INFLUENCE OF SOIL TREATMENT WITH MICROBIAL PLANT BIOSTIMULANT ON TOMATO YIELD AND QUALITY

F. Oancea[1,2], I. Răut[1], V. Zamfiropol-Cristea[2,1]

[1]National Institute for Research & Development in Chemistry & Petrochemistry - ICECHIM, 202 Spl. Independentei, 060022 Bucharest, Romania

[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd., sector 1, 011464 Bucharest, Romania

Abstract

The influence of plant biostimulant based on Azospirillum lipoferum DO12 and Brevibacillus parabrevis B50 on tomato yield and quality was studied. Bacterial biomass was formulated as alginate microbeads and applied to soil, during seedling plantation. The applied A. lipoferum DO12 and B. parabrevis B50 increase tomato marketable yield, on the first two waves, by 12.7% and, respectively, by 9.2%. A. lipoferum DO12 determined an increase of tomato content on lycopene (by 18.7%), vitamin C (by 25.4%) and total polyphenols (by 12.3%). Tomato treated with B. parabrevis B50 have lower increase on lycopene (by 8,6%) and vitamin C (by 15.4%), but higher increase on total polyphenols (by 17,2%).

Key words: *tomato, microbial plant biostimulant, yield and quality*

1. INTRODUCTION

Plant biostimulants are an emerging class of agricultural inputs. Their effects on plants are different from those exerted by the traditional inputs, fertilizers, pesticides (including plant growth regulators) and amendments. Under this umbrella term, which was relative recent introduced into scientific community, are included agricultural inputs that increase nutrient uptake and nutrient use efficiency, improve plant tolerance to abiotic stress and enhance crop quality (du Jardin, 2015). Plant biostimulants are divided into two major classes: microbial plant biostimulants and non-microbial plant biostimulants (Calvo et al., 2014). On the classes of microbial plant biostimulants are included plant growth promoting rhizobacteria (Ruzzi & Aroca, 2015); *Trichoderma* strains (López-Bucio et al., 2015) and arbuscular mycorrhizal fungi (Rouphael et al., 2015). Non-microbial plant biostimulants are organic, i.e. humic and fulvic acids (Canellas et al., 2015), seaweed extracts (Battacharyya et al., 2015), protein hydrolysates (Nardi et al., 2016, Colla et al., 2015), chitosan and short chain chitin oligomers (Winkler et al., 2017, Pichyangkura & Chadchawan, 2015) or inorganic, i.e. soluble silicon / silicic acid (Savvas & Ntatsi, 2015), selenium salt (Seciu et al., 2016), titanium (Lyu et al., 2017, Kovacik et al., 2016) and phosphite (Gómez-Merino & Trejo-Téllez, 2015). In the case of the microbial plant biostimulants and several organic plant biostimulant which are complex mixtures, such as humic and fulvic acids, seaweed extracts or protein hydrolysates the actives ingredients were not clearly defined. Such active ingredients are interacting with plant signaling processes (Brown & Saa, 2015).

Plant biostimulants represent one of the few solutions for mitigation of the abiotic stress effects on cultivated plants (Meena et al., 2017, Bulgari et al., 2015). However, due to their effects on the activation of plant secondary metabolism and accumulation of the bioactives compounds into edible yields, plant biostimulants are also an useful tool to promote production of fresh functional food with improved properties (Oancea et al., 2015, Donno et al., 2013).

Our group focused on microbial plant biostimulants which are releasing polyamines (Oancea et al., 2014b, Sicuia et al., 2012, Oancea et al., 2009) and their use for the activation of the winter cover crops mulch (Hoza et al., 2010, Vilău et al., 2010, Oancea, 2011). Polyamines, i.e. cadaverine, putrescine and superior polyamines derived from putrescine, spermidine and spermine, are plant growth regulators involved in the plant response to abiotic stress (Liu et al., 2015, Minocha et al., 2014, Alcázar et al., 2010), nutrient uptake and their use for plant development (Nahar et al., 2016, Pottosin & Shabala, 2014, Papenfus et al., 2013) and enhanced yield quality (Khalil et al., 2016, Zhou et al., 2016). Exogenous

polyamines from soil solution are up-taken by a specific root transporter (Fujita & Shinozaki, 2014, Mulangi et al., 2012) and increase the level of endogenous polyamines (Ondo Ovono et al., 2010, Shi et al., 2010). Because exogenous polyamines are increasing the level of the endogenous polyamines acting as plant growth regulators on plant features directly related to the targeted actions of plant biostimulant exogenous polyamines are good candidates of plant biostimulants active ingredients which are interacting with plant signaling processes.

Several plant growth promoting microbial strains isolated from rhizosphere were demonstrated to release polyamines as one of their main mechanism for crop biostimulation – *Azospirillum brasilense* Az39 (Rivera et al., 2014, Cassán et al., 2009); *Beijerinckia derxii* ICB-10 (Thuler et al., 2003); *Bacillus amyloliquefaciens* SQR9 (Chen et al., 2017); *Bacillus megaterium* BOFC15 (Zhou et al., 2016); *Bacillus pumilus* OS15 (Sicuia et al., 2012); *Bacillus subtilis* OKB105 (Xie et al., 2014); *Candida tropicalis* HY (Amprayn et al., 2012); *Paenibacillus graminis* FL400 (Oancea et al., 2013); *Streptomyces griseoluteus* WT (Nassar et al., 2003). Such plant growth promoting rhizobacterial (PGPR) strains are representing a good source for microbial plant biostimulants. As was already presented, the exogenous polyamines from soil solutions, including those produced by PGPR, are influencing the level of endogenous polyamines and, consequently, the plant signaling cascading.

We previously shown that microbial plant biostimulant based on bacteria which release polyamines increased the yield and the quality of the tomato grown on a green mulch, produced from hairy vetch (*Vicia villosa*) grown as a winter cover crop (Sicuia et al., 2010). Polyamine releasing strains of *Azospirillum brasilense* Sp001 and *Bacillus amyloliquefaciens* B165 accelerate first stages of tomato plant development, promoting earlier fruit setting. The fruit production significantly increased, by 30.76% (till 6.8 $kg.m^{-2}$) for the experimental treatment with Sp 001 strain, and by 23.07% on the experimental treatment with B165 strain (6.4 $kg.m^{-2}$), compared with the production from the control, hairy vetch mulch untreated with bacteria releasing polyamines, which was 5.2 $kg.m^{-2}$. Accumulation of the dry matter was significantly higher on B165 strain treatment (with 15.8%) and Sp 001 strain treatment (with 12.4%), compared to control, untreated mulch.

Our focus on tomato crop was in direct relationship with its importance. Tomato are among the most consumed fresh vegetable in the world and represent an important source of vitamin C, carotenoids and especially lycopene and antioxidant polyphenols (Perveen et al., 2015). Their content on bioactive phytonutrients was related to the cancer prevention (Martí et al., 2016) and to reduced risks for cardiovascular diseases (Canene-Adams et al., 2005).

Our goal on this study was to determine the effects on tomato crop of bacterial plant biostimulants based on polyamine releasing strains applied alone, without the support of the hairy vetch mulch, which is a source proteins / amino acids that is further converted into polyamines. For a better characterization of the plant biostimulants effect of the tested polyamine releasing strains, we evaluated also the influence of the applied microorganisms on the level of several bioactive compounds important for the quality characteristics of tomato fruit - lycopene, vitamin C and total polyphenols. Determination of these bioactive compounds, which have a significant influence on biomarker related to oxidative stress and inflammation (Jacob et al., 2007) and, respectively, cancer prevention (Martí et al., 2016), shall substantiate the contribution of the plant biostimulant to the production of fresh functional food.

2. MATERIAL AND METHODS

2.1. Biological material

We used for the experiments two strains isolated from Romanian soils. *Azospirillum lipoferum* DO12 was isolated from the soils of South-East Dobrogea (Topraisar), from a typical chernozem soil, by using the technique of successive enrichments in a nitrogen-free semisolid medium (Bashan & Levanony, 1985) and subsequent screening for the plant biostimulant characteristics (Mara et al., 2016, Laslo et al., 2012). The isolate was identified as being a strain of *Azospirillum lipoferum* species by a polyphasic taxonomy approach, including morphological characterization, physiological traits determination by Biolog™ technique and 16S rRNA and *nifH* gene sequence analyses (Oancea, 2011). *Brevibacillus*

parabrevis B50 was isolated from a soil sample from South Muntenia (Borcea), being selected from more than 125 isolates based on: *in vitro* antagonism against plant pathogens; release of polyamines and others plant growth stimulation compounds; innocuity on a mini-host test based on *Galleria mellonella* test. Its identification as a *Brevibacillus parabrevis* strain was done based on partial sequencing of gene coding for 16S RNA and membrane fatty acids profile analysis done with a Sherlock® Microbial ID system (Oancea et al., 2014a). The used tomatoes (*Lycoperson esculentum*) in the experiment from this work was cultivar Menhir F1 (Nunhems BV, Haelen, The Netherlands).

2.2. Experimental site and agricultural practices

We used an experimental field from Movilița, Ialomița County, Romania, located at 44° 39' 15" N latitude, 26°48'17" E longitude and 66 m altitude. The averages values of multi-annual temperature, sunshine daily duration and total precipitations for this site are: 11.6°C, 6.9 h and, respectively, 515 mm. Soil on the experimental site is a typical chernozem, developed on a loess parental rock. The tomato crop was established from six-week-old seedlings, which were transplanted into rows placed 0.7 m apart within a row and 0.5 m between plants in the row, according to the recommended cultivation technology for Ialomița area. Each plot consisted of 17,5 sqm, with 5 rows and 10 transplants per rows, i.e. 50 plants per repetition. Between plots were maintained buffer zones of 2 m wide as bare soil, by repeated hoeing, which were done each two weeks. The experiment was organized in a Latin square design, with 3 experimental treatments on 3 repetitions. The soil was fertilized with inorganic fertilizers (N – 160 $kg.ha^{-1}$, P – 120 $kg.ha^{-1}$, K – 120 $kg.ha^{-1}$), applied 5 days before tomato seedlings transplantation. Plant were irrigated by drip irrigation, in accordance to calculated evapotranspiration. The climatic conditions for 2016 period of tomatoes vegetation were characterized in general by a higher monthly temperatures (+2.3°C in April; +4.5°C in June; +3.7°C in July; +3,9°C in August) than the average multi-annual. Standard production practices for pest and weed management were followed.

2.3. Bioproducts and treatment application.

The polyamine producing plant biostimulant bacterial strains were cultivated into a low cost medium, which use as a carbon source raw glycerol produced during biodiesel fabrication from rapeseed oil (Oancea et al., 2004). We formulated the resulted biomass as a granulated bioproducts, based on the slighty modified method for bacteria encapsulation into dry alginate beds (Bashan et al., 2002). We added activated magnesium silicate 100-200 mesh (Florisil®) as a mild adsorbent which enhance bacterial strain survival and improve the controlled release (Oancea, 2011). The resulted dry alginate beds were applied as rhizosphere inoculant during seedling transplantation, in a dose of 25 grams per sqm. The experimental treatments were: V1 – control, tomato not treated with microbial plant biostimulants; V2 – tomato treated during transplantation with bioproduct based on *A. lipoferum* DO12 $2x10^8$ $cfu.g^{-1}$, applied in a dose of 25 grams per sqm; V3 – tomato treated during transplantation with bioproduct based on *B. parabrevis* B50 $3x10^9$ $cfu.g^{-1}$, applied in a dose of 25 grams per sqm.

2.4. Determination of plant growth and yield parameters.

The determination of the morphological characteristics were done on five tomatoes plant, harvested from each repetition, 80 days after transplanting. Roots were washed and the following morphological traits were determined: root length, plant height, fresh and dry weight of roots and of aerial parts. Dry weight was determined after drying until constant weight into an oven at 70°C. Total leaf area was measured using a Portable Leaf Area Meter (model LI-3000C, LI-COR Environmental, Lincoln, Nebraska USA). On the first two waves of harvested tomatoes the following yield parameter were determined: fruit set (%); fruit number ($plant^{-1}$), fruit fresh weight (grams), fruit dimension (diameter and length, in mm), total yield and marketable yield (grams). Marketable fruits were defined as those that were firm, without visible symptoms of insects or diseases damage and free of cracks in skin.

2.5. Determination of tomatoes on compounds representatives for yield quality

We determined the content of tomatoes fruit for the following compounds: lycopene, vitamin C and total polyphenols. Lycopene was extracted from tomatoes pure by a hexane/acetone/ethanol (50:25:25 v/v/v) mixture and determined by HPLC with UV-VIS detection, using methanol/acrylonitril (90:10 v/v) + triethylamine 9 μM as mobile phase (Barba et al., 2006). As instrumentation we used a Zorbax Extend-

C18 column (Agilent Technologies, Santa Clara, California, USA) and Agilent 1260 Infinity with diode array and multiple wavelength detector (Agilent Technologies). Vitamin C was determined after extraction with 4.5% metaphosphoric solution, by a HPLC-UV method, using 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile as mobile phase (Odriozola-Serrano et al., 2007). We used Zorbax NH2 column and Agilent 1260 Infinity with diode array and multiple wavelength detector (Agilent Technologies) as instrumentation. Total phenols were determined with Folin-Ciocâlteu reagent (Singleton et al., 1999), using known concentration of gallic acid for the calibration curve.

2.6. Statistical analysis

The obtained data were statistically processed by analysis of variance (JMP® v13.0, SAS Institute Inc., Cary, NC, USA). The least-significant differences (LSD) were calculated and compared with the differences between the means, based on Tukey–Kramer method ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Microbial plant biostimulants influence on yield parameters

The applied microbial plant biostimulants based on *A. lipoferum* DO12 and *B. brevis* B50 increase tomato marketable yield, on the first two waves, by 12.7% and, respectively, by 9.2% - Table 1. Such a significant increase of marketable yield in the first two waves resulted from two overlapping plant biostimulant effects (*i*) enhanced precocity, with an accelerated growth and development during early tomato growth stages and a more rapid fruit setting, as is resulting from Table 2, and (*ii*) improved fruit commercial quality, which increase the weight of the marketable fruits into the total yield – Table 1.

Table 1. Influence of the microbial plant biostimulant treatments on the yield parameter for the first two waves of tomato (*Lycoperson esculentum* cv. Menhir F1) production*.

Experimental treatment	Fruit set (%)	Fruit number (plant^{-1})	Fruit fresh weight (grams)	Fruit dimension (mm)		Yield per plant (grams)	
				diameter	length	Total	Marketable
V1 Control	59.33a	4.16a	155.1a	49.3b	41.8a	644	425
V2 *A. lipoferum* DO12 $2x10^8$cfu.g^{-1}	67.83b	3.83a	171.3a	58.6a	46.3b	689	479
V3 *B. parabrevis* B50 $3x10^9$ cfu.g^{-1}	64.66b	4.16a	168.4a	56.3a	43.1ab	683	464

*Value followed for the same letter did not differ significantly for $P < 0.05$

Table 2. Influence of the microbial plant biostimulant treatments on the morphological traits of tomato (*Lycoperson esculentum* cv. Menhir F1)*

Experimental treatment	Root length (cm)	Plant height (cm)	Total leaf area (cm^2)	Root weight (grams)		Aerial parts weight (grams)	
				fresh	dry	fresh	dry
V1 Control	68.4a	197.8b	7924.3b	65.8b	15.7b	1284.5b	162.3b
V2 *A. lipoferum* DO12 $2x10^8$cfu.g^{-1}	74.2b	217.6a	8575.8a	78.4a	17.8a	1484.4a	192.7a
V3 *B. parabrevis* B50 $3x10^9$ cfu.g^{-1}	75.3a	214.3a	8487.5a	75.7a	17.3a	1420.5a	187.5a

*Value followed for the same letter did not differ significantly for P <0.05

Tomatoes plant growth promotion by the beneficial bacteria, with an acceleration of the development, especially in the first stages, was demonstrated also for other bacterial strains - *Azospirillum brasilense* Sp7, Sp7-S and Sp245 (Mangmang et al., 2015); *Promicromonospora* sp., SE188 (Kang et al., 2012); *Bacillus subtilis* BEB-13b (Mena-Violante & Olalde-Portugal, 2007); *Enterobacter cloacae* CAL3 (Mayak et al., 2001). The plant growth stimulation is generally related to modulation of the plant level of phytohormones and/or growth regulators by the introduced beneficial bacteria (Ruzzi & Aroca, 2015, Dodd et al., 2010). Such plant biostimulation significantly depends on plant cultivar (Mangmang et al., 2015) and specific pedo-climatic conditions (Ruzzi & Aroca, 2015, Dutta & Podile, 2010). Microbial plant biostimulant are intended, by definition, to mitigate the abiotic stress through an activation of the plant defence mechanisms (Meena et al., 2017).

In our specific experimental conditions, characterized by higher temperature than normal, the applied plant biostimulant microbial strains increased fruit setting. Fruit setting is well-known to be a physiological process which is affected by the higher temperature (Peet et al., 1997). Our tested polyamine releasing strain mitigate this temperature stress, thus are acting as plant biostimulant. Another indirect proof of their action as plant biostimulants is the stimulation of plant growth and development during the early stages, which is in direct correlation with an enhanced photosynthesis and an improved nutrient uptake and efficient use.

3.2. Microbial plant biostimulants effects on compounds representatives for yield quality

We tested also the effect of treatment with our bacterial plant biostimulants on compounds representatives for yield quality of tomatoes – Table 3. *A. lipoferum* DO12 determined an increase of tomato content on lycopene (by 18.7%), vitamin C (by 25.4%) and total polyphenols (by 12.3%). Tomato treated with *B. brevis* B50 have lower increase on lycopene (by 8.6%) and vitamin C (by 15.4%). but higher increase on total polyphenols (by 17.2%).

Table 3. Effects of the microbial plant biostimulant treatments on the tomato (*Lycoperson esculentum* cv. Menhir F1) content on compounds representative for yield quality*

Experimental treatment	Lycopene (mg. kg^{-1} fresh weight)	Vitamin C (mg. kg^{-1} fresh weight)	Total polyphenols (mg GAE. kg^{-1} fresh weight)
V1 Control	275.25a	18.37a	1248.7a
V2 *A. lipoferum* DO12 $2x10^8$cfu.g^{-1}	326.72b	23.13b	1402.3ab
V3 *B. parabrevis* B50 $3x10^9$ cfu.g^{-1}	298.92ab	21.20ab	1463.5b

*Value followed for the same letter did not differ significantly for P <0.05

Our strains have a similar effects, promoting accumulation of bioactives compounds, as was reported also for others bacterial strain applied to tomato crop – *Azotobacter* spp. Az (Kumar & Sharma, 2015); *Pseudomonas putida* S41, *Azotobacter chroococcum* S5 and *Azosprillum lipoferum* OF (Ordookhani et al., 2013); *Rhodopseudomonas* sp. KL9 (Lee et al., 2008). Accumulation of bioactive compounds under the action of applied bacterial strain enhance the functional food characteristics of the tomatoes.

4. CONCLUSION

The applied *A. lipoferum* DO12 and *B. parabrevis* B50 increase tomato marketable yield, on the first two waves, by 12.7% and, respectively, by 9.2%.

A. lipoferum DO12 determined an increase of tomato content on lycopene (by 18.7%), vitamin C (by 25.4%) and total polyphenols (by 12.3%). Tomato treated with *B. parabrevis* B50 have lower increase on lycopene (by 8,6%) and vitamin C (by 15.4%), but higher increase on total polyphenols - by 17,2%.

ACKNOWLEDGMENTS

This work was supported by a grant of the Romanian Ministry for Research and Innovation. CNCDI – UEFISCDI, project PN-II-PT-PCCA-2013-4-0846, Contract 159 / 2014. Authors thanks to eng. Valeriu Zamfir and his team for the support on organizing field experiment.

REFERENCES

Alcázar R, Altabella T, Marco F, *et al.*, 2010. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta* **231**, 1237-49.

Amprayn K-O, Rose MT, Kecskés M, Pereg L, Nguyen HT, Kennedy IR, 2012. Plant growth promoting characteristics of soil yeast (Candida tropicalis HY) and its effectiveness for promoting rice growth. *Applied Soil Ecology* **61**, 295-9.

Barba AIO, Hurtado MC, Mata MCS, Ruiz VF, Tejada MLSD, 2006. Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chemistry* **95**, 328-36.

Bashan Y, Hernandez J-P, Leyva LA, Bacilio M, 2002. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. *Biology and Fertility of Soils* **35**, 359-68.

Bashan Y, Levanony H, 1985. An improved selection technique and medium for the isolation and enumeration of Azospirillum brasilense. *Canadian Journal of Microbiology* **31**, 947-52.

Battacharyya D, Babgohari MZ, Rathor P, Prithiviraj B, 2015. Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae* **196**, 39-48.

Brown P, Saa S, 2015. Biostimulants in agriculture. *Frontiers in Plant Science* **6**, 671.

Bulgari R, Cocetta G, Trivellini A, Vernieri P, Ferrante A, 2015. Biostimulants and crop responses: a review. *Biological Agriculture & Horticulture* **31**, 1-17.

Calvo P, Nelson L, Kloepper JW, 2014. Agricultural uses of plant biostimulants. *Plant and Soil* **383**, 3-41.

Canellas LP, Olivares FL, Aguiar NO, *et al.*, 2015. Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae* **196**, 15-27.

Canene-Adams K, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW, 2005. The tomato as a functional food. *The Journal of Nutrition* **135**, 1226-30.

Cassán F, Maiale S, Masciarelli O, Vidal A, Luna V, Ruiz O, 2009. Cadaverine production by Azospirillum brasilense and its possible role in plant growth promotion and osmotic stress mitigation. *European Journal of Soil Biology* **45**, 12-9.

Chen L, Liu Y, Wu G, Zhang N, Shen Q, Zhang R, 2017. Beneficial rhizobacterium Bacillus amyloliquefaciens SQR9 induces plant salt tolerance through spermidine production. *Molecular Plant-Microbe Interactions* **30**, 423-32.

Colla G, Nardi S, Cardarelli M, *et al.*, 2015. Protein hydrolysates as biostimulants in horticulture. *Scientia Horticulturae* **196**, 28-38.

Dodd IC, Zinovkina NY, Safronova VI, Belimov AA, 2010. Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology* **157**, 361-79.

Donno D, Beccaro GL, Mellano MG, Canterino S, Cerutti AK, Bounous G, 2013. Improving the nutritional value of kiwifruit with the application of agroindustry waste extracts. *Journal of Applied Botany and Food Quality* **86**, 11-5.

Du Jardin P, 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* **196**, 3-14.

Dutta S, Podile AR, 2010. Plant Growth Promoting Rhizobacteria (PGPR): the bugs to debug the root zone. *Critical Reviews in Microbiology* **36**, 232-44.

Fujita M, Shinozaki K, 2014. Identification of polyamine transporters in plants: paraquat transport provides crucial clues. *Plant and Cell Physiology* **55**, 855-61.

Gómez-Merino FC, Trejo-Téllez LI, 2015. Biostimulant activity of phosphite in horticulture. *Scientia Horticulturae* **196**, 82-90.

Hoza G, Oancea F, Sicuia O, Draghici E, 2010. Researches regarding the tomatoe culture in biocomposit mulching system. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture* **66**, 672.

Jacob K, Periago MJ, Böhm V, Berruezo GR, 2007. Influence of lycopene and vitamin C from tomato juice on biomarkers of oxidative stress and inflammation. *British Journal of Nutrition* **99**, 137-46.

Kang SM, Khan AL, Hamayun M, *et al.*, 2012. Gibberellin-producing Promicromonospora sp SE188 improves Solanum lycopersicum plant growth and influences endogenous plant hormones. *Journal of Microbiology* **50**, 902-9.

Khalil SA, Kamal N, Sajid M, *et al.*, 2016. Synergism of polyamines and plant growth regulators enhanced morphogenesis, stevioside content, and production of commercially important natural antioxidants in Stevia rebaudiana Bert. *In Vitro Cellular & Developmental Biology - Plant* **52**, 174-84.

Kovacik P, Simansky V, Wierzbowska J, Renco M, 2016. Impact of foliar application of the biostimulator mg-tytanit on the formation of winter oilseed rape phytomass and titanium content. *Journal of Elementology* **21**, 1235-51.

Kumar R, Sharma MK, 2015. Effect of different growing media and bio-fertilizers on tomato production in plastic greenhouses. *Indian Journal of Horticulture* **72**, 510-3.

Laslo É, György É, Mara G, Tamás É, Ábrahám B, Lányi S, 2012. Screening of plant growth promoting rhizobacteria as potential microbial inoculants. *Crop Protection* **40**, 43-8.

Lee KH, Koh RH, Song HG, 2008. Enhancement of growth and yield of tomato by Rhodopseudomonas sp under greenhouse conditions. *Journal of Microbiology* **46**, 641-6.

Liu J-H, Wang W, Wu H, Gong X, Moriguchi T, 2015. Polyamines function in stress tolerance: from synthesis to regulation. *Frontiers in Plant Science* **6**.

López-Bucio J, Pelagio-Flores R, Herrera-Estrella A, 2015. Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. *Scientia Horticulturae* **196**, 109-23.

Lyu S, Wei X, Chen J, Wang C, Wang X, Pan D, 2017. Titanium as a beneficial element for crop production. *Frontiers in Plant Science* **8**, 597.

Mangmang JS, Deaker R, Rogers G, 2015. Optimal plant growth-promoting concentration of Azospirillum brasilense inoculated to cucumber, lettuce and tomato seeds varies between bacterial strains. *Israel Journal of Plant Sciences* **62**, 145-52.

Mara G, Becze A, Varga O, Laslo É, Oancea F, 2016. Screening of multifunctional bacterial inoculants with lignocellulose degradation ability for agricultural applications. *Studia Universitatis Vasile Goldis Seria Stiintele Vietii (Life Sciences Series)* **26**.

Martí R, Roselló S, Cebolla-Cornejo J, 2016. Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *Cancers* **8**, 58.

Mayak S, Tirosh T, Glick BR, 2001. Stimulation of the growth of tomato, pepper and mung bean plants by the plant growth- promoting bacterium Enterobacter cloacae CAL3. *Biological Agriculture & Horticulture* **19**, 261-74.

Meena KK, Sorty AM, Bitla UM, *et al.*, 2017. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Frontiers in Plant Science* **8**, 172.

Mena-Violante HG, Olalde-Portugal V, 2007. Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): Bacillus subtilis BEB-13bs. *Scientia Horticulturae* **113**, 103-6.

Minocha R, Majumdar R, Minocha SC, 2014. Polyamines and abiotic stress in plants: a complex relationship. *Frontiers in Plant Science* **5**, 17.

Mulangi V, Chibucos MC, Phuntumart V, Morris PF, 2012. Kinetic and phylogenetic analysis of plant polyamine uptake transporters. *Planta* **236**, 1261-73.

Nahar K, Hasanuzzaman M, Rahman A, *et al.*, 2016. Polyamines confer salt Tolerance in mung bean (Vigna radiata L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. *Frontiers in Plant Science* **7**, 1104.

Nardi S, Pizzeghello D, Schiavon M, Ertani A, 2016. Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Scientia Agricola* **73**, 18-23.

Nassar AH, El-Tarabily KA, Sivasithamparam K, 2003. Growth promotion of bean (Phaseolus vulgaris L.) by a polyamine-producing isolate of Streptomyces griseoluteus. *Plant growth regulation* **40**, 97.

Oancea AO, Gaspar A, Seciu AM, *et al.*, 2015. Development of a new technology for protective biofortification with selenium of Brassica crops. *Agrolife Scientific Journal* **4**, 80-5.

Oancea F, 2011. Conservation agriculture system based on bioactive mulch produced from winter cover crop. *Scrisul Romanesc, Craiova*, 67-87.

Oancea F, Cornea P, Popa G, Sicuia O, Draganoiu M, Dinu S, 2009. Bacillus amyloliquefaciens strain antagonistic to Erwinia amylovora and elicitor of tomato defense response. *IOBC/WPRS Bull* **43**, 172.

Oancea F, Dinu S, Constantinescu F, Kupferberg S, 2004. Microbial conversion of glycerin waters resulted during the biodiesel production. *Analele Institutului de Cercetare-Dezvoltare pentru Protecția Plantelor* **33**, 319-28.

Oancea F, Dinu S, Popescu A, Mathe I, Abraham B, Lanyi S, 2013. Paenibacillus graminis strain promoting the nodulation of leguminous plant. *Patent RO125651 B1*.

Oancea F, Pairault AL, Doni M, Raut I, Calin M, Jecu ML, 2014a. Strain of Brevibacillus parabrevis and controlled release composition based on it. *Patent EP2765185 A2*.

Oancea F, Szabolcs L, Oancea A-O, *et al.*, 2014b. Selenium biofortification biotechnologies of wheat grain in south-eastern part of Romania for a better human health. *Studia Universitatis" Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series)* **24**, 47-56.

Odriozola-Serrano I, Hernández-Jover T, Martín-Belloso O, 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry* **105**, 1151-8.

Ondo Ovono P, Kevers C, Dommes J, 2010. Tuber formation and development of Dioscorea cayenensis–Dioscorea rotundata complex in vitro effect of polyamines. *In Vitro Cellular & Developmental Biology - Plant* **46**, 81-8.

Ordookhani K, Moezi A, Khavazi K, Rejali F, 2013. Effect of plant growth promoting rhizobacteria and mycorrhiza on tomato fruit quality. *Acta Horticulturae* **989**, 91-6.

Papenfus HB, Kulkarni MG, Stirk WA, Finnie JF, Van Staden J, 2013. Effect of a commercial seaweed extract (Kelpak®) and polyamines on nutrient-deprived (N, P and K) okra seedlings. *Scientia Horticulturae* **151**, 142-6.

Peet MM, Willits DH, Gardner R, 1997. Response of ovule development and post-pollen production processes in male-sterile tomatoes to chronic, sub-acute high temperature stress. *Journal of Experimental Botany* **48**, 101-11.

Perveen R, Suleria HaR, Anjum FM, Butt MS, Pasha I, Ahmad S, 2015. Tomato (Solanum lycopersicum) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims - a comprehensive review. *Critical Reviews in Food Science and Nutrition* **55**, 919-29.

Pichyangkura R, Chadchawan S, 2015. Biostimulant activity of chitosan in horticulture. *Scientia Horticulturae* **196**, 49-65.

Pottosin I, Shabala S, 2014. Polyamines control of cation transport across plant membranes: implications for ion homeostasis and abiotic stress signaling. *Frontiers in Plant Science* **5**, 16.

Rivera D, Revale S, Molina R, *et al.*, 2014. Complete genome sequence of the model rhizosphere strain Azospirillum brasilense Az39, successfully applied in agriculture. *Genome Announcements* **2**.

Rouphael Y, Franken P, Schneider C, *et al.*, 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae* **196**, 91-108.

Ruzzi M, Aroca R, 2015. Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae* **196**, 124-34.

Savvas D, Ntatsi G, 2015. Biostimulant activity of silicon in horticulture. *Scientia Horticulturae* **196**, 66-81.

Seciu A-M, Oancea A, Gaspar A, *et al.*, 2016. Water use efficiency on cabbage and cauliflower treated with a new biostimulant composition. *Agriculture and Agricultural Science Procedia* **10**, 475-84.

Shi J, Fu XZ, Peng T, Huang XS, Fan QJ, Liu JH, 2010. Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidative capacity and stomatal response. *Tree Physiology* **30**, 914-22.

Sicuia O-A, Oancea F, Constantinescu F, Dinu S, Cornea CP, 2012. Bacillus strains useful in improving vegetal mulch technology trough bio-activation. *Romanian Biotechnological Letters* **17**, 7611.

Sicuia O, Oancea F, Dinu S, *et al.*, 2010. Weed bio-control and tomato plants growth promotion by applying an alternative cultivation system into bio-composite mulch. *Romanian Journal for Plant Protection* **3**, 23-36.

Singleton VL, Orthofer R, Lamuela-Raventós RM, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocâlteu reagent. *Methods in Enzymology* **299**, 152-78.

Thuler DS, Floh EIS, Handro W, Barbosa HR, 2003. Beijerinckia derxii releases plant growth regulators and amino acids in synthetic media independent of nitrogenase activity. *Journal of Applied Microbiology* **95**, 799-806.

Vilău N, Vilău F, Oancea F, Dinu S, 2010. Soybeans planted in an alternative system based on bioactive plant mulch. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series* **40**, 255-9.

Winkler A, Dominguez-Nuñez J, Aranaz I, *et al.*, 2017. Short-chain chitin oligomers: promoters of plant growth. *Marine Drugs* **15**, 40.

Xie S-S, Wu H-J, Zang H-Y, Wu L-M, Zhu Q-Q, Gao X-W, 2014. Plant growth promotion by spermidine-producing Bacillus subtilis OKB105. *Molecular Plant-Microbe Interactions* **27**, 655-63.

Zhou C, Ma Z, Zhu L, *et al.*, 2016. Rhizobacterial strain Bacillus megaterium BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance. *International Journal of Molecular Sciences* **17**, 976.