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Biochar and zeolite as carriers of PGPB strains: Impact on the physiology, productivity and quality characteristics of industrial tomato

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Abstract: Plant Growth Promoting Bacteria (PGPB) are emerging as a key solution for enhancing agricultural productivity in response to the growing global food demand. While PGPB enhance crop productivity, low bacterial survival in soil environments often reduces their field effectiveness. This study evaluated the impact of six PGPB strains, applied through three different carriers (liquid, zeolite and biochar) on the physiology, growth, yield and quality of industrial tomato, cultivated under Mediterranean field conditions. Results showed that, in comparison to the control, Bacillus subtilis strains 548, 557 and Z3 significantly increased the dry weight of tomato plants by up to 32.93% and yield by up to 23.8%. Photosynthetic rate was enhanced by up to 33.7% by the use of PGPB treatments and among carriers, biochar presented increased values compared to liquid and zeolite. Due to Carrier-PGPB interaction, certain combinations improved quality parameters, such as antioxidant capacity (up to 42% higher with B. subtilis 557 and zeolite) and total phenolic content (48% increase with B. subtilis 557 and Zeolite as a carrier). Overall, these findings highlight that optimizing the interaction between PGPB strains and suitable carriers is key to enhancing both yield and quality of industrial tomato, providing a mechanistic basis for the development of more efficient and sustainable biostimulant strategies.

Keywords: plant growth promoting bacteria; strains; biostimulants; carriers; biochar; zeolite; industrial tomato; quality characteristics.

1. Introduction

With the global population expected to grow by around 2 billion by 2050, agricultural and food production will need to increase by two-thirds to meet the rising demand and ensure adequate nutrition[1]. This poses a major challenge in modern agriculture and has led to the adoption of alternative cultivation practices to increase agricultural productivity. In recent years, the use of biostimulant formulations has

gained the interest of the research community as they improve the yield and quality characteristics of plants, promoting also sustainable agriculture. Plant biostimulants can be substances (organic and inorganic) as well as microorganisms, such as beneficial bacteria, that promote plant growth, nutrient uptake and protect crops from abiotic stresses[2,3]. Plant Growth Promoting Bacteria (PGPB) are a category of beneficial bacteria and are considered a promising agricultural practice as it enhances crop yield in an environmentally friendly way without causing adverse side effects[4]. These bacteria mainly colonize around plant roots in the soil (rhizosphere) but can also be found inside plant tissues (endosphere) and on the surface of aerial parts of the plant, such as leaves or stems (phyllosphere)[5,6]. This colonization plays a dynamic role in plant vigor, directly promoting growth and yield through several mechanisms (e.g., facilitating resource acquisition or modulating plant hormone levels) and indirectly by suppressing the harmful effects of various pathogens on plant growth and development, acting as biocontrol agents[7].

The implementation of PGPB as a means to increase crop growth, yield and quality characteristics has been investigated over the years by many researchers, in different crops with interesting results leading to case-specific questions[8–10]. Questions like, how effective is the inoculation method to their activity[11], or which strains of a specific genus can be potentially used as biostimulants to improve crop growth[12] have been in the center of attention. For *Bacillus* spp. strains, there are a lot of experimental data demonstrating their plant growth promoting abilities. For example, it has been found that a mix of *Bacillus subtilis* (CNPMS B2084-BRM034840) and *Bacillus megaterium* (CNPMS B119-BRM033112) when inoculated at *Phaseolus vulgaris* L. crop increased its yield by 12% and dry matter up to 7.80 g per plant[13]. These experiments show the importance of the use of PGPB in different crops, however, there is an increasing interest in more effective methods of application of the microbial biostimulants.

It has been observed that the effectiveness of free-living bacteria and their availability to plants decrease due to the complexity of the soil environment[14]. This issue of bacterial survival creates a major challenge for researchers seeking solutions to optimize their release rate and enhance effectiveness[15]. To address this challenge, researchers are investigating a wide range of soil amendments as microbial carriers since, due to their high porosity, they can provide a suitable habitat with stable conditions, ensuring high survival of bacteria[16,17]. The origin of the carriers can be organic, such as biochar, peat and sawdust, or inorganic, such as zeolite, vermiculite and palygorskite[18,19]. These materials differ in form and exhibit various properties, such as composition of organic matter, water-holding capacity, processing method, friability and vulnerability.

A promising carrier could be biochar, a multi-functional solid product obtained by heating biomass in an oxygen-limited environment (pyrolysis). Its composition and properties mainly depend on the type of biomass used, as well as the pyrolysis temperature and residence time[20]. Biochar is characterized by high porosity and moisture-holding capacity, which benefits microbes and is enriched with organic carbon and contains nitrogen, phosphorus and potassium. According to Ajeng et al.[4], enriched biochar can function as both a nutrient source and a microbial carrier for soil application. Additionally, biochar's functional groups, including hydroxyl, carboxyl, sulfonic acid, amino, imino, and acylamino groups, promote microbial cell adhesion and proliferation. Research has shown that using biochar as a carrier

enhances the persistence, survival and colonization of inoculated microbes in soil and plant roots which play a crucial role in soil biochemical processes, nutrient and carbon cycling, and soil contamination remediation[18,21,22]. Another potential carrier, zeolite are hydrated aluminosilicates of alkaline and alkaline earth elements, existing in more than 50 natural and 150 synthetic forms. These minerals are considered to contribute significantly to improve soil physical and chemical properties, Cation Exchange Capacity (CEC), saturated hydraulic conductivity (Ks), water holding capacity and because of their unique porous structures, they can efficiently retain water and nutrients[23]. High porosity and large surface area of zeolite have made them useful for the immobilization of microorganisms[24]. Furthermore, zeolite have been found to increase soil pH, while their effect on the cation exchange capacity, and subsequently the nutrient availability, triggers microbial metabolic activity stimulation (e.g., increase in dehydrogenase activity) and soil organic matter alteration[25].

Researchers have applied PGPB inoculated onto biochar and zeolite to evaluate their effect on plant growth, soil properties and microbial population under different environmental conditions. For example, bamboo biochar inoculated with *Pseudomonas putida* Rs-198 was found to improve grape quality traits, soil nutrient content as well as microbial community[26]. Similarly, bacterial community structure was favored by the application of wheat straw-based biochar inoculated with *Alcaligenes faecalis* WZ-2 to tebuconazole-contaminated soils[27]. Hale et al.[15] used four different types of biochar (palm fronds, pinewood, peanut shell, stone fruit pits) with *Enterobacter cloacae* UW5 and reported that pinewood-based biochar was the greatest carrier as it increased the bacterial population density. Moreover, the application of zeolite carrier with strains of *Pseudomonas putida*, *Serratia ficaria* and *Pseudomonas fluorescens* increased the water use efficiency of wheat crops under salinity conditions in a pot experiment[19]. It has also been shown that immobilized nitrogen-fixing bacteria in zeolite stimulated plant growth in maize plants and improved nitrogen content as well as organic matter in the soil[28].

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and economically important vegetable species worldwide, with millions of hectares under cultivation and a global production of up to 180 million tons[29,30]. Tomatoes fruits are considered as nutrient-dense food because they contain vitamins, proteins, minerals and dietary fibers in addition to bioactive compounds like lycopene, β-carotene and other antioxidants that contribute to human health[31]. However, in order to maintain high yields and fruit quality, tomato cultivation requires significant inputs of fertilizers and amounts of irrigation water[32], which leads to resource waste and environmental effects[33]. To encounter these needs and the climate change, which results abiotic stresses, the application of PGPB has emerged as a sustainable strategy to reduce these challenges, with research already proven that these beneficial bacteria help not only the resilience of the tomato plants but also, they have positive effects on the quality characteristics of tomato fruits[8,34–36].

Despite the proven individual benefits of PGPB and soil amendments, a substantial research gap still exists concerning the comparative field evaluation of particular PGPB, especially *Bacillus* strains, combined with various carriers in the soil. Hence, the aim of this study was to assess the effect of six different strains of PGPB in interaction with three carriers (liquid, biochar and zeolite) on industrial tomato (*Solanum lycopersicum* L.) crop under Mediterranean soil and climatic conditions.

Measurements of plant growth, physiology, yield and quality traits were conducted to evaluate the effect of the different strains combined with the carriers.

2. Materials and Methods

2.1 Experimental Site and Plant Material

The field experiment was conducted in Oropos, Attica, Greece (38°18′ N, 23°45′ E) at an altitude of 45 m. The industrial tomato used was hybrid H1015 (Heinz, D. Sandros S.A. Commerce of Agricultural Supplies, Thessaloniki, Greece), which is a productive, resilient, firm, with high Brix levels, and good color hybrid. The tomato seedlings were transplanted on 13 April 2022, and the harvest was conducted on 3 August 2022. The in-field conditions (air temperature, relative humidity and precipitation) were logged by a weather station Weatherhub (TFA Dostmann GmbH & Co. KG, Wertheim-Reicholzheim, Germany) and they are presented in Figure 1.

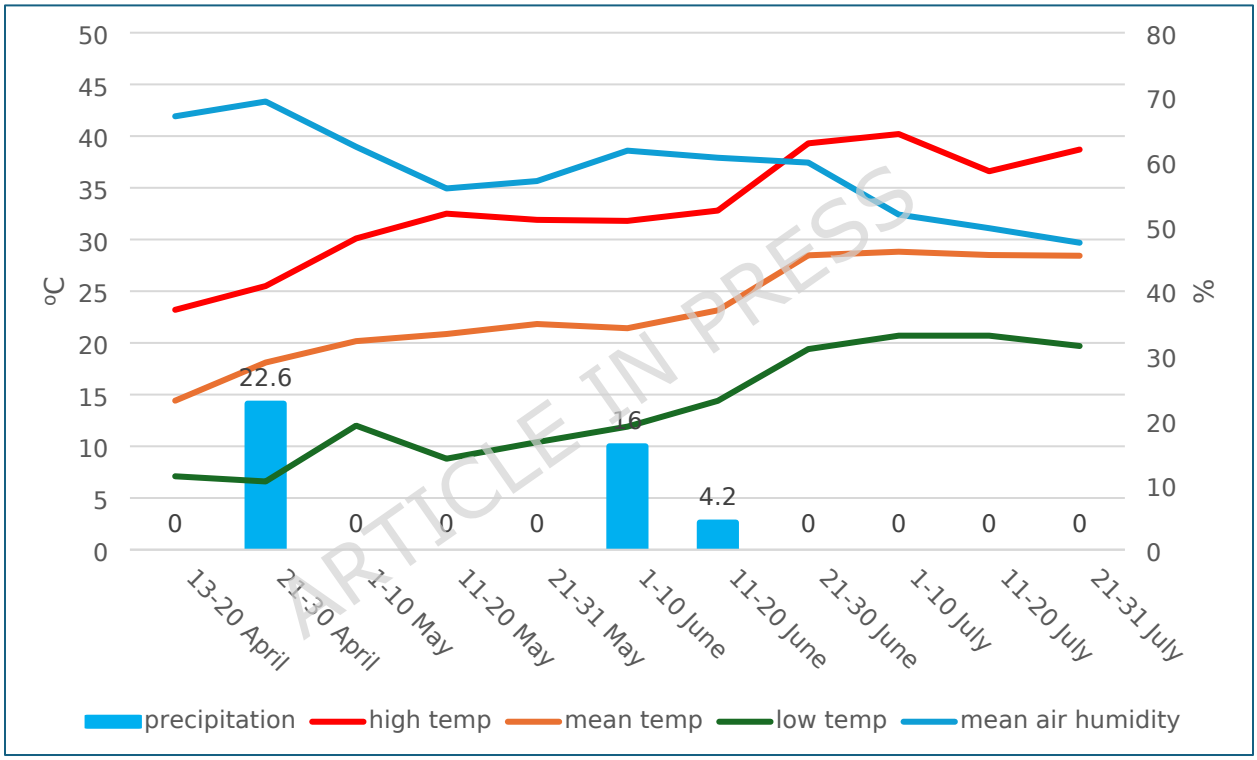

Figure 1. Daily mean, high and low temperature, mean air humidity and precipitation at the experimental field at Oropos during the experimental period.

2.2. Soil Analysis and Field Management

Two weeks before transplanting, a composite soil sample was collected from four representative points of the experimental field at a depth of 0–30 cm. The parameters studied and the analytical methods that were used are presented in Table 2. The results indicated that the soil of the experimental site has a clay loam texture and a slightly alkaline pH. Electrical conductivity and the concentration of total soluble salts were within normal ranges, suggesting satisfactory salinity levels and overall favorable conditions for tomato cultivation. The organic matter content was adequate for typical Greek soils, classifying the field as fertile but the total

nitrogen content was found to be low. In contrast, available phosphorus and exchangeable cations (potassium, calcium, and magnesium) were present at high levels. Regarding micronutrients, available copper was found at high concentrations, while available iron and manganese were at satisfactory levels. Conversely, zinc and boron concentrations were low, likely due to the elevated levels of available phosphorus in the soil. After collecting the soil samples and before transplanting, a basal fertilization was applied using a 12-10-20 (N-P-K) fertilizer at a rate of 1200 kg ha-1. This application was performed to simulate common local agricultural practices for industrial tomato cultivation under real field conditions.

Table 2. Soil physical and chemical properties before the agricultural practices

Parameters	Values	Method
Sand (%)	34	Bouyoucos[37]
Silt (%)	28	
Clay (%)	38	
Soil texture	Clay loam	
pH	7.7	pH-meter
Saturation percentage (%)	52	furnace at 105 °C
Electrical conductivity (mS $cm^{-1)}$	1.41	ISO 11265:1994[38]
Total salts (%)	0.05	calculation
Organic matter (%)	4.2	ISO 14235:1998[39]
Total nitrogen (mg g^{-1})	1.1	ISO 11261:1995[40]
Available K (cmo$_c$ kg^{-1})	1.5	atomic absorption - spectrometry[41]
Available Ca (cmo$_c$ kg^{-1})	17	
Available Mg (cmo$_c$ kg^{-1})	14	
Available P (mg kg^{-1})	43	ISO 11263:1994[42]
Fe-DTPA (mg kg^{-1})	13	DTPA[43]
Cu-DTPA (mg kg^{-1})	5.9	
Zn-DTPA (mg kg^{-1})	2.4	
Mn-DTPA (mg kg^{-1})	17	
Available B (mg kg^{-1})	0.57	Bingham[44]

2.3 Bacterial Strains and Inoculum Preparation

The bacterial strains originating from the corn rhizosphere were collected and belong to Agrounik d.o.o. (Belgrade-Zemun, Serbia). The bacteria were isolated by the streaking method. The identification was conducted by sequencing 16S rDNA, following the process described by Katsenios et al.[45] and the cultivation was carried out as described previously by Efthimiadou et al.[46]. The liquid carrier, which was tap water, was diluted at a ratio of 1:100 to achieve a bacterial concentration of 10^7 CFU/ml. The PGPB treatments were soil-applied at a rate of 7 L ha^{-1} for the liquid carrier, targeting the rhizosphere zone of each tomato plant. The application rates for the other two carriers were calculated to provide the plants with the same amount of microorganisms in CFU per plant. For the control treatments, pure water, zeolite and biochar were used.

2.4 Carrier Characteristics and Immobilization Process

Regarding the solid carriers, zeolite granulation was 2.5-5 mm, while biochar (Basna d.o.o., Čačak, Serbia) was made of hardwood only and had an internal surface area of 350 - 400 m^2/g. The biochar's particle distribution was characterized by diameters of 3.43 µm at 10% (D_{10}), 16.94 µm at 50% (D_{50}) and 45.34 µm at 90% (D_{90}) with a mean diameter of 21.10 µm. Both materials were obtained as thermally processed products. Zeolite was activated at temperatures of >400 °C and biochar was produced under high-temperature pyrolysis at 800°C. According to ISO 11465:2002, the initial moisture content was 2-3% for zeolite and 1-3% for biochar and to guarantee absolute sterility for both the inoculated treatments and the non-inoculated controls, both carriers were further sterilized by baking in an oven at 160°C for 2 hours prior to the immobilization process.

The bacterial culture was inoculated in a Luria-Bertani medium for 24 hours. For immobilization, 10% (v/w) of the bacterial culture was transferred to the sterile carriers and mixed thoroughly to ensure uniform distribution between zeolite - biochar and bacteria. The mixture was then incubated at room temperature for 24 hours until the bacteria were attached to the zeolite. Following immobilization, the resulting zeolite-bacteria and biochar-bacteria mixtures were rinsed with sterile distilled water to remove any unattached bacteria and after this process the final moisture content of the products was precisely adjusted to approximately 8–9%. The zeolite and biochar carriers were then dried at room temperature before being used in the subsequent experiment. To ensure that they do not significantly alter soil properties, allowing us to focus on the effects of microbial activity, smaller quantities were used compared to their application as soil amendments.

2.5 Experimental Design and Treatments

The experiment followed a Completely Randomized Design (CRD) with two factors: Carrier (Liquid, Biochar, Zeolite) and PGPB (six PGPB strains and one control) (Table 1), each with three replications. The six PGPB strains included five strains of *Bacillus subtilis* (subgroup *B. subtilis*) and one strain of *Bacillus thuringiensis* (subgroup *B. cereus*), while the control involved no microbial inoculation. More specifically, the seven treatments of Microorganisms factor were the following: 1) *B. subtilis*, subgroup *B. subtilis*, strain 548, 2) *B. mojavensis*, subgroup *B. subtilis*, strain 5B2, 3) *B. subtilis*, subgroup *B. subtilis*, strain 557, 4) *B. subtilis*, subgroup *B. subtilis*, strain Z3, 5) *B. amyloliquefaciens*, subgroup *B. subtilis*, strain RS-3, 6) *B. thuringiensis*, subgroup *B. cereus*, strain 109/18 and 7) *Control*. This resulted in a total of 21 treatment combinations (3 Carriers × 7 PGPB). The experimental plot size was 6 m^2 (2*3 meters), with row spacing of 80 cm and plant spacing within rows of 50 cm, resulting 12 plants per plot. To prevent cross-contamination between treatments buffer zones of 1 meter were maintained between the plots. Finally, applications were performed 48 Days After Transplanting (DAT) and all physiological and yield measurements were conducted on the middle plant of each plot to eliminate the edge effect.

Table 1. Description of strain, pH, auxin, colony forming unit (CFU), colony forming unit (CFU) per g of carrier and NCBI accession number

Strain	pH	Auxin (ppm)	CFU/ml	Zeolite (CFU/g)	Biochar (CFU/g)	Identification	NCBI Accession Number
548	5.7	11.5	$6.4*10^9$	$3.4*10^7$	$1.86*10^7$	*B. subtilis*	OQ727514

5B2	5.79	16.38	$2.46*10^9$	$2.48*10^7$	$1.7*10^7$	*B. mojavensis*	MZ433323
557	5.96	25	$2.1*10^9$	$2.94*10^7$	$1.01*10^7$	*B. subtilis*	OQ727549
Z3	6.09	30.52	$3.03*10^9$	$2.57*10^7$	$2.18*10^7$	*B. subtilis*	MW396734.1
RS-3	6.97	23.62	$1.78*10^9$	$2.55*10^7$	$2.9*10^7$	*B. amyloliquefaciens*	OQ727551
109/18 1	5.84	22.93	$3.21*10^9$	$2.88*10^7$	$1.6*10^7$	*B. thuringiensis*	-

2.6 Agronomic and Physiological Measurements

Dry weight measurements were conducted at 62 and 83 DAT (Days After Transplanting). A precision balance was used to measure the dry weight after the samples (whole plants) underwent a three-day period of oven-drying at 70°C. The photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), and stomatal conductance (mol m^{-2} s^{-1}) of plants were quantified using the LCi Leaf Chamber Analysis System (ADC Bioscientific, Hoddesdon, UK) at 62 and 83 DAT, during midday hours on sunny days. This system measures these plant physiology parameters in the field during midday hours under clear skies, on fully expanded leaves, providing immediate on-site results. It is important to note that the collection of plant material complied with all relevant institutional, national, and international guidelines and legislation.

2.7 Fruit Quality and Bioactive Compounds Analysis

All samples were characterized in terms of their moisture content (g H_2O/100 g), ash content (g/100 g), pH-value, total soluble solids (°Brix) and chroma. Moisture content was determined by drying at 110ºC (Memmert, B50 type, Memmert GmbH + Co. KG) until constant weight (AOAC, 1990). Ash content was also determined by drying (Nabertherm, Nabertherm GmbH, Germany) at 550-600□C for 5 h. A digital refractometer (°Brix- KERN Digital Refractometer, KERN & SOHN GmbH, Germany) was used to measure the total soluble solids. For pH-measurements, an ORION pH-meter (ORION 188 ion analyzer model EA 940, ORION-scientific, Limena (PD), Italy), was used. Chroma analysis of samples was conducted using a Minolta CR-300 colorimeter (Minolta Company, Chuo-Ku, Osaka, Japan). The measurements were expressed in CIELab colorscale (Commission International de l' Eclairage) (CIE, 1978) where L*-value represents the change from black to white (0-100), a*-value the change from green to red ((-)60 - (+)60) and b*-value the change from blue to yellow ((-)60 - (+)60). All measurements were performed five times on ten different fruits.

The major intracellular bioactive compounds of tomatoes that were determined were the concentration of total carotenoids (mg/g dw), total phenolic compounds (mg caffeic acid/g dw) and the antioxidant capacity (mg Trolox/g dm) of tomato samples. For all studied samples, tomatoes were homogenized, deep frozen (-80°C), dried (−52°C, 0.080 mbar for 48 h using a Thermo Savant MODULYOD-230 freeze dryer (Thermo Fisher Scientific, US) and then stored until further analysis. Extraction of total carotenoids from dried tomato samples was performed by using as solvent a mixture solution hexane:acetone:ethanol (50:25:25) following the procedure described by Andreou et al. (2020). The content of Total Carotenoids (TC) was

determined spectrophotometrically at 470 nm (A_{470}), 663 nm (A_{663}), and 647 nm and was estimated using the Lichtenthaler equations[47]. The extraction procedure for total phenolic compounds and antioxidant activity for dried tomato samples was carried out as described by Andreou, et al.[48], with some modifications. A solid-liquid extraction was carried out by combining 1 g of the dried sample with 10 mL of an 80:20 (v/v) methanol:water solution. The mixture was incubated in an ultrasonic bath for 1 h, followed by filtration. The resulting extract was stored at −20 °C until further analysis. Total phenolic compounds were estimated following the Folin-Ciocalteu method. Antioxidant capacity in dried tomato samples was determined spectrophotometrically as described by Chanioti and Tzia[49]. Total phenol concentration was expressed as mg of caffeic acid equivalence/g dm (CAE/g dm) and antioxidant activity was reported as mg Trolox/g dm. Samples were analyzed in triplicate.

2.8 Machine Learning Modeling

To determine the predictive significance of this dataset, Python 3.12, the Scikit Learn library[50] and the Pinguin library[51] were used, testing twelve different Machine Learning (ML) models in five-fold cross validation. In total over 10,000 different combinations of ML models and hyper-parameters were tested and estimated in order to find the best one for our dataset.

2.9 Statistical analysis

A two-way analysis of variance (ANOVA) was used to evaluate the main effects of the carrier type (Factor 1), the PGPB inoculation (Factor 2), and their interaction. Statistical analysis was conducted in IBM SPSS version 24 (IBM Corp., Armonk, NY, USA), with post-hoc mean comparisons performed using the Tukey Honestly Significant Difference (HSD) test ($p \leq 0.05$).

3. Results

3.1 Plant Growth

At both measurements of dry weight (g/plant) of the tomato plants (62 and 83 DAT) the analysis of the results indicated that there were statistically significant differences for the factor of PGPB, while there were no statistically significant differences for the factor carrier, nor interaction of the two factors (Figure 2). Specifically, the three carriers at 62 DAT presented values around 309 g/plant and at 83 DAT approximately 388 g/plant without significant differences in both measurements. However, at 62 DAT, *B. subtilis* str. 557, str. Z3 and str. 548 showed significantly higher mean values (348.9, 354.3 and 341.3 g/plant, respectively) than the rest of the PGPB. Significantly lower values were observed for *B. mojavensis* str. 5B2 (306.7 g/plant) followed by *B. amyloliquefaciens* str. RS-3 (282.9 g/plant) and *B. thuringiensis* (274 g/plant), with the last two without statistically significant differences compared to the control (256.9 g/plant). The same trend was observed during the second measurement (83 DAT) with *B. subtilis* str. 557, str. Z3 and str. 548 having the highest values of dry weight without significant differences between them (435.1, 431.3 and 430 g/plant, respectively) followed by *B. mojavensis* str. 5B2 (387.8 g/plant). On the other hand, *B. amyloliquefaciens* str. RS-3 (351.1 g/plant) and *B. thuringiensis* str. 109/18 1 (350.7 g/plant) had the lowest values, along with the control (327.3 g/plant), without significant differences among them.

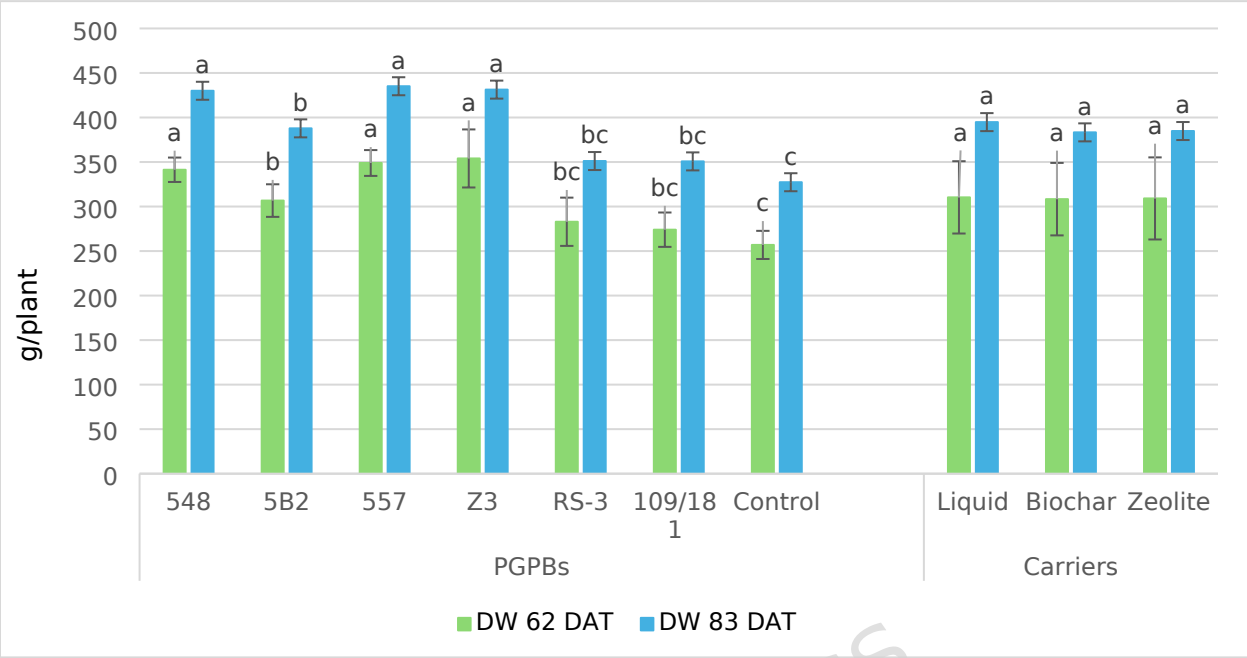

Figure 2. Effect of Carriers and PGPB on dry weight (g/plant) of tomato plants 62 and 83 DAT. Means followed by the same letter for treatments are not significantly different according to Tukey Honestly Significant Difference (HSD) test ($p < 0.05$). Values presented are mean values of three replicates ± standard deviation. F values for DAT62: F_{PGPB}:26.91***; $F_{Carrier}$:0.04^{ns} F_{PxC}:0.55^{ns}. F values for DAT83: F_{PGPB}:27.78*** $F_{Carrier}$:1.23^{ns} F_{PxC}:0.34^{ns}. Significance levels: ***: $p < 0.001$; ns: not significant ($p > 0.05$).

3.2 Physiological measurements

In most of the physiology measurements (Table 3) interaction between the two factors was observed, except from the measurement of photosynthetic rate, where statistically significant differences were observed among the PGPB applications for both measurements, while the differences were statistically significant for the factor of carriers only for the second measurement (Figure 3).

At the measurement of photosynthetic rate during the first measurement (62 DAT), *B. thuringiensis* str. 109/18 1 (17.38 μmol CO_2 $m^{-2}s^{-1}$) along with *B. subtilis* str. 557 (16.57 μmol CO_2 $m^{-2}s^{-1}$) presented the highest values among the treatments followed by *B. subtilis* str. 548 (16.29 μmol CO_2 $m^{-2}s^{-1}$) and *B. mojavensis* str. 5B2 (15.7 μmol CO_2 $m^{-2}s^{-1}$). The lowest values of photosynthetic rate were observed on *B. subtilis* str. Z3 (15.29 μmol CO_2 $m^{-2}s^{-1}$) and *B. amyloliquefaciens* str. RS-3 (13.88 CO_2 μmol $m^{-2}s^{-1}$), however, it should be noted that all PGPB treatments except *B. amyloliquefaciens* str. RS-3 presented significantly higher values than the control (13.02 μmol CO_2 $m^{-2}s^{-1}$). On the second measurement (83 DAT), *B. thuringiensis* str. 109/18 1 (18.09 μmol CO_2 $m^{-2}s^{-1}$) and *B. subtilis* str. 557 (17.07 μmol CO_2 $m^{-2}s^{-1}$) had the highest values. Lower values were presented for *B. subtilis* str. 548 (16.76 μmol CO_2 $m^{-2}s^{-1}$) and *B. mojavensis* str. 5B2 (16.44 μmol CO_2 $m^{-2}s^{-1}$) without statistically significant differences among them. However, all PGPB treatments except *B. subtilis* str. Z3 (14.33 μmol CO_2 $m^{-2}s^{-1}$), gave values higher than the control (13.53 μmol CO_2 $m^{-2}s^{-1}$). Furthermore, at 83 DAT, among the carriers, biochar presented significantly

higher values (16.76 µmol CO_2 $m^{-2}s^{-1}$) than liquid (15.39 µmol CO_2 $m^{-2}s^{-1}$) and zeolite (15.6 µmol CO_2 $m^{-2}s^{-1}$).

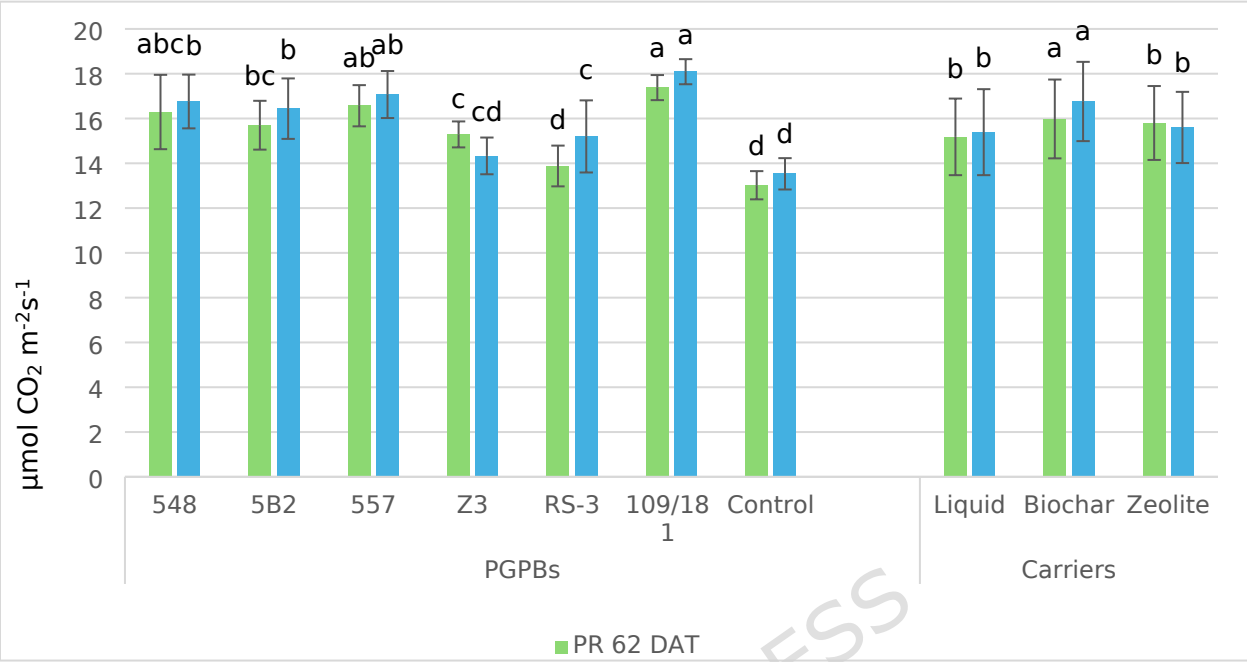

Figure 3. Effect of Carriers and PGPB on photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) of tomato plants 62 and 83 DAT. Means followed by the same letter for treatments are not significantly different according to Tukey Honestly Significant Difference (HSD) test ($p < 0.05$). Values presented are mean values of three replicates ± standard deviation. F values for DAT62: F_{PGPB}: 36.11^{***}; $F_{Carrier}$: 7.57^{**}; F_{PxC}: 1.20^{ns}. F values for DAT83: F_{PGPB}: 34.61^{***} $F_{Carrier}$: 16.61^{***}; F_{PxC}: 1.99^{ns}. Significance levels: ***: $p < 0.001$; **: $p < 0.01$; ns: not significant ($p > 0.05$).

Table 3. Interaction of Carriers and PGPB on transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}) and chlorophyll content (SPAD values) of tomato plants 62 and 83 DAT. Means followed by the same letter for treatments are not significantly different according to Tukey Honestly Significant Difference (HSD) test ($p < 0.05$). Values presented are mean values of three replicates ± standard deviation. Significance levels: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: not significant ($p > 0.05$).

	Transpiration rate (mmol H_2O m^{-2} s^{-1})		Stomatal conductance (mol m^{-2} s^{-1})		Chlorophyll (SPAD
t	**62 DAT**	**83 DAT**	**62 DAT**	**83 DAT**	**62 DAT**
8	4.1 ± 0.21^{bc}	4.3 ± 0.18^{ab}	0.39 ± 0.03^{abcd}	0.42 ± 0.02^{bcd}	17.1 ± 0.36^{defg}
2	4.3 ± 0.22^{ab}	4.6 ± 0.17^{a}	0.41 ± 0.02^{abc}	0.45 ± 0.03^{abcd}	20.2 ± 1.26^{ab}
7	4.7 ± 0.26^{a}	4.8 ± 0.32^{a}	0.40 ± 0.01^{abcd}	0.47 ± 0.04^{abcd}	18.9 ± 1.4^{abcde}
3	4 ± 0.13^{bcd}	4.2 ± 0.26^{ab}	0.37 ± 0.04^{bcd}	0.43 ± 0.07^{abcd}	18 ± 1.08^{bcdef}
-3	3.5 ± 0.09^{de}	3.6 ± 0.11^{bc}	0.39 ± 0.03^{abcd}	0.44 ± 0.04^{abcd}	18.9 ± 0.1^{abcde}
18 1	4.1 ± 0.07^{b}	4.4 ± 0.08^{a}	0.39 ± 0.04^{abcd}	0.44 ± 0.05^{abcd}	16.7 ± 0.2^{defg}
rol	2.7 ± 0.23^{fg}	2.9 ± 0.3^{de}	0.35 ± 0.03^{cd}	0.38 ± 0.02^{cd}	15 ± 0.32^{g}
48	4.4 ± 0.12^{ab}	4.6 ± 0.26^{a}	0.42 ± 0.02^{abc}	0.49 ± 0.02^{abc}	20 ± 1.5^{abc}
32	4.1 ± 0.07^{b}	4.4 ± 0.09^{a}	0.42 ± 0.03^{abc}	0.49 ± 0.06^{abc}	19.5 ± 0.87^{abcd}
57	4 ± 0.17^{bcd}	4.3 ± 0.17^{ab}	0.42 ± 0.02^{abc}	0.48 ± 0.02^{abc}	17 ± 1.51^{defg}

3	4.3±0.23^{ab}	4.5±0.32^{a}	0.45±0.01^{ab}	0.53±0.02^{ab}	20.9±0.97^{a}
-3	3.2±0.13^{ef}	3.4±0.2^{cd}	0.46±0.02^{a}	0.52±0.04^{ab}	20.1±0.5^{ab}
18 1	4.2±0.27^{ab}	4.7±0.3^{a}	0.45±0.02^{ab}	0.55±0.07^{a}	15.8±0.81^{fg}
trol	2.5±0.19^{g}	2.6±0.25^{e}	0.32±0.04^{d}	0.35±0.04^{d}	15.3±0.44^{fg}
8	4.2±0.22^{ab}	4.5±0.26^{a}	0.46±0.04^{a}	0.55±0.06^{a}	17.2±0.68^{cdefg}
2	4.1±0.22^{bc}	4.3±0.06^{ab}	0.39±0.03^{abcd}	0.43±0.04^{abcd}	17.4±0.67^{bcdefg}
7	4.2±0.14^{ab}	4.5±0.17^{a}	0.45±0.04^{ab}	0.50±0.05^{abc}	19.2±1.32^{abcde}
3	4±0.11^{bcd}	4.3±0.27^{ab}	0.38±0.01^{abcd}	0.42±0.04^{bcd}	18±0.75^{bcdef}
-3	3.6±0.14^{cde}	3.7±0.37^{bc}	0.41±0.03^{abc}	0.46±0.03^{abcd}	18.1±1.15^{abcdef}
18 1	4.2±0.12^{ab}	4.5±0.22^{a}	0.39±0.03^{abcd}	0.42±0.03^{bcd}	16.5±0.8^{efg}
trol	2.6±0.12^{g}	2.7±0.22^{de}	0.35±0.01^{cd}	0.40±0.02^{bcd}	15.8±0.25^{fg}
	114.34***	78.7***	10.39***	7.5***	22.94***
	2.35^{ns}	0.41^{ns}	8.61**	9.4***	5.27**
	3.43**	2.24*	3.17**	3.28**	5.32***

Regarding transpiration rate, an interaction was observed between the factors on both measurements (62 and 83 DAT). The treatment of *B. subtilis* str. 557 with Liquid carrier presented the highest value among all the combinations tested (4.7 mmol H_2O $m^{-2}s^{-1}$) in 62 DAT. Most of PGPB and carriers' combinations had values between 3.5-4.4 mmol H_2O $m^{-2}s^{-1}$, except for *B. amyloliquefaciens* str. RS-3 with Biochar that recorded the lowest transpiration rate (3.2 mmol H_2O $m^{-2}s^{-1}$). It should be noted that the control application of all carriers did not exceed 2.7 mmol H_2O $m^{-2}s^{-1}$ with no significant differences between them. At 83 DAT, there was an increase in all combinations with many of them recording values above 4.2 mmol H_2O $m^{-2}s^{-1}$. Exception was for *B. amyloliquefaciens* str. RS-3 with all three carriers, recording the lowest values among the treatments (3.7, 3.6 and 3.4 mmol H_2O $m^{-2}s^{-1}$ with Zeolite, Liquid and Biochar carriers respectively), still higher than the control (<2.9 mmol H_2O $m^{-2}s^{-1}$).

For stomatal conductance at 62 DAT *B. subtilis* str. 548 with Zeolite as a carrier and *B. amyloliquefaciens* str. RS-3 with Biochar as a carrier had the highest values (0.46 mol $m^{-2}s^{-1}$). Furthermore, the rest combinations of PGPB with Biochar carrier recorded values between 0.42-0.45 mol $m^{-2}s^{-1}$ without significant differences observed between them. Notable high values were also recorded on *B. mojavensis* str. 5B2 with Liquid carrier (0.41 mol $m^{-2}s^{-1}$) and *B. subtilis* str. 557 with Zeolite as a carrier (0.45 mol $m^{-2}s^{-1}$). Control treatments did not exceed 0.35 mol $m^{-2}s^{-1}$. A similar tendency was observed at 83 DAT, with *B. subtilis* str. 548 with Zeolite as a carrier having the highest value of stomatal conductance along with *B. thuringiensis* str. 109/18 1 with Biochar as a carrier (0.55 mol $m^{-2}s^{-1}$), followed by *B. subtilis* str. 557 with Zeolite as a carrier, the rest PGPB with Biochar as a carrier had values between 0.48-0.53 mol $m^{-2}s^{-1}$ that did not differ significantly. The rest of the combinations did not exceed 0.47 mol $m^{-2}s^{-1}$.

Regarding chlorophyll content (SPAD values) at 62 DAT, the highest values were recorded for *B. subtilis* str. Z3 and 548, *B. mojavensis* str. 5B2, *B. amyloliquefaciens* str. RS-3 with Biochar as a carrier and *B. mojavensis* str. 5B2 with Liquid carrier, with SPAD values between 19.5-20.9 and without significant differences between

them. A slight increase was observed at 83 DAT, still the same combinations as the previous measurement had the highest SPAD values (20.5-22.4).

3.3 Tomato Fruit Yield

Regarding yield (fresh tomatoes), only the factor of PGPB had a statistically significant impact on production, while carriers and the interaction between the two factors showed no significant effect (Figure 4). Most of the PGPB evaluated showed higher values than the control. Specifically, *B. subtilis* str. 557 (3367 g/plant), str. Z3 (3511 g/plant), str. 548 (3522 g/plant) and *B. thuringiensis* str. 109/18 1 (3306 g/plant) did not present significant differences between them; however, they produced a higher yield than *B. mojavensis* str. 5B2 (2983 g/plant) and *B. amyloliquefaciens* str. RS-3 (2861 g/plant) that produced a similar yield to the control (2844 g/plant).

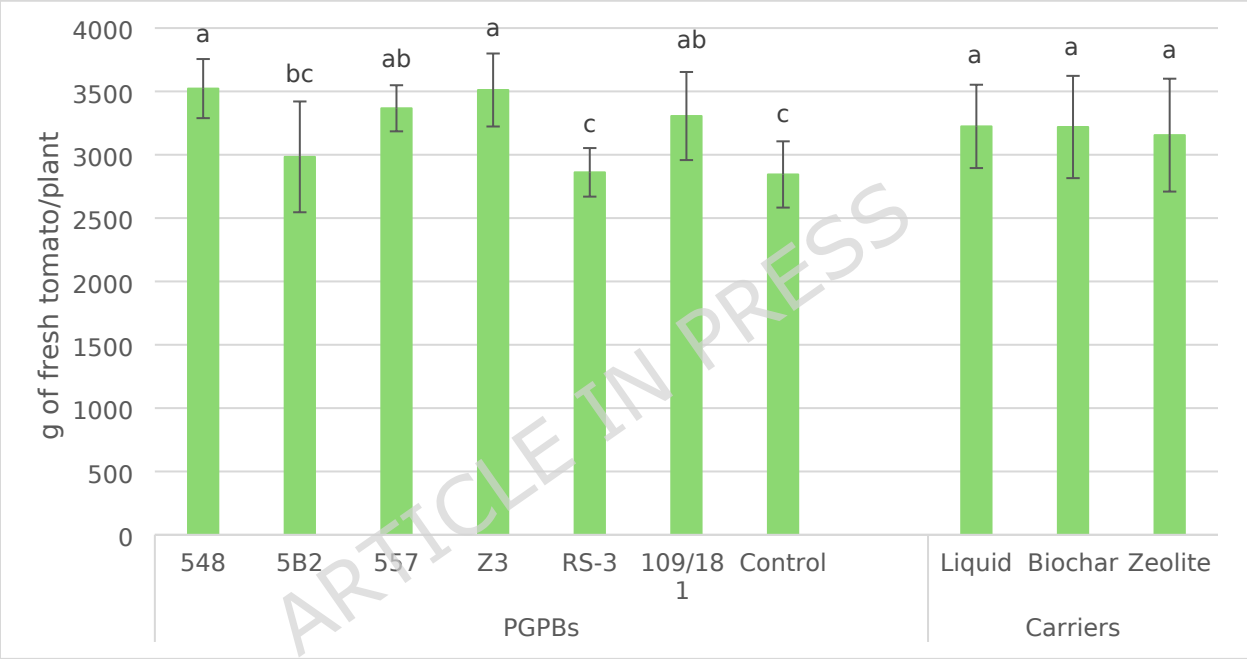

Figure 4. Effect of Carriers and PGPB on yield (g of fresh tomato/plant). Means followed by the same letter for treatments are not significantly different according to Tukey Honestly Significant Difference (HSD) test ($p < 0.05$). Values presented are mean values of three replicates ± standard deviation. F_{PGPB}:10.32***; $F_{Carrier}$:0.40^{ns}; F_{PxC}:1.54^{ns}. Significance levels: ***: $p < 0.001$; ns: not significant ($p > 0.05$).

3.4 Quality Characteristics of the Harvested Tomatoes

The effect of all techniques applied, on several quality characteristics of harvested tomatoes was studied and presented in Tables 4, 5 and 6. Moisture content and total soluble solids were not significantly ($p>0.05$) affected by the application of all studied treatments and their combinations. Moisture content and total soluble solids ranged from 95.07 to 95.62% and from 4.72 to 4.94% for all studied samples, respectively. No significant effect was also observed for total carotenoids of samples (values ranged from 1.15 to 1.27 mg/g dw for all samples). Chroma values (L, a and b) of harvested tomatoes also did not show statistically significant differences among all treatments. As demonstrated in the experiment, treatments with all carriers did

not exhibit statistically significant differences for pH-values, except for the use of *B. subtilis* strain Z3 which appeared to provide a slight increase in pH-value. In contrast, the interaction of carriers with PGPB did not result in statistically significant differences. The biochar application also led to a slight decrease in ash content with statistically significant differences. The results obtained also showed that treatments with all PGPB and carriers, as well as their interactions led to statistically significant differences in total phenolic content and antioxidant activity of samples. Specifically, the *Zeolite 557* sample showed the highest values among the other treatments, achieving values equal to 5.51 mg CAE/g dw and 8.65 mg Trolox/g dw for total phenolic compounds and antioxidant activity, respectively.

Table 4. Effect of Carriers and PGPB on chroma (L, a, b) and moisture content (%) of the harvested tomatoes

	L	a	b	Moisture (%)
PGPB				
548	45.67±2.14	30.01±2.10	32.68±2.82	95.62±0.64
5B2	44.47±1.36	30.26±2.17	32.32±4.12	95.44±0.58
557	44.18±1.95	29.80±1.77	36.01±11.65	95.58±0.35
Z3	44.48±1.26	30.21±2.25	31.98±1.73	95.07±0.57
RS-3	45.25±2.01	30.85±2.29	32.63±3.08	95.51±0.44
109/18 1	45.50±2.42	30.35±2.62	32.96±2.95	95.54±0.40
Control	44.68±1.13	31.22±2.21	31.77±0.78	95.24±0.60
Carrier				
Liquid	45.15±1.77	30.32±2.00	32.55±2.03	95.36±0.49
Biochar	44.63±2.00	30.09±2.15	33.74±8.03	95.55±0.58
Zeolite	44.90±1.69	30.75±2.34	32.43±1.88	95.38±0.51
F_{PGPB}	0.845^{ns}	0.410^{ns}	0.719^{ns}	1.381^{ns}
$F_{Carrier}$	0.403^{ns}	0.452^{ns}	0.431^{ns}	0.872^{ns}
F_{PxC}	0.771^{ns}	0.776^{ns}	0.851^{ns}	1.203^{ns}

Values presented are mean values of three replicates ± standard deviation. Significance levels: ns: not significant.

Table 5. Effect of Carriers and PGPB on total soluble solids (°Brix), pH-value, ash content (%) and concentration of total carotenoids (mg/ g dm) of the harvested tomatoes.

	°Brix	pH	Ash (%)	TC (mg/g dm)
PGPB				
548	4.78±0.39	$4.10±0.07^{b}$	0.58±0.25	1.15±0.13
5B2	4.94±0.24	$4.16±0.07^{ab}$	0.61±0.19	1.27±0.10
557	4.82±0.29	$4.15±0.32^{ab}$	0.61±0.17	1.23±0.12
Z3	4.79±0.42	$4.34±0.20^{a}$	0.52±0.08	1.15±0.34
RS-3	4.59±0.34	$4.12±0.07^{ab}$	0.64±0.13	1.14±0.29
109/18 1	4.75±0.53	$4.12±0.09^{b}$	0.68±0.15	1.20±0.15
Control	4.75±0.33	$4.14±0.07^{ab}$	0.63±0.17	1.16±0.31

Carrier				
Liquid	4.85±0.32	4.15±0.09	0.69±0.22^{a}	1.21±0.22
Biochar	4.75±0.43	4.15±0.07	0.54±0.09^{b}	1.18±0.21
Zeolite	4.72±0.35	4.19±0.27	0.61±0.15^{ab}	1.16±0.23
F_{PGPB}	0.809^{ns}	2.523*	0.907^{ns}	0.44^{ns}
$F_{Carrier}$	0.843^{ns}	0.543^{ns}	4.541*	0.304^{ns}
F_{PxC}	1.928^{ns}	1.18^{ns}	0.898^{ns}	1.570^{ns}

Means followed by the same letter for treatments are not significantly different according to Tukey Honestly Significant Difference (HSD) test at $p < 0.05$. Values presented are mean values of three replicates ± standard deviation. Significance levels: *p < 0.05, ns: not significant.

Table 6. Interaction of Carriers and PGPB on total phenolic compounds (mg/g dm) and antioxidant activity (mg/g dm) of the harvested tomatoes.

Treatment	**TPC (mg/g dm)**	**Antioxidant Activity (mg/g dm)**
Liquid 548	3.65±0.80^{d}	6.89±0.78^{abc}
Liquid 5B2	4.06±0.26^{bcd}	6.82±0.55^{bc}
Liquid 557	4.11±0.07^{bcd}	6.01±0.37^{c}
Liquid Z3	4.37±0.28^{abcd}	7.26±0.01^{abc}
Liquid RS-3	4.24±0.35^{abcd}	6.03±0.00^{c}
Liquid 109/18 1	4.60±0.02^{abcd}	7.36±0.18^{abc}
Liquid Control	3.73±0.17^{cd}	6.07±0.62^{c}
Biochar 548	4.47±0.11^{abcd}	7.84±0.77^{ab}
Biochar 5B2	4.62±0.12^{abcd}	7.12±0.13^{abc}
Biochar 557	4.66±0.05^{abcd}	7.75±0.60^{abc}
Biochar Z3	4.40±0.13^{abcd}	7.38±0.49^{abc}
Biochar RS-3	4.29±0.44^{abcd}	7.15±0.34^{abc}
Biochar 109/18 1	4.14±0.11^{bcd}	7.09±1.23^{abc}
Biochar Control	5.19±0.15^{ab}	8.60±0.87^{a}
Zeolite 548	4.81±0.40^{abcd}	7.42±0.05^{abc}
Zeolite 5B2	4.43±0.27^{abcd}	7.12±0.24^{abc}
Zeolite 557	5.51±0.46^{a}	8.65±0.00^{a}
Zeolite Z3	4.88±0.05^{abcd}	7.12±0.88^{abc}
Zeolite RS-3	5.00±0.74^{abc}	7.83±0.43^{ab}
Zeolite 109/18 1	4.36±0.50^{abcd}	7.06±0.75^{abc}
Zeolite Control	4.44±1.03^{abcd}	7.31±0.07^{abc}
F_{PGPB}	1.246^{ns}	0.864^{ns}
$F_{Carrier}$	14.343***	17.47***
F_{PxC}	2.936**	4.146***

Means followed by the same letter for treatments are not significantly different according to Tukey Honestly Significant Difference (HSD) test at $p < 0.05$. Values presented are mean values of three replicates ± standard deviation. Significance levels: **p < 0.01, ***p < 0.001, ns: not significant.

3.5 Statistical Analysis Using Machine Learning Models

In order to find which of the physiological and agronomic measurements were correlated with the yield and the quality characteristics of the tomato fruits, 12

different Machine Learning (ML) algorithms were tested. The dataset used to train the model, consisted of 5 different field measurements (PR, TR, SC, Chl and DW) in two different timestamps (62, 83 DAT) during the cultivation period, as well as 8 variables related to the quantity and the quality of production (Yield, Brix, pH, Ash, DPPH, TPC, PG, PME). In order to find the best algorithm and its hyperparameters for our dataset, we tested the models in 7 different metrics, and their STDs, to determine which combination of algorithm and hyperparameters is better in terms of accuracy and efficiency. As we can see in Figure 5, the Ridge algorithm has the best results both in terms of speed and accuracy.

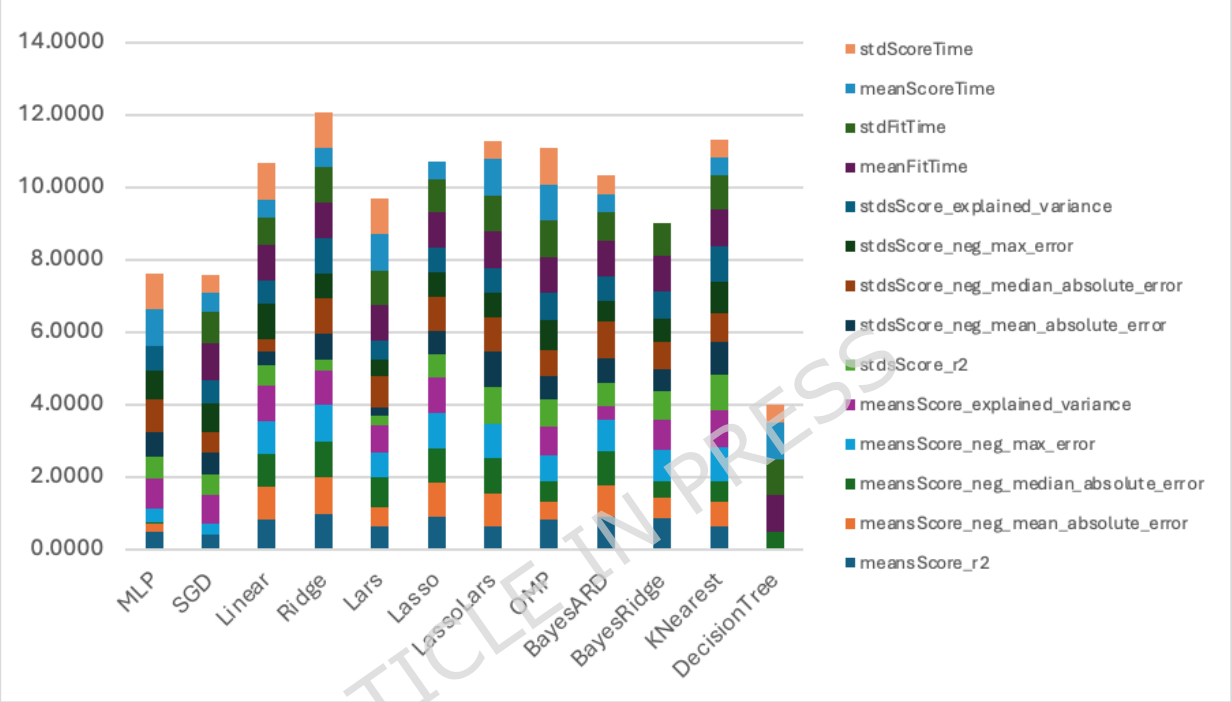

Figure 5. Comparison of normalized error metrics and their standard deviations across the 12 algorithms tested.

Using the Ridge algorithm, we extracted the feature importance between the physiology measurements and the target variables (yield and quality characteristics). The reason we are using feature importance and not feature correlation is that the Ridge algorithm is based on linear regression. Feature importance represents the weight distribution assigned to each variable by the algorithm. Positive values show that the variables have a positive impact on the model's predictions, while negative values demonstrate an inverse relationship with the predicted results. As we can see in Figure 6, TR 83 has a positive impact both on yield and on brix, PR 62 has the most positive impact on yield but a negative, close to no impact, on brix, while PR 83 has a positive impact on brix and a negative on yield.

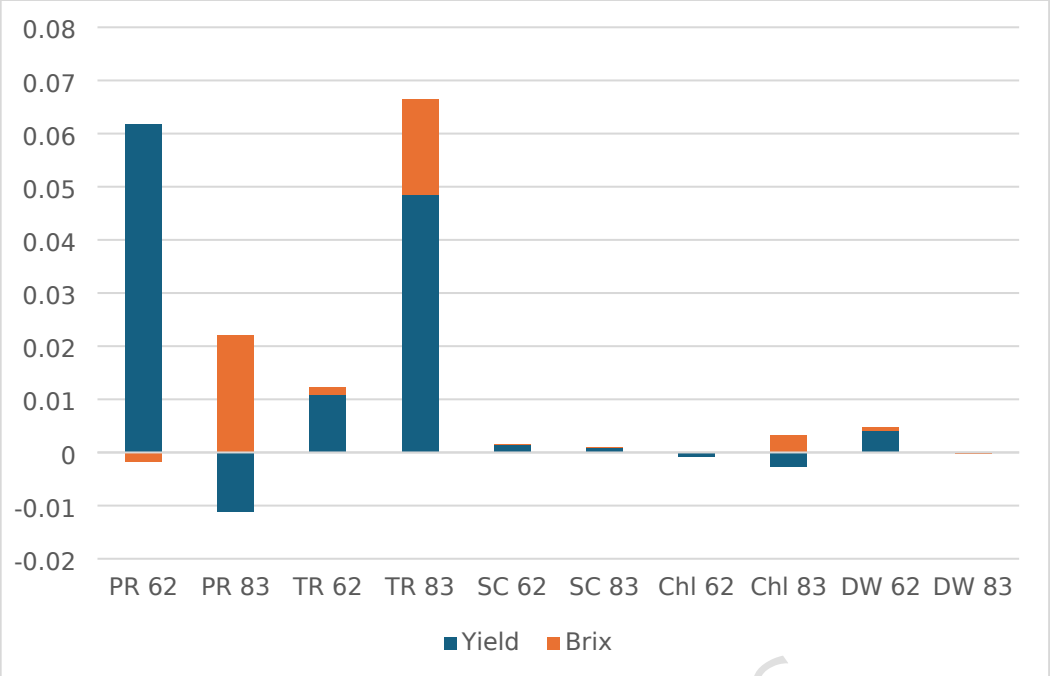

Figure 6. Feature importance of the Ridge Algorithm for the Yield and Brix variables. PR, photosynthetic rate; TR, transpiration rate; SC, stomatal conductance; Chl, chlorophyll content; DW, dry weight.

Figure 7 shows the feature importance for all the target variables. In total TR 83 is an important variable for all the target variables. PR 62 and PR 83 have a positive impact on most of the target variables. Lastly, TR 62 also has a positive impact but not at the same level as the above-mentioned variable.

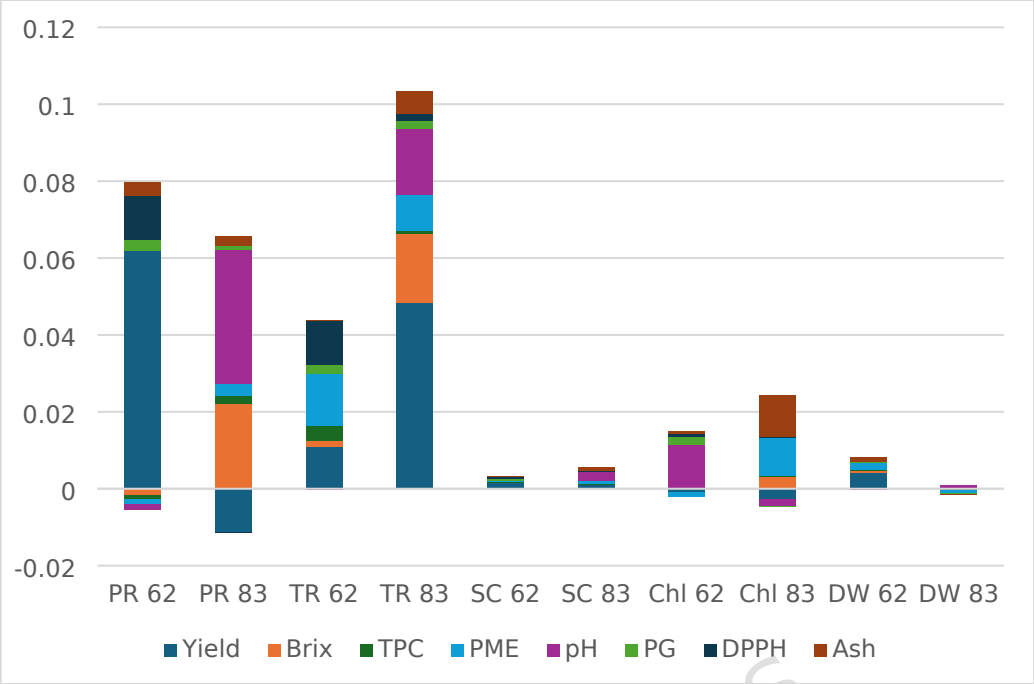

Figure 7. Feature importance of the Ridge Algorithm for all the target variables. PR, photosynthetic rate; TR, transpiration rate; SC, stomatal conductance; Chl, chlorophyll content; DW, dry weight.

4. Discussion

The survival of PGPB in soil is one of the major challenges for their efficacy. Therefore, the use of carriers like biochar and zeolite have been shown to be effective, as they provide a stable substrate that supports their survival in the soil[52]. As demonstrated in the experiment, the treatment of biochar as a carrier presented an increase at both measurements of photosynthetic rate, while at the same time there was Carrier-PGPB interaction for the rest of the physiology measurements. Similar increases in photosynthetic rate and gas-exchange parameters under biochar application have been widely reported in different crops and conditions[53,54]. Mechanistically, the positive impact of biochar can be explained by gas exchange parameters and its highly porous structure and water-holding capacity, which create a stable microenvironment in the rhizosphere[55,56]. This improved moisture retention allows plants to maintain stomata open for longer periods, thereby increasing with this way the CO_2 assimilation and the photosynthetic efficiency[57]. In a recent study, a novel approach implemented *Bacillus* spp. PGP5 with two types of biochar (corn stover and rice husk-derived) and found that this use not only remediates cadmium and lead contaminated soil but also increases lettuce yield significantly by up to 65%[58]. Our approach, even though it utilizes the synergistic effect of biochar and PGPB, differs from the previous experiment as the bacterial strains were mixed with our carriers following a specific procedure to ensure an even distribution and attachment.

The interaction of carriers and specific PGPB that was recorded in physiology measurements in our results, shows the potential of synergistic effect. It should be noted for the tomato plants used in this experiment that the stomatal conductance and transpiration rate confirm the effect of this interaction on the plant growth

parameters, but in the end for the dry weight and yield of tomato plants only PGPB effect was significant. In a pot experiment with tomatoes, *Burkholderia* sp. strain L2 and *Bacillus megaterium* strain A30 were mixed with Biochar and showed that this combination could increase shoot dry weight and yield significantly compared to the control[59]. Yield results indicate that only the bacterial strains significantly influenced tomato production without a direct effect from the carriers. There are indications that these treatments have an impact on tomato yield, but at the final measurement of the yield, the factor that clearly affected the production was the PGPB used. The significant optimization in plant dry weight and final yield was driven primarily by *B. subtilis* strains (Z3, 557 and 548). While strains like Z3 and 557 exhibit strong *in vitro* production of auxins (30.52 and 25 ppm respectively), which directly stimulates root growth and nutrient absorption, the high field performance of 548 despite its lower *in vitro* auxin production (11.5 ppm) proves that a single physiological trait cannot fully explain field capacity but its more complicated[60]. Under real soil conditions, the success of these specific strains is likely attributed not only to hormonal regulation but also to their ability to colonize rhizosphere, creating biofilm formation and enhancing nutrient solubilization, which allow them to secure resources and boost tomato yield more effectively than strains with high *in vitro* auxin but poorer field adaptation[61].

Furthermore, differences between pot and field experiments are expected to happen since field experiments are exposed to real cultivating conditions. The use of different carriers in combination with various PGPB have been tested for ameliorate effect from biotic and abiotic stress as well as nitrogen fixation and other soil nutrient enhancement[62,63]. In all these studies, however, there is no comparison among the factors themselves towards the growth of the crops. Our results show an interaction with a potential improvement in plant physiology characteristics such as transpiration rate, chlorophyll content and stomatal conductance without that being expressed in better yield or tomato dry biomass. This clearly indicates that while carrier materials like biochar and zeolite provide a stable microenvironment that improves mid-season physiological functions, they do not act as direct yield enhancers on their own in this context. As already discussed, these measurements were significantly improved using *B. subtilis* strains 557, Z3 and 548 without any effect from carriers. However, the growth results from photosynthetic rate show that there was an effect from the carriers, and specifically biochar showed higher values than the liquid and zeolite carriers, indicating a promising growth to tomato crop that needs further investigation.

Regarding the quality characteristics of the fruits, the results show that the application of PGPB strains with different carriers did not significantly affect moisture content, total soluble solids (°Brix), carotenoids and color parameters (L, a, b). This suggests that these quality traits are unaffected by these treatments, which aligns with previous studies where microbial inoculants had minimal influence on fruit characteristics[36]. However, a slight increase in pH was observed with *B. subtilis* Z3 treatment, while biochar application led to a statistically significant decrease in ash content, which may be related to nutrient uptake of tomato plants[20]. Furthermore, the most noteworthy finding was the interaction between PGPB and carriers, which significantly influenced total phenolic content and antioxidant activity with the Zeolite 557 treatment showing the highest values. The synergistic effect of the Zeolite 557 treatment on bioactive compounds could be linked to the unique physiochemical properties of zeolite, such as its Cation Exchange Capacity

(CEC) and porosity, which improve nutrient retention and create a favorable rhizosphere microenvironment[64]. When combined with metabolic activity of B. subtilis 557, this specific microenvironment likely acts as a mild elicitor, improving plant defenses. This priming is consistent with activation of induced systemic resistance and upregulation of phenylpropanoid-pathway genes, leading to greater accumulation of phenolic compounds and increased total antioxidant capacity, as reported for bacterial endophytes and rhizobacteria that stimulate phenolic and antioxidant production in various crops[65]. These results show that specific combinations of carrier-PGPB can enhance the bioactive compounds of tomatoes, even though other physicochemical properties remained unaffected. Such results are similar to studies reporting PGPB-mediated increases in secondary metabolites[45,48], though the carrier-dependent enhancement observed here offers a novel perspective for optimizing delivery systems.

The correlations based on Machine Learning models have increased application over the last years in different scientific sectors[66,67]. The reason for selecting Machine Learning models instead of traditional statistical methods is that in Machine Learning models extract weights based on the input dataset, meaning that correlation is extracted specifically for each case[68]. Agricultural traits, such as yield and brix, can be affected by various features. Depending on the dataset, different algorithms are more suitable both in terms of accuracy and efficiency. For our dataset, the Ridge algorithm had the best results, however in published research with similar datasets, some other algorithms were selected. In a recent study K-means algorithm was used in maize yield for the extraction of the feature correlation[69]. In another study regarding corn yield, a dataset of 598 features was used, testing eight algorithms in 4 different metrics. Random Forest was the one with the best average score[70]. Machine Learning models can find the variables that are highly correlated with increased yield and other important variables. For our dataset, the second measurement of transpiration rate had a positive impact on yield and brix, while the first measurement of photosynthetic rate had a positive impact on yield and the second measurement of photosynthetic rate had a positive impact on brix. In a study, regarding sweet corn using BayesianRidge algorithm, stomatal conductance at 98 days, was the variable with most positive impact on yield and fiber[8]. BayesianRidge algorithm was also used in another study regarding maize, in which Nitrogen at 70 days has a positive correlation for fat, while stomatal conductance at 65 and 106 have a negative correlation for fat, yield, protein and starch[71]. In sugarcane research, regarding the extraction of important variable of the yield, 11 algorithms were tested in a dataset consisting of 32 features and the algorithms with the best results were Random Forest[72]. Moreover, 4 different Machine Learning models were tested to find the most important variable regarding blueberry yield. The model selected was Multiple Linear Regression, which correlated the density of Bumblebees as the most important variable[73]. Lastly, in a study regarding the yield of industrial tomato, Ridge algorithm was selected, out of the 12 tested, and the low temperature of June and May had the highest correlation with the yield[74].

5. Conclusions

This study confirms that the effectiveness of Plant Growth Promoting Bacteria (PGPB) in industrial tomato production depends primarily on strain-specific functionality and the mechanisms governing microbe-carrier interactions. Among

the tested strains, *Bacillus subtilis* 548, 557 and Z3 significantly enhanced plant growth and yield, highlighting that traits such as rhizosphere colonization, nutrient solubilization and microbial persistence are critical determinants of field performance. Carriers such as biochar and zeolite contributed differently to the processes. Biochar primarily improved physiological performance by creating a favorable rhizosphere microenvironment, while zeolite played a more pronounced role in modifying biochemical pathways. However, carriers alone did not directly increase yield, highlighting that their role is supportive rather than determinative, acting by enhancing microbial survival and functional expression rather than replacing microbial effects.

Specific PGPB strains, such as *Bacillus subtilis* 548, 557 and Z3, significantly improved plant dry weight and yield by up to 32.93% and 23.8% compared to control plants, respectively. Photosynthetic rate was enhanced by up to 33.7% using PGPB treatments and among carriers, biochar presented increased values compared to liquid and zeolite. The carrier-PGPB strain interaction improved some physiological parameters such as transpiration rate, stomatal conductance and chlorophyll content, with the Liquid 557, Zeolite 548 and Biochar Z3 to show the highest values respectively. This indicates that carriers may create a more favorable environment for certain PGPB, while their impacts on their own are limited. Additionally, the quality traits of the tomato fruits were also positively impacted. More specifically, the combination of *Bacillus subtilis* 557 with zeolite increased antioxidant capacity by up to 42% and total phenolic content by 48%, showing that modified carrier-PGPB systems could improve the bioactive compounds. The key outcome of this work is that the optimal performance is achieved through the synergistic alignment of PGPB strain selection, carrier properties and plant responses. This mechanistic perspective provides a foundation for designing more reliable and efficient biostimulant strategies for sustainable crop production.

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Data Availability Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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