated (control) plants fertilized according to the conventional practise; CRD – uninoculated (control) plants with reduced fertilization; Myc + 19Fv1T - plants inoculated with a mix of arbuscular mycorrhizal fungi (AMF – see below for details) and *Pseudomonas* sp. strain 19Fv1T and grown with reduced fertilization; Myc + C7 – plants inoculated with the same AMF mix and with *Pseudomonas fluorescens* C7 and grown with reduced fertilization; Myc + 19Fv1T + C7– plants inoculated with AMF and with *Pseudomonas* sp. 19 Fv1T and *P. fluorescens* C7 and grown with reduced fertilization.

The experiment was performed, between April and August, in a field located in Torre Garofoli (latitude 44°88′84″ N, longitude 8°79′92″ W, altitude 90 a.s.l.), close to Alessandria (Italy). According to its texture, the soil was classified as clay-loam (silt, 40%; clay, 28%; and sand, 32%) and had the following physical/chemical parameters: pH 8.2, soil organic matter content 1.5%, Cation Exchange Capacity (CEC) 19 meq/100 g, N 1.1 g/kg, P 8.7 ppm, K 177.7 ppm.

Tomato seeds of *Solanum lycopersicum* L., var. CXD 219 F1 (Velia S.r.l., San Valentino Torio – SA, Italy) were pre-germinated in 100 ml alveolar boxes on sterilized soil (100 °C at flowing steam for 1 h) and grown in a greenhouse: 20 ml of mycorrhizal inoculum and 10 ml of bacterial suspension (density about 10^8 CFU/ml) were provided to plantlets to be inoculated. After three weeks, 99 tomato plantlets per treatment were transplanted in open field and after two weeks (when they were well acclimatized), the bacterial inoculum was replicated, watering each plant with 200 ml of bacterial suspension (density about 10^8 CFU/ml). One week before transplanting, basal fertilization was homogeneously distributed in the field; after transplanting plants were fertigated weekly as described in Table 1 and watered when necessary using drip irrigation until harvesting (at tomato ripening, after four months). The fertigation was provided by Greenhas Italia (Canale, CN, Italy), according to a fertilization plan modulated in the different phenological phases of the tomato plants.

2.2. Microorganisms

The mycorrhizal inoculum consisting of fragments of colonized roots, spores, and hyphae of *Rhizophagus intraradices* (N.C. Schenck and G.S. Sm.), *Rhizophagus aggregatus* (N.C. Schenck and G.S. Sm.) C. Walker 2016, *Septoglomus viscosum* (T.H. Nicolson) (Redecker et al., 2013), *Claroideoglomus etunicatum* (W. N. Becker and Gerd.), and *Claroideoglomus claroideum* (N.C. Schenck and G.S. Sm.), provided by Mybasol s.r.l. (Alessandria, Italy), was used. The inoculum potential, tested by the provider before the experiment, was about 85,000 infective propagules/L of inoculum.

Two bacterial strains were used to inoculate the plants, in combination with the mycorrhizal inoculum. *Pseudomonas* sp. 19Fv1T (abbreviated: 19Fv1T) was isolated from the rhizosphere of *Vaccinium myrtillus* L. grown in a larch woodland located in Bellino (CN, Italy) and characterized as described in Bona et al. (2015). The 16S rDNA reference sequences of *Pseudomonas* sp. 19Fv1T are available at the NCBI World Wide Web database GenBank with the accession numbers KF752592.

Pseudomonas fluorescens strain C7 (briefly: C7) was kindly provided by Dr. Philippe Lemanceau (ECOLDUR, INRA, Dijon, France). The fluorescent *Pseudomonas* C7 was isolated from the rhizospheric soil of *Linum usitatissimum* from Châteaurenard as reported in Eparvier et al., (1991). Its beneficial effect in the biological control of *Fusarium* diseases has been described in different papers (Lemanceau and Alabouvette, 1991; Olivain et al., 2004).

19Fv1T and C7 physiological traits are fully described in Bona et al. (2017). Briefly, 19Fv1T synthesized siderophores (++), solubilized tricalcium phosphate (+) and produced the phytohormone indole acetic acid (IAA) (++++) and C7 synthesized siderophores (+/−), solubilized dicalcium and tricalcium phosphate (+) and produced the phytohormone IAA (+).