Title

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.

Keywords

Tomato, PGPB, pseudomonads, arbuscular mycorrhizae, fertilization, fruit quality

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 and 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; Castellanos-Morales et al., 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 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.

Materials and methods

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/100g, 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 1h) 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.

Table 1 Total fertilization inputs in the different treatments

		CFD	CRD	Myc+19Fv1T	Myc+C7	Myc+19Fv1T+C7
Basal fertilization[1]	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[2]	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

[1] Basal fertilization was homogeneously distributed in the field one week before transplanting
[2] 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.
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.

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 (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 (+).

Mycorrhizal colonization

174 Forty randomly chosen 1 cm-long pieces were cut from each root system, fixed in
175 70% ethanol, and then stored at 4 °C until analysis. Root pieces were cleared in 10%
176 KOH for 45 min at 60 °C, stained with 1% methyl blue in lactic acid and mounted on
177 a slide. Mycorrhizal colonization was estimated according to Trouvelot et al. (1986).
178
179 *Growth parameters, flower and fruit production analysis*
180 At the harvesting time, the number of flowers, allegation percentage (number of
181 flowers that became fruits), number of fruits, total fruit weight per plant, total weight
182 of mature fruits, percentage of not marketable fruit were evaluated in 25 plants per
183 treatments, randomly chosen. Root fragments from each plant sampled were collected
184 for the evaluation of AM colonization, as previously described.
185
186 *Fruit analyses*
187 Qualitative analyses of fruits were performed according to Bona et al. (2017). For the
188 biochemical analyses, eight pools of three plants for each treatment were built,
189 sampling one plant per row, per treatment. Therefore, each analysis consisted of eight
190 replicates. Fruit water percentage, dry biomass, concentration of sugars (sucrose,
191 glucose and fructose), organic acids (malic acid, citric acid) and vitamins (ascorbic
192 acid), pH value, titratable acids and carotenoids were analysed in order to investigate
193 the effect of bacteria and AMF on fruit quality. Analytical methods are described in
194 Bona et al. (2017).
195
196 *Statistical analysis*
197 Statistical analyses were performed with StatView 4.5 (Abacus Concepts) and
198 RStudio vs. 1.1.383. To assess differences between treatments for yield parameters

(number of flowers, percentage fruits/flowers, number of fruits, total fruit weight/plant, total weight of mature fruits, percentage of non-marketable fruits and collar diameter) and mycorrhization parameters, data were analysed using RStudio by Nested-ANOVA with cut-off significance at $P<0.05$.

To assess differences between treatments for fruits qualitative analysis, data were statistically analyzed by one-way ANOVA, followed by Fisher's probable least-squares difference test with cut-off significance at $P<0.05$.

Results and Discussion

In the present work, biostimulants (AM fungi and PGPB used in combination), were applied in the field, in a real industrial tomato farm. This approach gives direct agronomic information about application of biostimulants in order to reduce chemical inputs and on the assessment of their impact on tomato production and nutritional quality. Inoculation significantly increased mycorrhizal colonization and the abundance of arbuscules if compared to uninoculated plants (Table 2), that were very poorly colonized (about 0.2-0.8%). Arbuscular percentage present in mycorrhized root fragments (a%) showed that the mycorrhizal colonization was very active (close to 100% of arbuscule formation in all the cases).

Table 2 Flower and fruit production and AM colonization parameters of tomato plants

Parameters	CFD	CRD	Myc+ 19Fv1T	Myc+C7	Myc+19Fv1T+C7	P-value
Collar diameter	2.15 ± 0.06 a	1.73 ± 0.07 b	2.08 ± 0.06 a	2.02 ± 0.05 a	2.09 ± 0.05 a	< 0.0001 [a)]
N. Flowers	298.3 ± 17.8 a	246.1 ± 10.6 b	221.8 ± 13.3 b	270.0 ± 9.8 ab	303.3 ± 15.6 a	0.0001 [a)]
Percentage Fruits/flowers	36.2 ± 1.8 a	37.2 ± 1.2 a	38.0 ± 1.6 a	36.2 ± 1.2 a	35.9 ± 1.1 a	0.8051 [a)]
N. Fruits	103.1 ± 5.3 a	87.7 ± 3.6 b	79.0 ± 4.0 b	92.7 ± 3.4 ab	101.5 ± 4.2 a	0.0003 [a)]
Total Fruit Weight/plant (g)	5337.6 ± 249.8 a	4329.3 ± 194.2 b	3975.4 ± 232.6 b	4792.2 ± 201.4 ab	4789.3 ± 261.7 ab	0.0008 [a)]
Total Weight of Mature fruits	4284.3 ± 216.2 a	3613.5 ± 180.1 b	3460.5 ± 231.5 b	4330.2 ± 185.3 a	4125.7 ± 251.2 a	0.0103 [a)]
Percentage of non-marketable fruits	29.5 ± 3.9 a	20.2 ±1.7 bc	18.2 ± 1.7 b	15.6 ± 1.5 b	25.7 ± 2.5 ac	0.0006 [a)]
F %	5.24 ± 1.76 a	7.62 ± 2.38 a	30.00 ± 5.22 b	25.56 ± 2.57 b	22.22 ± 2.14 b	<0.0001 [a)]
M %	0.17 ± 0.09 a	0.70 ± 0.34 a	5.90 ± 1.70 b	4.84 ± 0.93 b	5.24 ± 1.25 b	0.0056 [a)]
A %	0.19 ± 0.11 a	0.80 ± 0.38 a	5.86 ± 1.70 b	4.83 ± 0.93 b	5.24 ± 1.25 b	0.0152 [a)]
V%	0	0	0	0	0	
a%	100.00 ± 0.00 a	95.00 ± 5.00 a	97.46 ± 1.36 a	99.85 ± 0.11 a	100.00 ± 0.00 a	0.1932 [a)]

Collar diameter, total number of flowers, percentage of flowers turning into mature fruit, total number of fruit, total weight of fruit production/plant, fresh weight, total weight of mature fruits and percentage of fruits not marketable of tomato plants inoculated or not with AMF, the two *Pseudomonas* strains, and fertilised with different amounts of nutrients. Frequency, intensity of mycorrhizal colonization, percentage of arbuscule and vesicles in tomato roots (according to Trouvelot et al., 1986). Different letters indicate significantly different values.

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)] Nested-ANOVA P-Values for the factor “treatment”.

Data related to M% were in agreement to those previously obtained by Bona and coworkers (2017) on another tomato variety, where no effect on M% was observed in double-inoculated plants, in contrast with results obtained by Gamalero et al. (2008) in which an increase in mycorrhizal colonization by other PGPB (*P. putida* UW4) was observed in cucumber plants. In our opinion this low level of mycorrhizal colonization could depend on two factors. The first is the field soil fertilization: high levels of N and P occurring in the field before the transplanting are known to negatively affect the AM symbiosis establishment (Bonneau et al. 2013). The second is the plant phenology at the moment of the mycorrhization check; it is well known that the extent of mycorrhizal colonization can be modulated by the plant phenological stage. Johnson et al. (1982) reported that AM colonization is reduced during chrysanthemum flowering due to the low amount of metabolites available in the roots for the fungal growth. Also, as reported in Bona et al. 2017, fruit production is a major sink for carbon, and a decrease of the carbohydrates available for the fungal partner can result in a decrease of colonization. Anyway, in our experimental conditions, we measured and confirmed a systemic effect induced by the presence of microorganisms (AMF and PGPB): this resulted in three main effects: i) restoring the yield (in terms of number of fruits) to the value of CFD plants and increasing the average tomato size, in spite of the reduced chemical fertilization, ii) increasing the sweetness of tomato fruits, and iii) boosting the concentration of molecules with high nutritional value in the fruits (citrate, ascorbate and carotenoids concentration).

Concerning the effects on plant morphology induced by the biostimulants, we observed that the collar diameter (Table 2) reduction occurred in CRD plants was restored at the CFD treatment level by the presence of microorganisms. This result is partially in agreement with those obtained in Bona et al. (2017) in a different tomato

258 variety, in which the fertilization reduction did not significantly reduced this
259 parameter. Plants co-inoculated with AMF and the two pseudomonads
260 (Myc+19Fv1T+C7) or only with *P. fluorescens* C7 (Myc+C7) produced a number of
261 flowers and fruits (and their total weight) comparable with that of CFD plants (table
262 2). On the contrary, the association Myc+19Fv1T didn't balance the reduction of
263 fertilization and resulted in the production of an amount of flowers similar to CRD
264 plants. Moreover, the percentage of not marketable fruits was significantly higher in
265 CFD plants if compared to Myc+19Fv1T and Myc+C7 ones, in which a reduction of
266 38% and 47% was observed, respectively.
267 Inoculation with soil microorganisms significantly increased fruit size (both length
268 and diameter) and weight (Table 3), in particular in Myc+C7 and in
269 Myc+19Fv1T+C7 (about 8% more than in CFD).
270 In agreement with the results obtained by Bona et al. (2017), the use of AM fungi and
271 PGPB (Myc+19Fv1T+C7 and Myc+C7) in tomato farm allowed to spare 30% of
272 chemical fertilizers without any yield reduction. AM fungi and PGPB are known to
273 influence on one hand plant nutrient balance, especially that of carbohydrates (Boldt
274 et al., 2011), and on the other hand hormone production (Torelli et al., 2000), these
275 two factors affect flowering and fruiting. Production of larger and heavier fruits is of
276 high economical interest (even more at reduced chemical inputs) and the influence of
277 AMF and PGPB in this direction is well documented in the literature: AMF and *P.*
278 *fluorescens* Pf4 or *Pseudomonas* sp. 5Vm1K induce a production of bigger
279 strawberries (Bona et al., 2015); Kapulnik et al. (2010) reported higher number of
280 fruits and an increase of oil yields in olive plants grown under field conditions and
281 colonized by *Glomus intraradices* (alone or in combination with *G. mosseae*); Wang
282 et al. (2008) observed an increased weight in cucumber fruits in plants inoculated

283 with *G. mosseae*. Finally, it is important to consider that the inoculation with the
284 beneficial microorganisms (Myc+19Fv1T and Myc+C7) significantly reduced the
285 percentage of non-marketable fruits, a result that is reported here for the first time,
286 underlying the positive influence of the bioinoculants also on the waste reduction in
287 agriculture.

Table 3 Fruit measurement, nutrient content and carotenoids of tomato in several treatments.

Parameters	CFD	CRD	Myc+ 19Fv1T	Myc+C7	Myc+19Fv1T+C7	P-Value
Average Fruit Fresh Weight (g)[a)]	64.3 ± 0.9 a	64.4 ± 0.9 a	67.1 ± 0.8 b	71.3 ± 0.6 c	69.6 ± 0.7 c	<0.0001
Average Fruit Length (cm)[b)]	5.81 ± 0.03 a	5.49 ± 0.03 b	5.88 ± 0.03 a	6.05 ± 0.02 c	5.97 ± 0.02 c	<0.0001
Average Fruit Diameter (cm)[c)]	4.62 ± 0.03 a	4.24 ± 0.02 b	4.64 ± 0.03 a	4.78 ± 0.02 c	4.77 ± 0.02 c	<0.0001
Percentage of water	94.79 ± 0.58 a	94.71 ± 0.17 a	94.72 ± 0.17 a	94.34 ± 0.09 a	93.76 ± 0.87 a	0.5477
Percentage of dry biomass	5.20 ± 0.58 a	5.29 ± 0.17 a	5.28 ± 0.17 a	5.66 ± 0.09 a	6.24 ± 0.87 a	0.5477
Glucose (g/kg)	10.45 ± 0.33 a	11.00 ± 0.35 a	10.66 ± 0.23 a	10.66 ± 0.27 a	11.83 ± 0.17 b	0.0046
Fructose (g/kg)	10.77 ± 0.51 a	11.14 ± 0.41 ab	11.70 ± 0.25 ab	11.91 ± 0.31 b	12.86 ± 0.15 b	0.0005
Sucrose (g/kg)	---	---	---	---	---	
Malate (mg/100g)	18.68 ± 0.50 ab	17.31 ± 0.37 a	20.19 ± 0.71 b	19.04 ± 0.82 b	19.31 ± 0.26 b	0.0071
Ascorbate (mg/100g)	5.47 ± 0.25 b	7.12 ± 0.44 c	7.36 ± 0.40 c	4.30 ± 0.31 a	10.75 ± 0.32 d	<0.0001
Citrate (g/100g)	0.15 ± 0.01 ab	0.14 ± 0.01 a	0.16 ± 0.01 b	0.16 ± 0.01 b	0.18 ± 0.01 c	<0.0001
Nitrite (mg/kg)	0	0	0	0	0	
Nitrate (mg/kg)	41.62 ± 8.17 a	63.27 ± 7.68 a	63.17 ± 6.15 a	46.85 ± 5.68 a	49.11 ± 15.37 a	0.4003
pH	4.3 ± 0.02 a	4.4 ± 0.04 ab	4.4 ± 0.09 ab	4.5 ± 0.05 b	4.3 ± 0.05 a	0.0233
Titratable acids (%CA)	0.2 ± 0.03 a	0.2 ± 0.01 a	0.2 ± 0.01 a	0.2 ± 0.01 a	0.2 ± 0.01 a	0.4112
β-carotene (µg/100g FW)	2.224 ± 0.051 a	2.178 ± 0.087 a	2.491 ± 0.071 b	2.829 ± 0.069 c	2.167 ± 0.062 a	<0.0001
Lycopene (µg/100g FW)	2799.90 ± 19.834 a	2889.28 ± 21.171 b	2652.97 ± 22.361 c	2408.24 ± 22.366 d	2708.93 ± 17.817 c	<0.0001
Luteine (µg/100g FW)	1.381 ± 0.014 a	1.377 ± 0.018 a	1.454 ± 0.014 b	1.501 ± 0.009 c	1.387 ± 0.016 a	<0.0001

Fresh weight, length, diameter, percentage of water, percentage of dry biomass, glucose concentration, fructose concentration, sucrose concentration, malate, ascorbate, citrate and nitrate concentration, pH, titratable acids, colors and carotenoids concentration of tomato of plants inoculated or not with AMF, the two *Pseudomonas* strains, and fertilised with different amounts of nutrients.

Plant treatments: CFD: Control 100, uninoculated plants with traditional 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.

299 Table 3 reports several qualitative parameters of tomato fruits (juice). Application of
300 the considered biostimulants didn't affect the percentage of dry biomass and water, in
301 contrast with the results obtained on another tomato variety where fruits of plants
302 treated with biostimulants showed levels of dry biomass double than those of
303 uninoculated plants (Bona et al., 2017). Concentrations of sugars differed in the
304 various treatments. In particular, sucrose was absent in the fruits of all treatments:
305 glucose and fructose concentrations were highest in the combined treatment
306 (Myc+19Fv1T+C7) (+ 13% glucose and + 19% fructose, if compared to CFD).
307 Fructose is the most important sugar for sweetness perception because is 2.30 times
308 sweeter than glucose (Keutgen and Pawelzik, 2007). This is an important result
309 because sweetness is particularly appreciated in tomatoes for industrial use.
310 Malate and citrate are the main organic acids in tomatoes, with citric acid as
311 predominant (Marconi et al., 2007). The concentration of these compounds increased
312 in inoculated plants (Myc+C7, Myc+19Fv1T and Myc+19Fv1T+C7). Moreover, the
313 combination of AMF with both pseudomonads induced also an increase of ascorbate
314 concentration, if compared to the other treatments (Table 3). These findings are
315 partially in accordance with what previously observed in another tomato cultivar
316 (Bona et al., 2017) where the Myc+19Fv1T+C7 treatment was not tested yet.
317 Nitrites were absent in the tomato juice and nitrates were not statistically different
318 between the various treatments and ranged from 41 to 63 mg/kg. pH was about 4.3 in
319 all treatments, except Myc+C7 that showed the highest values (4.5). Titratable acidity
320 was about 0.2% in all the treatments, but this parameter was not affected either by the
321 fertilization reduction or by the use of biostimulants. Moreover, the concentration of
322 ascorbic acid significantly increased in fruits of Myc+19Fv1T+C7 plants. Our results
323 are in agreement with those reported in the literature. For example, the concentration

of citric, ascorbic and succinic acids was significantly higher in fruits of pepper plants inoculated with *Azospirillum* and *Pantoea* and subjected to reduced N supply (del Amor et al., 2008). The accumulation of organic acids in fruits could be linked with nitrate metabolism and the synthesis of organic acids is essential for $N\text{-}NO_3$ assimilation (Stitt 1999). Nitrate levels can affect carbohydrate metabolism; in fact, during nitrate assimilation, carbohydrate synthesis decreased and more carbon is converted to organic acids (Stitt et al., 2002). Moreover, the balance between sugar and organic acid concentration is an important industrial parameter linked with consumer perception of the tomato taste (Baldwin et al., 2008).

Lycopene was the most abundant carotenoid of all samples of tomato fruits, followed by β-carotene. Lycopene concentration decreased in tomato juice from plants grown with Myc+C7 and Myc+19Fv1T if compared to that recorded in control plants (CFD and CRD), while β-carotene and luteine concentrations were higher in Myc+C7 and Myc+19Fv1T and not in Myc+19Fv1T+C7. These results showed the importance of mixed soil microorganisms in maintaining the concentration of these antioxidant compounds in fruits of tomato var. CXD 219 F1, under the regime of reduced fertilization, as previously reported by Ordookhani et al., 2010.

Conclusion

The results of the present study show that inoculation with beneficial 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 and the ecosystem services provided by the microbes.

Moreover, on the basis of the present and previous results obtained by Bona and coworkers on different tomato varieties, we can conclude that the microorganisms have different effects on plant if applied alone or in combination and that the use of mixed microorganisms (PGPB and AM fungi) seems to be more effective in increasing the production of secondary metabolites and consequently in affecting the fruit nutritional value. Finally, it's fundamental to test selected microorganisms on different plant varieties in order to assess predictability of the microbial effect.

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Conflict of interest

No conflict of interest declared.

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