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Response of Sweet Pepper Varieties to Low-Input Conditions and Microbial Biostimulant Application

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Abstract

The excessive use of irrigation water and fertilizers in agriculture raises serious environmental concerns, emphasizing the need for more sustainable practices. Screening genotypes with reduced nutrient and water requirements, combined with favorable responses to plant growth-promoting rhizobacteria (PGPR), offers a promising strategy for developing more sustainable farming systems. Seven sweet pepper genotypes (*Capsicum annuum* L.) were evaluated under six treatments, involving two fertilization levels (100% and 50% standard dose), two irrigation regimes (100% and 75% full irrigation), and PGPR inoculation applied under reduced fertilization. Yield, fruit weight, rhizosphere enzymatic activities, and soluble sugars in green and red fruits were evaluated. The genotype effect contributed significantly to all traits. Combined reductions in fertilizer and irrigation decreased average yield by 21.7%, while PGPR did not fully compensate for these losses. Alkaline phosphomonoesterase activity increased by 22.9% under low fertilization, whereas averaged catalase and dehydrogenase remained relatively stable regardless of PGPR. In green fruits, PGPR inoculation under combined stress conditions increased glucose and fructose concentrations by 11.6% and 13.9%, respectively, compared to uninoculated stressed plants, although sucrose decreased. At fully ripe stage, sugar composition was less responsive to treatments. These findings underscore the importance of genotype evaluation and the exploitation of genotype × treatment interactions in peppers breeding for sustainable farming.

Keywords: *Capsicum annuum*; low fertilization; reduced irrigation; plant growth-promoting rhizobacteria; yield; soil enzymatic activity; rhizosphere; sugar profile; sustainable agriculture

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1. Introduction

Sweet peppers (*Capsicum annuum* L.) possess a remarkable economic value for their flavor and distinctive taste [1]. They are also appreciated due to their antioxidant profile, being an important source of vitamins such as C and A, phenolic compounds and carotenoids [2]. Their cultivation has high environmental and nutritional demands [3], and they are suitable for being cultivated all year round in plastic greenhouses, especially in Spain and the Mediterranean basin. Consequently, peppers play an important role in the horticultural sector of Spain, since the country is the leading producer of peppers in the EU, particularly concentrated in the southeast [4].

Intensive farming practices typically involve high inputs of water, fertilizers and pesticides to maximize productivity. However, their excessive use leads to soil and water degradation, which, in combination with the negative effects of climate change, represents an unsustainable farming system in the long term [5]. As a result, agriculture is gradually shifting toward more environmentally friendly farming systems, which include the use of high-frequency fertigation, biological pest control, and research about the optimization of fertilizers application and the implementation of sustainable strategies, such as plant biostimulants [4]. In this context, selecting pepper cultivars with a positive response to reduced inputs is crucial, as pepper development is highly sensitive to water and nutrient deficits, which directly affect yield [6].

Plant biostimulants have emerged as promising tools for sustainable agriculture, both in organic [7] and conventional systems such as greenhouse cultivation [8]. According to European Regulation (EU 2019/1009), plant biostimulants are defined as EU fertilizing products that can enhance plant nutrition processes. The aim is to improve one or more of the following aspects of the plant or its rhizosphere: (i) nutrient use efficiency, (ii) tolerance or alleviation to abiotic stresses, (iii) quality traits, and/or (iv) the availability of immobilized nutrients in the soil or the rhizosphere [9].

Plant biostimulants have been classified into six non-microbial and three microbial categories. Microbial biostimulants involve non-toxigenic, non-pathogenic microorganisms, corresponding to: (i) bacterial plant biostimulants, belonging to genera like *Azospirillum* spp., *Rhizobium* spp., or *Azotobacter* spp.; (ii) arbuscular mycorrhizal fungi; and (iii) *Trichoderma* spp. [10]. Microbial biostimulants are of particular interest since plants host a diverse and complex array of microorganisms in their endosphere, phyllosphere and rhizosphere. The establishment of symbiotic interactions between plants and microorganisms is a fundamental and prevalent aspect of plants, coevolving with these microbial symbionts, which play important roles in nutrient uptake, plant performance and yield [11].

Bacterial plant biostimulants can be further subcategorized into plant growth-promoting bacteria, with plant growth-promoting rhizobacteria (PGPR) being the most extensively studied group. PGPR can colonize plant root systems and adhere to the rhizosphere [12]. These microorganisms are able to enhance plant development through nitrogen fixation, phosphate solubilization, siderophore production and phytohormone synthesis, among other effects [13]. Their enzymatic activities support soil functioning, serving in parallel as markers of microbial efficiency and soil vitality [14].

Among the commonly measured enzymatic activities, alkaline phosphomonoesterase (ALP) is involved in organic phosphorus mineralization and it is considered a valuable microbial indicator due to its predominantly microbial origin [15]. Catalase (CAT), which is present in plants, animals and microorganisms, mitigates oxidative stress by breaking down hydrogen peroxide (H_2O_2) into water and oxygen [16]. In the rhizosphere, CAT activity also reflects microbial activity, as many soil microorganisms contribute to the degradation of organic matter and the regulation of oxidative stress [17]. Dehydrogenase (DH) is a marker of microbial respiration, linked to microbial metabolic activity, particularly in redox processes that are essential for maintaining soil fertility. DH activity is also involved in humus decomposition and organic matter [18].

Hence, the main aim of this work was to assess the impact of reduced fertilization and irrigation on a collection of sweet pepper varieties, as well as the potential effects of applying a commercial PGPR biostimulant. The main agronomic traits (yield and fruit weight) were assessed throughout the reproductive stage of the crop. The effect of treatments at the rhizosphere level was studied through the determination of ALP, CAT and DH enzymatic activities. In addition, given the relevance of sugars to organoleptic fruit quality in sweet peppers [19], glucose, fructose and sucrose concentrations were quantified

in both unripe/green-ripe and fully ripe fruits. The genotype had a significant influence on all evaluated traits. By contrast, the effects of the stress treatments and PGPR inoculation were more limited, particularly regarding agronomic traits. For sugars, the ripening stage also had a significant influence, with some effects observed in specific treatments and a relative enhancement associated with PGPR application, especially at the green-ripe stage. These results provide valuable insights into specific genotype responses under low-input conditions and support the identification of promising candidates for sustainable pepper cultivation.

2. Materials and Methods

2.1. Plant Material

A collection of seven sweet pepper genotypes (*Capsicum annuum* L.) was evaluated, including three traditional varieties, two experimental F1 hybrids developed at the COMAV Institute, and two commercial F1 hybrids (Table 1, Figure 1).

Table 1. Description and origin of the *Capsicum annuum* L. genotypes used in this study.

Accession	Description	Origin
BGV13004	Local ecotype—Coarse pepper for roasting	Basque Country (Spain)
Najerano	Traditional variety—Protected Geographical Indication (P.G.I)	La Rioja (Spain)
Piquillo	Traditional variety—Protected Designation of Origin (P.D.O)	Navarra (Spain)
H1	Experimental F1 hybrid—BGV13004 x experimental line with L_4 R gene	Comunitat Valenciana (Spain)
H2	Experimental F1 hybrid—Najerano x experimental line with L_4 R gene	Comunitat Valenciana (Spain)
Isabel F1	Commercial F1 hybrid—Semillas Ramiro Arnedo S.A.	La Rioja (Spain)
Cabañeros F1	Commercial F1 hybrid—Semillas Ramiro Arnedo S.A.	La Rioja (Spain)

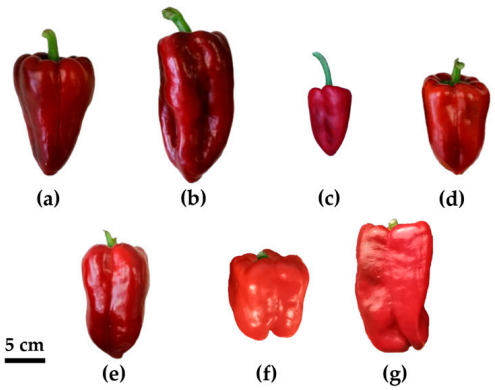

Figure 1. Appearance of the evaluated pepper genotypes at the fully ripe stage. Traditional varieties: (**a**) BGV13004, (**b**) Najerano, (**c**) Piquillo; experimental F1 hybrids: (**d**) H1, (**e**) H2; and commercial F1 hybrids: (**f**) Isabel F1 and (**g**) Cabañeros F1.

2.2. Pepper Cultivation Conditions and Experimental Design

The experiment was conducted at the Torreblanca experimental farm (IMIDA Institute, Murcia, Spain; GPS coordinates: 37°46′34.6″ N, 0°53′47.7″ W). The main soil properties of the site are summarized in Table 2.

The pepper plants were grown in a multi-tunnel greenhouse measuring 400 m^2 during the 2021 winter-summer growing season. Irrigation was carried out using a localized system with pressure-compensating drippers at 2.2 L h^{-1} per plant, and the plants were spaced 0.4 m apart and 1 m between rows.

Table 2. Main soil parameters at the Torreblanca Experimental Farm (IMIDA, Murcia, Spain).

Parameter	Value
Soil texture	Loam-silty (44.55% sand, 52.50 silt, 2.95% clay)
pH (1:2 soil–water, at 23.9 °C)	8.2 (Alkaline)
Organic matter (%)	4.21
Organic carbon (%)	2.4
Total nitrogen (%)	0.212
Nitrates (mg kg^{-1})	375
Available phosphorus (Olsen, mg kg^{-1})	11.5
Available potassium (mg kg^{-1})	317
Available calcium (mg kg^{-1})	23,900
Available magnesium (mg kg^{-1})	1170
Available sodium (mg kg^{-1})	1380
Electrical conductivity (mS cm^{-1}, 1:2 soil–:water, at 25 °C)	7.71

A total of six treatments were evaluated for each genotype (Figure 1), combining two fertilization and two irrigation levels, and the application of PGPR. PGPR inoculation was applied only to the treatments receiving reduced fertilization. Abbreviations were used as follows: 100F + 100I (control): 100% fertilization + 100% irrigation, 50F + 100I: 50% fertilization + 100% irrigation, 50F + 100I+PGPR: 50% fertilization + 100% irrigation + PGPR, 100F + 75I: 100% fertilization + 75% irrigation, 50F + 75I: 50% fertilization + 75% irrigation, 50F + 75I + PGPR: 50% fertilization + 75% irrigation + PGPR. In each combination of genotype and treatment, twenty plants were established, organized into two blocks of ten plants, under a completely randomized design.

Agronomic management and cultural practices depended on the environmental conditions throughout the crop cycle and were carried out according to the technical specifications for integrated pepper production, in accordance with the Order of 10 May 2012 [20]. Fertilization rates corresponding to the 100% dose were 195 kg ha^{-1} N, 162 kg ha^{-1} P_2O_5, 292 kg ha^{-1} K_2O, 120 kg ha^{-1} Ca, and 20 kg ha^{-1} Mg. Irrigation was applied at a total rate of 110 L $plant^{-1}$ (equivalent to 0.28 m^3 m^{-2}) for the 100% dose throughout the entire growing cycle. PGPR was a commercial formulation based on a mixture of plant growth-promoting bacteria. Bactogreen® (Thader Biotechnology, S.L., Murcia, Spain) included phosphorus-solubilizing bacteria (*Pseudomonas fluorescens*, $\geq 1 \times 10^9$ CFU mL^{-1}), potassium-solubilizing bacteria (*Bacillus megaterium* and *Bacillus circulans*, each $\geq 1 \times 10^9$ CFU mL^{-1}), and nitrogen-fixing bacteria (*Azospirillum brasilense*, $\geq 1 \times 10^9$ CFU mL^{-1}). Bactogreen® was applied monthly at a dose of 3 mL $plant^{-1}$ throughout the entire crop cycle.

2.3. Agronomic Traits Analysis

To evaluate the agronomic performance of the studied genotypes, all fruits per cultivar and treatment were harvested and weighed individually when they reached the commercial maturity stage. The variables evaluated included total yield (kg $plant^{-1}$) and mean fruit weight (g).

2.4. Rhizosphere Analyses

2.4.1. Soil Sampling and Preparation

Soil sampling was carried out at the end of the growing cycle. Four replicates per cultivar and treatment were collected by sampling plants in the second and fourth positions within each of the two greenhouse locations, following a similar approach to Morales-Manzo et al. [21]. Samples were collected at the rhizosphere level by taking soil in direct contact with the roots of the plants, maintaining a similar position and depth, about 30 cm,

in each case. Soil samples were preserved in plastic tubes at 4 °C in dark for subsequent enzymatic analyses.

2.4.2. Alkaline Phosphomonoesterase (ALP)

ALP, classified as EC 3.1.3.1. according to the Enzyme Commission, was measured using a protocol based on the method described by Tabatabai et al. [22]. ALP activity was quantified by spectrophotometry, using a UviLine 9400 UV-Vis spectrophotometer (Schott Instruments, Mainz, Germany). 1 g of soil was mixed with 4 mL of Modified Universal Buffer (MUB) at pH 11, 0.2 mL of pure toluene (Sigma-Aldrich, Burlington, MA, USA) and 1 mL of p-nitrophenyl phosphate solution (0.025 M, pH 11), homogenized and incubated in a digital Incuterm stove (Raypa, Terrassa, Spain) during 1h at 37 °C. MUB (pH 11) and p-nitrophenyl phosphate solution (0.025 M, pH 11) were prepared as described by Kumari et al., [23]. Then, 1 mL of 0.5 M $CaCl_2$ (Sigma-Aldrich) and 4 mL of 0.5M NaOH (Sigma-Aldrich) were added, and each sample was homogenized and filtered with a 0.11 µm FILTER-LAB® filter paper (Filtros Anoia, Barcelona, Spain). The absorbance of p-nitrophenol, produced by the hydrolysis of the substrate due to ALP action, was measured at 400 nm. For ALP activity quantification, a calibration curve was performed for the external standard of p-nitrophenol (Sigma-Aldrich), including 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.5M NaOH, and applying the following formula:

$$EA = \frac{C \times V}{M_{W\,(\mathrm{p-nitrophenol})} \times G \times T} \quad (1)$$

where EA is ALP activity (µmol p-nitrophenol g^{-1} dry soil h^{-1}), C is the amount of p-nitrophenol that was detected (µg), V is the dilution factor, M_W is the molecular weight of p-nitrophenol, G is the factor related to the amount of dry soil and T is the factor related to the incubation time.

2.4.3. Catalase (CAT)

CAT activity (EC 1.11.1.6.) analysis was based on a method described by Johnson et al. [24], with slight modifications. Determination of CAT activity was carried out by spectrophotometry, using a UviLine 9400 UV-Vis spectrophotometer (Schott Instruments). One soil sample of 0.5 g per replicate was mixed with 40 mL of Milli-Q® water and homogenized using an Orbital Vibrax shaker (OVAN, Suministros Grupo Esper, Barcelona, Spain) at 300 rpm for 30 min. 5 mL of H_2O_2 solution (1:100, v/v) were added, the tubes were covered with parafilm and shaken for 10 min at 300 rpm. Then, each replicate was mixed with 5 mL of H_2SO_4 and filtered with a 0.11 µm FILTER-LAB® filter paper (Filtros Anoia, Barcelona, Spain). One control per replicate was prepared, following the same procedure, except for changing the 5 mL of H_2O_2 solution per 5 mL of Milli-Q® water and the blank was also prepared with every reagent but without adding soil. 25 mL of each filtered sample were titrated with 0.01M $KMnO_4$ (Sigma-Aldrich), until a constant pink color was obtained, recording the volume of $KMnO_4$ used. For CAT activity quantification, the following formula was applied:

$$EA = \frac{\left[\mathrm{mL_{blank}} - \left(\mathrm{mL_{sample}} - \mathrm{mL_{control}}\right)\right] \times N_{\mathrm{KMnO_4}} \times V \times T \times D}{\mathrm{Sampleweight}} \quad (2)$$

where EA is CAT activity (mmol H_2O_2 g^{-1} dry soil h^{-1}), $\mathrm{mL_{blank}}$ is the volume of $KMnO_4$ used in the titration of the blank, $\mathrm{mL_{sample}}$ is the volume of $KMnO_4$ used in the titration of the sample, $\mathrm{mL_{control}}$ is the volume of $KMnO_4$ used in the titration of the control, N_{KMnO4} is the normality of $KMnO_4$, V is the reaction factor of $KMnO_4$ with H_2O_2, T is the factor

related to the incubation time, D is the dilution factor and sample weight is the amount of dry soil.

2.4.4. Dehydrogenase (DH)

DH (EC 1.2.1.3) activity was quantified following the method of García et al. [25], with some modifications. DH activity was measured spectrophotometrically, utilizing a UviLine 9400 UV-Vis spectrophotometer (Schott Instruments). 1 g of each soil sample was mixed with distilled water until reaching 60% of field capacity. 0.2 mL of 0.4% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (Sigma-Aldrich) were added, and each sample was homogenized, covered with parafilm, and incubated during 20 h at 20 °C in dark. The iodonitrotetrazolium formazan (INTF) (Sigma-Aldrich) formed in each soil sample after the incubation was mixed with 9.8 mL of methanol 100% (ITW Reagents, Monza, Italy), vigorously shaken using an Orbital Vibrax shaker (OVAN) for 15 min and filtered with a 0.11 µm FILTER-LAB® filter paper. One control per soil sample was prepared following the same protocol, adding 0.2 mL of distilled water instead of 0.4% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride. The absorbance of INTF was measured at 490 nm and DH activity was quantified using a calibration curve for the external standard of 60 µg mL^{-1} INTF and the following equation:

$$EA = \frac{C \times V}{M_{W(INTF)} \times G \times T} \quad (3)$$

where EA is DH activity (µmol INTF g^{-1} dry soil h^{-1}), C is the amount of INTF of each sample (µg mL^{-1}), V is the final volume, M_W is the molecular weight of INTF, G is the factor related to the weight of the dry soil used, and T is the factor related to the incubation time.

2.5. Fruit Analyses

2.5.1. Fruit Sampling and Preparation

Pepper fruits were harvested at their two main commercial maturity stages: (i) green-ripe (final size but entirely green color) and (ii) fully ripe (final size and completely red). For each combination of genotype × treatment × ripening stage, three biological replicates were prepared. Each replicate consisted of a bulk of fruits collected from different plants of the two blocks, combining at least 5 fruits per block (i.e., each replicate included at least 10 fruits merged from both blocks). Fruits were cut into small pieces and seeds were eliminated. Fruits were processed to obtain liquid extract, using a BAPI 1000 PLUS INOX domestic blender (Taurus, Oliana, Spain). Liquid extracts were subsequently preserved at −80 °C for sugars analysis.

2.5.2. Soluble Sugars

Determination of sugars (fructose, glucose and sucrose) was performed by high-performance liquid chromatography (HPLC), based on the methodology described by Rosa-Martínez et al. [26], with some modification. Analyses were carried out using an Agilent 1220-Infinity LC System (Agilent Technologies, Santa Clara, CA, USA), equipped with an automatic injector, a binary pump and a Varian ProStar 350 RI detector (Varian, CA, USA). Liquid extract samples were centrifuged at 12,000 rpm for 5 min. The supernatant was diluted (1:2) with Milli-Q® water, homogenized with a vortex agitator and centrifuged at 12,000 rpm for 5 min. Then, the supernatant was filtered using a 0.20 µm Phenex-PTFE 15 mm syringe filter (Phenomenex, Torrance, CA, USA). The sugar separation was carried out using a Luna Omega SUGAR LC column (3 µm; 150 × 4.6 mm; Phenomenex, CA, USA). The mobile phase consisted of acetonitrile 100% (A) and HPLC-grade water (B), using an isocratic elution of 75% A:25% B and a flow rate of 0.8 mL·min^{-1}. The

injection volume was 10 µL, and the column temperature was maintained at 35 °C. For the sugar quantification, a calibration curve for external standards of fructose (≥99%), glucose (≥99.5%) and sucrose (≥99.5%) (Sigma-Aldrich) was used. Total sugars were calculated as the sum of glucose, fructose and sucrose. The contents of all sugars were expressed as mg kg^{-1} fresh weight (fw).

2.6. Statistical Analysis

Data were analyzed using Statgraphics Centurion 18 software (StatPoint Technologies, Warrenton, VA, USA). Results were expressed as the average value for each genotype and treatment, differentiating between ripening stages in the case of sugars. To assess normality of data, the Kolmogorov–Smirnov Test was used for analyzing global differences between genotypes or treatments ($n > 50$), and W of Shapiro-Wilks Test was used for analyzing data among genotypes within each treatment and between treatments within each genotype ($n < 50$). Data were transformed when their distribution did not correspond with a normal distribution. To test the effect of the genotype, treatment and ripening stage and their interactions, the results were analyzed using a three-way and a two-way analysis of variance (ANOVA). Then, Duncan post hoc multiple range test at $p < 0.05$ was applied.

3. Results

3.1. Analysis of Variance (ANOVA) for the Studied Traits

A general ANOVA was performed to evaluate the effects of genotype and treatment on yield traits and rhizosphere enzymatic activities. This two-way ANOVA included Genotype (G), Treatment (T) and their interaction G × T as factors (Table 3). Both genotype and treatment had significant effects on yield and fruit weight, as well as on all the enzymatic activities evaluated. The only exception was DH activity, which was not significantly influenced by the treatment. According to the mean square values, genotype was the factor contributing most to the variation observed in all the evaluated traits, while treatment contributed slightly to yield and ALP. The G × T interaction was significant for fruit weight and for two of the three enzymatic activities, ALP and CAT, but showed no effect on yield or DH (Table 3).

For fruit sugar concentration, ripening stage (R) was included in a separate three-way ANOVA. This analysis revealed that genotype, and particularly ripening stage, contributed most to the variation in the sugar profile, while treatment only significantly affected the accumulation of individual sugars, and not the total sugar concentration (Table 3). Regarding the interactions found between the main factors, G × T was not significant for any of the evaluated sugars. Conversely, the mean squares showed a significant contribution of the ripening stage in combination with other factors, particularly in G × R, while the T x R interaction showed a lower level of significance for glucose and sucrose, with no significant effect on fructose accumulation (Table 3).

The significant influence of ripening stage on the sugar profile in the general ANOVA could hide relevant effects of the other main factors and their interactions. For this reason, individual ANOVAs were also performed, considering the green-ripe and fully ripe stages separately. The ANOVA analysis of the green-ripe stage showed significant effects of both genotype and treatment on sugar accumulation, with genotype having the largest impact. In contrast, G × T was only slightly significant for glucose content. ANOVA analysis of fully ripe fruits also showed significant effects of genotype and treatment on sugar accumulation. Genotype showed the highest contribution to all sugars except sucrose, whereas treatment had minimal effects on fructose and total sugars. In contrast to the green-ripe stage, a significant G × T interaction was observed for fructose, sucrose and total sugars, but not for glucose (Table 3).

Table 3. General ANOVA for total yield, fruit weight, alkaline phosphomonoesterase (ALP), catalase (CAT) and dehydrogenase (DH) activities. General and individual ANOVA for green-ripe and fully ripe stages for glucose, fructose, sucrose and total sugar concentration.

General ANOVA		**Yield**	**Fruit Weight**		**ALP**	**CAT**	**DH**
Effect	**df** [1]	**MS** [2]	**MS**	**df**	**MS**	**MS**	**MS**
Main effect							
Genotype (G)	6	15.034 ***	0.132 ***	6	0.729 **	3.442 ***	18.500 ***
Treatment (T)	5	2.693 *	0.004 ***	5	0.617 *	0.153 **	0.636 ^{NS}
Interaction							
G × T	30	1.343 ^{NS}	0.001 *	30	0.831 ***	0.117 ***	0.353 ^{NS}
Error	321	0.899	0.001	126	0.236	0.026	0.408
		Glucose	**Fructose**	**Sucrose**	**Total sugars**		
Effect	**df**	**MS**	**MS**	**MS**	**MS**		
Main effect							
Genotype (G)	6	10.2 ***	29.6 ***	46.9 ***	90.3 ***		
Treatment (T)	5	12.8 **	17.7 **	43.2 ***	14.8 ^{NS}		
Ripening stage (R)	1	11,128.4 ***	23,500.0 ***	804.7 ***	53,095.3 ***		
Interactions							
G × T	30	3.3 ^{NS}	7.1 ^{NS}	5.2 ^{NS}	18.7 ^{NS}		
G × R	6	58.3 ***	105.8 ***	60.4 ***	197.2 ***		
T × R	5	16.5 **	10.2 ^{NS}	12.2 *	64.3 ***		
Error	198	3.5	4.9	3.0	16.3		
Green-ripe stage ANOVA							
Main effect							
Genotype (G)	6	38.7 ***	32.7 ***	101.0 ***	85.8 ***		
Treatment (T)	5	6.1 ***	5.1 ***	45.0 ***	27.3 **		
Interaction							
G × T	30	2.0 *	1.2 ^{NS}	6.5 ^{NS}	8.8 ^{NS}		
Error	84	1.2	0.9	4.3	6.3		
Fully ripe stage ANOVA							
Main effect							
Genotype (G)	6	29.7 ***	102.7 ***	6.3 ***	201.7 ***		
Treatment (T)	5	23.3 **	22.8 *	10.3 ***	51.8 *		
Interaction							
G × T	30	6.5 ^{NS}	14.6 **	3.9 ***	37.8 *		
Error	84	5.2	7.5	0.9	22.0		

[1] df: degrees of freedom. [2] MS: mean square. ^{NS} *, ** and *** indicate non-significant at $p > 0.05$, or significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, according to the Duncan test.

3.2. Yield and Fruit Weight

In accordance with the ANOVA, genotype produced a significant effect on both yield and fruit weight (Table 3). Among the genotype means, yield varied widely in our collection, ranging from 1.69 to 3.24 kg plant^{-1} in BGV13004 and H2, respectively. Regarding fruit weight, the lowest value was recorded in Piquillo, with 27 g, while the highest fruit weight was observed in Cabañeros F1, with 197 g (Table 4).

Considering the treatment means, the control treatment (100F + 100I) showed the highest average total yield, with 2.72 kg plant^{-1} (Table 4). Total yield under reduced fertilization (50F + 100I) and reduced irrigation (100F + 75I), with 2.48 and 2.55 kg plant^{-1}, respectively, were slightly lower than under 100F + 100I, although no significant differences were detected. In contrast, the combined stress treatment (50F + 75I) caused a significant yield reduction of 21.7%, with 2.13 kg plant^{-1} obtained. Regarding PGPR influence,

combined reduced fertilization with PGPR inoculation (50F + 100I + PGPR) did not differ significantly from the control, quantifying 2.50 kg plant^{-1}. However, a significant decrease of 16.5% was observed under combined fertilizer and irrigation reductions with PGPR inoculation (50F + 75I + PGPR), with 2.27 kg plant^{-1}, compared to 100F + 100I (Table 4).

Table 4. Total yield and fruit weight of the evaluated genotypes and treatments.

Yield (kg Plant^{-1})																			
Genotype	**Genotype Mean**		**100F + 100I**			**50F + 100I**			**50F + 100I + PGPR**			**100F + 75I**			**50F + 75I**			**50F + 75I + PGPR**		
BGV13004	1.69	A [1]	2.02	a [2]	B [3]	1.31	a	AB	1.97	a	B	1.97	ab	B	1.17	a	A	1.70	a	AB
Najerano	2.41	B	1.90	a	A	2.72	b	AB	2.09	a	A	3.17	c	B	2.13	b	A	2.49	ab	AB
Piquillo	1.88	A	1.95	a	A	1.75	a	A	1.87	a	A	1.60	a	A	2.14	b	A	1.94	a	A
H1	2.43	B	3.20	b	C	2.71	b	BC	2.22	a	AB	2.25	abc	AB	2.07	b	AB	1.72	a	A
H2	3.24	C	3.33	b	A	3.16	b	A	3.62	b	A	3.04	bc	A	3.00	b	A	3.30	b	A
Isabel F1	2.56	B	2.89	ab	A	2.84	b	A	2.31	a	A	2.59	abc	A	2.29	b	A	2.37	ab	A
Cabañeros F1	2.96	C	3.82	b	C	2.90	b	ABC	3.53	b	BC	3.03	bc	ABC	2.09	b	A	2.26	ab	AB
Treatment Mean			2.72	B [1]		2.48	AB		2.50	AB		2.55	AB		2.13	A		2.27	A	
Fruit weight (g)																			
BGV13004	124	C	135	cd	A	115	b	A	112	bc	A	135	c	A	123	bc	A	125	b	A
Najerano	110	B	110	b	A	120	bc	A	110	bc	A	105	b	A	99	b	A	113	b	A
Piquillo	27	A	26	a	A	27	a	A	29	a	A	26	a	A	27	a	A	25	a	A
H1	123	C	143	cd	B	143	c	B	104	b	A	112	bc	A	114	bc	A	108	b	A
H2	129	C	132	c	A	133	bc	A	132	cd	A	122	bc	A	128	cd	A	128	b	A
Isabel F1	148	D	156	d	B	166	d	B	144	d	AB	137	c	AB	152	d	AB	124	b	A
Cabañeros F1	197	E	231	e	C	203	e	ABC	183	e	AB	175	d	A	213	e	BC	176	c	A
Treatment Mean			132	B		127	AB		116	AB		116	AB		119	AB		109	A	

[1] Significant differences between overall treatment means and overall genotype means are indicated by capital letters; [2] significant differences between genotypes within the same treatment are indicated by lowercase letters; and [3] significant differences between treatments within the same genotype are indicated by capital letters. All statistical significance tests were performed using Duncan's test at the 5% level.

In terms of fruit weight, the 100F + 100I treatment resulted again in the highest average fruit weight across treatments, with 132 g. Although fruit weight values were lower under stress treatments (50F + 100I, 100F + 75I, 50F + 75I) compared to 100F + 100I, no significant differences were observed. Regarding PGPR effect, the 100F + 100I treatment showed a slightly higher, though not significant, fruit weight compared to fertilizer reduction with PGPR inoculation (50F + 100I + PGPR). However, fruit weight under control was significantly higher than under combined fertilizer and irrigation reduction with PGPR (50F + 75I + PGPR), which averaged 109 g, representing a 17.4% decrease. No significant differences were identified between PGPR treatments and their corresponding stress treatments (50F + 100I, 50F + 75I) (Table 4). These results align with the ANOVA, which revealed a significant effect of the treatments on both traits (Table 3). However, the absence of a clear separation in mean values suggested that variation may be more pronounced in certain genotypes. To further investigate these effects, the responses of individual genotypes were also examined.

Thus, under fertilizer reduction (50F + 100I), none of the genotypes showed a statistically significant decrease in yield or fruit weight compared to 100F + 100I (Table 4). In contrast, irrigation reduction (100F + 75I), although it did not significantly influence yield and fruit weight in some genotypes, some of them showed significant differences compared to 100F + 100I (Table 4). For example, Najerano had a significant yield increase of 66.8% under 100F + 75I compared to 100F + 100I. Conversely, H1 exhibited a significant yield reduction of 29.7% under 100F + 75I, while both Cabañeros F1 and H1 showed significantly lower fruit weight compared to 100F+100I, with reductions of 24.2% and 21.7%, respectively (Table 4). Combined fertilization and irrigation reduction (50F + 75I) decreased yield and fruit weight in some genotypes. Significant yield reductions of 42.1%, 45.3% and 35.3% were detected in BGV13004, Cabañeros F1 and H1, respectively, while fruit weight was significantly reduced by 20.3% under 50F + 75I only in H1 (Table 4).

The potential role of PGPR inoculation was assessed by comparing its effect under reduced fertilization (50F + 100I + PGPR) and combined fertilization and irrigation reduction (50F + 75I + PGPR) with both 100F + 100I treatment and their corresponding stress treatments (50F + 100I and 50F + 75I). Across the evaluated genotypes, the application of PGPR under 50F + 100I + PGPR did not produce significant differences in yield or fruit weight compared to either 100F + 100I or 50F + 100I. Only BGV13004 and Cabañeros F1 showed slight yield increases under PGPR inoculation relative to 50F+100I, reaching values closer to those of 100F + 100I, although these differences were not statistically significant. A similar trend was observed for the 50F + 75I + PGPR treatment, where yield and fruit weight were comparable to or lower than those observed under the 100F + 100I or 50F + 75I treatments. Slight increases in yield were only observed in BGV13004 and Najerano under PGPR application compared to the 50F + 75I, with values similar to or exceeding those of the control, but again, these differences were not statistically significant (Table 4).

The remarkable stability observed in most genotypes across treatments, in terms of yield and fruit weight, was in agreement with the non-significant G × T interaction detected by the ANOVA for the former, and with the slight but significant interaction found for the latter (Tables 3 and 4).

3.3. Rhizosphere Enzymatic Activity

As indicated by the ANOVA results, significant differences in soil enzymatic activities among genotypes were observed, particularly for CAT and DH (Table 3). Genotype means for ALP ranged from 1.71 to 2.22 µmol p-nitrophenol g^{-1} dry soil h^{-1} in Najerano and Piquillo, respectively. CAT activity varied from 0.75 to 1.83 mmol H_2O_2 g^{-1} dry soil h^{-1}, with Piquillo and BGV13004 showing the lowest and highest values, respectively. DH activity also varied widely, ranging from 0.36 to 2.65 µmol INTF g^{-1} dry soil h^{-1} in Piquillo and H2, respectively (Table 5).

In contrast, treatment means showed low variability (Table 5), consistent with the ANOVA results, which indicated that treatments had a relatively minor effect on enzymatic activities (Table 3). The control treatment (100F + 100I) mean showed the lowest ALP activity, 1.79 µmol p-nitrophenol g^{-1} dry soil h^{-1}. A general increase in ALP activity was observed under stress treatments (50F + 100I, 100F + 75I and 50F + 75I), although only fertilizer reduction (50F + 100I) resulted in a statistically significant increase, reaching 2.20 µmol p-nitrophenol g^{-1} dry soil h^{-1}, which represented a 22.9% rise (Table 5). A similar, though non-significant, trend was observed in both treatments involving PGPR inoculation, with ALP values of 1.94 and 2.03 µmol p-nitrophenol g^{-1} dry soil h^{-1} under reduced fertilization with PGPR (50F + 100I + PGPR) and combined fertilizer and irrigation reduction with PGPR (50F + 75I + PGPR), respectively (Table 5). CAT and DH activities did not show significant differences across treatment means (Table 5). However, due to the significant G × T interactions revealed by the ANOVA (Table 3), individual genotype responses, particularly for ALP and CAT, were further analyzed.

Under fertilizer reduction (50F + 100I), ALP activity strongly varied among genotypes. Significant increases of 71.3% and 114.7% were observed in Cabañeros F1 and Isabel F1, respectively, under 50F + 100I compared to 100F + 100I (Table 5). In contrast, ALP activity remained unchanged in Piquillo, H1 and H2, while BGV13004 and Najerano showed significant decreases of 27.2% and 35.6%, respectively (Table 5). Under water reduction (100F + 75I), only BGV13004 and Najerano showed significant ALP reductions of 31.3% and 27.9%, respectively (Table 5). Regarding PGPR application, no consistent effect on ALP activity was observed in our collection. No significant differences were detected between PGPR treatments and their corresponding stress conditions (50F + 100I and 50F + 75I),

except for H1, which showed significant increases of 94.9% and 45.2% under 50F + 100I + PGPR compared to 100F + 100I and 50F + 100I, respectively (Table 5).

Table 5. Rhizosphere alkaline phosphomonoesterase, catalase and dehydrogenase activities of the evaluated genotypes and treatments.

Alkaline Phosphomonoesterase Activity (μmol p-Nitrophenol g^{-1} Dry Soil h^{-1})																		
Genotype	**Genotype Mean**		**100F + 100I**			**50F + 100I**			**50F + 100I + PGPR**			**100F + 75I**			**50F + 75I**			**50F + 75I + PGPR**		
BGV13004	1.85	AB [1]	2.46	c [2]	B [3]	1.79	abc	A	1.61	a	A	1.69	ab	A	1.58	a	A	1.97	ab	AB
Najerano	1.71	A	2.19	bc	B	1.41	a	A	1.63	a	A	1.58	ab	A	1.70	ab	A	1.76	a	A
Piquillo	2.22	B	2.16	bc	A	2.55	bcd	A	2.16	a	A	2.35	c	A	2.12	bc	A	2.00	ab	A
H1	1.86	AB	1.17	a	A	1.57	ab	AB	2.28	a	C	1.42	a	A	2.14	bc	BC	2.59	b	C
H2	2.04	AB	1.39	a	A	1.98	abc	AB	1.89	a	AB	1.89	bc	AB	2.31	c	AB	2.76	b	B
Isabel F1	2.11	AB	1.56	ab	A	3.35	d	C	2.37	a	BC	1.95	bc	AB	1.79	abc	AB	1.61	a	AB
Cabañeros F1	1.90	AB	1.60	ab	A	2.74	cd	B	1.65	a	A	1.86	abc	A	2.03	abc	A	1.51	a	A
Treatment Mean			1.79	A [1]		2.20	B		1.94	AB		1.82	AB		1.95	AB		2.03	AB	
Catalase activity (mmol H_2O_2 g^{-1}dry soil h^{-1})																				
BGV13004	1.83	D	1.10	c	A	1.70	c	B	1.98	c	BC	2.04	b	BC	2.00	d	BC	2.18	e	C
Najerano	0.80	AB	0.72	a	A	0.89	ab	A	0.85	ab	A	0.95	a	A	0.69	a	A	0.73	a	A
Piquillo	0.75	A	0.69	a	A	0.73	a	A	0.71	a	A	0.80	a	A	0.77	ab	A	0.79	ab	A
H1	0.81	AB	0.66	a	A	0.71	a	AB	0.80	ab	ABC	0.87	a	BC	0.87	bc	C	0.94	bc	C
H2	0.85	BC	1.00	bc	D	0.95	b	CD	0.90	b	BCD	0.82	a	BC	0.77	ab	AB	0.65	a	A
Isabel F1	0.93	C	0.93	bc	A	0.97	b	A	0.86	ab	A	0.85	a	A	0.96	c	A	1.00	cd	A
Cabañeros F1	0.94	C	0.86	ab	A	0.83	ab	A	0.83	ab	A	0.93	a	AB	1.02	c	BC	1.14	d	C
Treatment Mean			0.85	A		0.97	A		0.99	A		1.04	A		1.01	A		1.06	A	
Dehydrogenase activity (μmol INTF g^{-1}dry soil h^{-1})																				
BGV13004	0.70	B	0.96	abc	A	0.56	a	A	0.72	bc	A	0.54	a	A	0.69	ab	A	0.71	ab	A
Najerano	0.63	B	0.69	ab	A	0.60	a	A	0.56	b	A	0.54	a	A	0.74	ab	A	0.64	ab	A
Piquillo	0.36	A	0.25	a	A	0.52	a	A	0.23	a	A	0.45	a	A	0.27	a	A	0.42	a	A
H1	2.28	E	1.52	cd	A	2.62	c	AB	2.50	d	AB	1.66	c	AB	3.19	c	B	2.19	d	AB
H2	2.65	E	2.20	d	A	3.35	c	A	2.59	d	A	2.60	c	A	2.45	c	A	2.73	d	A
Isabel F1	1.35	D	1.24	bc	A	1.84	c	B	1.02	c	A	1.49	c	AB	1.24	b	A	1.28	c	A
Cabañeros F1	0.93	C	0.71	ab	A	0.97	b	A	0.84	bc	A	0.95	b	A	1.13	b	A	1.02	bc	A
Treatment Mean			1.08	A		1.49	A		1.21	A		1.17	A		1.39	A		1.28	A	

[1] Significant differences between overall treatment means and overall genotype means are indicated by capital letters; [2] significant differences between genotypes within the same treatment are indicated by lowercase letters; and [3] significant differences between treatments within the same genotype are indicated by capital letters. All statistical significance tests were performed using Duncan's test at the 5% level.

CAT activity generally remained stable between the control (100F + 100I) and reduced fertilization (50F + 100I) for most genotypes. An exception was observed in BGV13004, which showed a significant 54.6% increase under 50F + 100I (Table 5). Responses to water reduction (100F + 75I) varied among genotypes, as BGV13004 and H1 displayed significant increases of 85.5% and 31.8%, respectively, compared to 100F + 100I, whereas H2 showed a significant decrease of 18% under water stress (Table 5). PGPR inoculation had no significant effect on CAT activity across 100F + 100I, 50F + 100I and fertilizer reduction with PGPR (50F + 100I + PGPR) in most genotypes. However, BGV13004 showed an increase under 50F + 100I + PGPR compared to 50F + 100I, though not statistically significant. Both 50F + 100I and 50F + 100I + PGPR were significantly higher than 100F + 100I, with increases of 54.6% and 80%, respectively (Table 5). Additionally, BGV13004, Cabañeros F1 and H1 showed higher CAT activity under reduced fertilization and irrigation with PGPR (50F + 75I + PGPR) compared to 50F + 75I, although the increases were not significant. However, values under both 50F + 75I and 50F + 75I + PGPR were significantly higher than in 100F + 100I, with increases of 81.8% and 98.2% for BGV13004, 18.6% and 32.6% for Cabañeros F1, and 31.8% and 42.4% for H1, respectively (Table 5). In contrast, H2 showed similar CAT activity under 50F + 75I and 50F + 75I + PGPR, both significantly lower than 100F + 100I (23% and 35% reductions, respectively) (Table 5).

Regarding DH activity, only Isabel F1 exhibited a significant increase of 48.4% under fertilizer reduction (50F + 100I). For water reduction (100F + 75I), no significant differences were observed between 100F + 100I and 100F + 75I in any genotype (Table 5). In addition, no significant PGPR effect on DH activity was detected across most genotypes (Table 5). Isabel F1 was the only genotype showing a significant DH decrease (44.6%) under reduced fertilization with PGPR (50F + 100I + PGPR) compared to 50F + 100I. H1 also displayed lower DH activity under combined reduced fertilization and irrigation with

PGPR (50F + 75I + PGPR) compared to 50F + 75I, but the difference was not statistically significant (Table 5).

Overall, the significant and genotype-specific trends observed in some genotypes across treatments for ALP and CAT activities, supported the significant G × T interactions detected by the ANOVA. By contrast, genotypes barely changed their DH activity among treatments, in agreement with the non-significant G × T interaction in this trait (Tables 3 and 5).

3.4. Sugars Content

As indicated by the ANOVA results for green-ripe and fully ripe fruits (Table 3), significant differences were found in the sugar profile across genotypes. At the green-ripe stage, genotype means showed the lowest glucose concentration in Piquillo peppers, with 9.6 g kg^{-1} fw, whereas Cabañeros F1 had the highest content, 14.3 g kg^{-1}. A similar trend was observed for fructose, with Piquillo showing the lowest concentration, 8.3 g kg^{-1}, and Cabañeros F1 the highest content, 12.2 g kg^{-1}. For sucrose, the lowest average contents were quantified in Najerano and Cabañeros F1, with 1.8 and 2.1 g kg^{-1}, respectively, while Isabel F1 showed the highest value, 8.7 g kg^{-1}. In contrast, variability in total sugar content among genotypes was less pronounced, with Piquillo and Najerano showing the lowest concentrations, 24.2 and 25.1 g kg^{-1}, respectively, while the others ranged from 28.4 to 30 g kg^{-1} (Table 6).

Table 6. Glucose, fructose, sucrose and total sugar concentrations in green-ripe pepper fruits of the evaluated genotypes and treatments.

Genotype	Genotype Mean		100F + 100I			50F + 100I			50F + 100I + PGPR			100F + 75I			50F + 75I			50F + 75I + PGPR		
Glucose (g kg^{-1} fw)																				
BGV13004	13.1	CD [1]	13.7	de [2]	A [3]	13.0	b	A	12.1	bc	A	13.7	cd	A	12.7	cd	A	13.4	ab	A
Najerano	12.3	BC	13.3	cde	BC	12.0	b	ABC	12.1	bc	ABC	11.1	b	A	11.5	bc	AB	13.6	ab	C
Piquillo	9.6	A	8.7	a	A	9.8	a	AB	10.1	a	AB	9.0	a	AB	8.3	a	A	11.8	a	B
H1	13.3	D	12.7	bcd	A	12.4	b	A	13.5	c	AB	12.6	bcd	A	14.0	d	B	14.5	ab	B
H2	12.7	BCD	12.0	b	A	12.1	b	A	13.0	bc	A	12.5	bc	A	13.7	cd	A	12.8	ab	A
Isabel F1	11.8	B	12.4	bc	B	12.1	b	B	11.8	b	B	12.3	bc	B	10.0	ab	A	12.3	a	B
Cabañeros F1	14.3	E	14.1	e	AB	13.2	b	A	13.5	c	AB	14.6	d	AB	14.2	d	AB	16.0	b	B
Treatment Mean			12.4	AB [1]		12.1	A		12.3	A		12.3	A		12.1	A		13.5	B	
Fructose (g kg^{-1} fw)																				
BGV13004	11.4	C	12.2	d	A	11.8	b	A	10.9	bc	A	11.0	b	A	11.2	b	A	11.4	ab	A
Najerano	11.0	C	11.4	cd	AB	11.0	ab	AB	11.2	c	AB	10.3	b	A	10.3	b	A	12.1	ab	B
Piquillo	8.3	A	7.4	a	AB	8.7	a	AB	8.9	a	AB	7.5	a	AB	6.9	a	A	10.2	a	B
H1	11.5	CD	11.6	cd	BC	11.0	ab	AB	12.2	c	C	10.3	b	A	11.6	b	BC	12.3	ab	C
H2	11.0	C	10.8	bc	A	10.8	ab	A	11.6	c	A	10.7	b	A	10.9	b	A	11.4	ab	A
Isabel F1	9.5	B	10.4	b	C	9.0	a	B	9.8	ab	BC	9.7	b	BC	7.9	a	A	10.1	a	BC
Cabañeros F1	12.2	D	12.1	d	A	11.6	b	A	12.0	c	A	12.7	c	A	11.9	b	A	12.8	b	A
Treatment Mean			10.8	AB		10.6	AB		10.9	AB		10.3	A		10.1	A		11.5	B	
Sucrose (g kg^{-1} fw)																				
BGV13004	4.2	B	2.1	a	A	5.5	bcd	AB	0.6	a	A	7.8	bc	B	4.9	ab	AB	4.1	bc	AB
Najerano	1.8	A	2.2	a	AB	0.5	a	A	1.0	ab	A	4.9	ab	B	1.7	a	AB	0.6	a	A
Piquillo	6.3	C	9.5	b	B	7.1	cd	B	2.6	ab	A	7.0	bc	B	8.6	cd	B	3.2	bc	A
H1	4.4	B	3.4	a	AB	4.8	bcd	AB	1.3	ab	A	6.6	bc	B	5.8	bc	B	4.6	bc	AB
H2	4.7	BC	8.0	b	B	3.6	abc	A	3.9	bc	A	5.6	ab	AB	5.1	ab	AB	2.0	ab	A
Isabel F1	8.7	D	10.0	b	A	8.5	d	A	7.7	c	A	9.2	c	A	10.0	d	A	6.6	c	A
Cabañeros F1	2.1	A	3.0	a	AB	2.6	ab	AB	0.2	a	A	2.3	a	AB	3.2	ab	B	1.5	ab	AB
Treatment Mean			5.4	C		4.7	BC		2.5	A		6.2	C		5.6	C		3.2	AB	
Total Sugars (g kg^{-1} fw)																				
BGV13004	28.7	B	28.0	ab	B	30.4	d	B	23.6	ab	A	32.5	c	B	28.8	b	B	28.9	ab	B
Najerano	25.1	A	26.9	a	A	23.4	a	A	24.3	ab	A	26.3	ab	A	23.5	a	A	26.2	ab	A
Piquillo	24.2	A	25.5	a	A	25.7	ab	A	21.6	a	A	23.6	a	A	23.8	a	A	25.2	a	A
H1	29.2	B	27.6	ab	A	28.2	bcd	A	27.0	bcd	A	29.5	bc	A	31.5	b	A	31.5	b	A
H2	28.4	B	30.9	ab	B	26.5	abc	AB	28.5	cd	AB	28.8	abc	AB	29.8	b	AB	26.2	ab	A
Isabel F1	30.0	B	32.8	b	B	29.7	cd	AB	29.3	d	AB	31.2	bc	AB	27.9	b	A	29.1	ab	AB
Cabañeros F1	28.6	B	29.2	ab	A	27.4	bcd	A	25.6	bc	A	29.5	bc	A	29.2	b	A	30.4	ab	A
Treatment Mean			28.7	B		27.3	AB		25.7	A		28.8	B		27.8	AB		28.2	B	

[1] Significant differences between overall treatment means and overall genotype means are indicated by capital letters; [2] significant differences between genotypes within the same treatment are indicated by lowercase letters; and [3] significant differences between treatments within the same genotype are indicated by capital letters. The statistical tests were performed using Duncan's test at the 5% level.

At the fully ripe stage, a marked increase in glucose, fructose and total sugar content was observed after ripening, although this increase varied across genotypes, reflecting a sig-

nificant G × R interaction (Table 3). Genotype means for glucose ranged from 23.7 g kg^{-1} in BGV13004 to 26.2–26.9 g kg^{-1} in H2, Isabel F1, Najerano and Piquillo. Fructose content was the lowest in Cabañeros F1, with 25.6 g kg^{-1}, and the highest in Piquillo, with 32.7 g kg^{-1}. Sucrose ranged from 0.3 g kg^{-1} in Piquillo to 2.0 g kg^{-1} in BGV13004. Total sugar content was the lowest in Cabañeros F1 and BGV13004, with 50.6 and 53.8 g kg^{-1}, respectively, and significantly higher in the other genotypes, with 57.4–59.9 g kg^{-1} (Table 7).

Table 7. Glucose, fructose, sucrose and total sugar concentrations in fully ripe pepper fruits of the evaluated genotypes and treatments.

Glucose (g kg^{-1} fw)																			
Genotype	**Genotype Mean**		**100F + 100I**			**50F + 100I**			**50F + 100I + PGPR**			**100F + 75I**			**50F + 75I**			**50F + 75I + PGPR**		
BGV13004	23.7	A [1]	23.5	a [2]	AB [3]	23.5	a	AB	25.2	a	B	23.1	ab	AB	25.0	a	B	21.8	a	A
Najerano	26.2	C	26.6	a	AB	27.7	bc	B	25.3	ab	AB	24.3	abc	A	26.9	a	AB	26.1	bc	AB
Piquillo	26.9	C	27.7	a	BC	28.9	c	C	28.1	b	BC	24.7	abc	A	26.5	a	ABC	25.7	bc	AB
H1	25.7	BC	23.7	a	A	29.0	c	B	27.3	ab	AB	24.9	abc	AB	23.2	a	A	26.0	bc	AB
H2	26.6	C	25.8	a	AB	29.2	c	B	27.7	ab	AB	26.5	bc	AB	22.1	a	A	28.2	c	AB
Isabel F1	26.8	C	26.2	a	A	27.7	bc	A	26.9	ab	A	27.8	c	A	26.6	a	A	25.8	bc	A
Cabañeros F1	24.3	AB	26.2	a	A	25.1	ab	A	26.0	ab	A	22.1	a	A	22.1	a	A	24.1	ab	A
Treatment Mean			25.7	ABC [1]		27.3	C		26.6	BC		24.8	A		24.6	A		25.4	AB	
Fructose (g kg^{-1}fw)																			
BGV13004	28.2	B	28.5	ab	B	27.9	ab	AB	29.4	ab	B	24.0	a	A	31.2	ab	B	28.3	ab	B
Najerano	31.3	CD	28.4	ab	A	31.3	cd	AB	31.2	abc	AB	32.1	b	AB	32.2	b	AB	32.7	c	B
Piquillo	32.7	D	32.5	b	B	34.4	de	B	33.5	c	B	28.9	b	A	33.9	b	B	32.9	c	B
H1	30.8	CD	27.2	a	A	34.9	e	B	31.8	bc	AB	30.3	b	AB	28.8	ab	AB	31.4	bc	AB
H2	31.5	CD	29.4	ab	AB	34.6	de	B	32.0	bc	AB	31.7	b	AB	26.0	ab	A	35.1	c	B
Isabel F1	30.0	BC	28.1	ab	A	30.6	bc	AB	30.4	abc	AB	32.6	b	B	30.8	ab	AB	27.5	a	A
Cabañeros F1	25.6	A	27.0	a	AB	25.4	a	AB	28.0	a	B	24.1	a	AB	23.7	a	A	25.6	a	AB
Treatment Mean			28.7	A		31.3	A		30.9	A		29.1	A		29.5	A		30.5	A	
Sucrose (g kg^{-1}fw)																			
BGV13004	2.0	C	2.2	a	B	0.1	a	A	1.5	bc	AB	7.5	d	C	0.3	a	A	0.1	a	A
Najerano	0.8	AB	0.5	a	A	1.1	a	A	0.3	ab	A	0.3	a	A	2.2	a	A	0.4	a	A
Piquillo	0.3	A	0.0	a	A	0.4	a	BC	0.0	a	A	0.7	ab	C	0.2	a	AB	0.4	a	BC
H1	1.6	BC	2.4	a	A	1.5	a	A	2.1	c	A	2.5	c	A	0.6	a	A	0.4	a	A
H2	1.3	ABC	1.9	a	A	0.5	a	A	1.3	bc	A	1.9	c	A	1.6	a	A	0.5	a	A
Isabel F1	0.6	AB	1.7	a	C	0.0	a	A	0.0	a	A	1.3	bc	BC	0.3	a	ABC	0.1	a	AB
Cabañeros F1	0.7	AB	1.9	a	B	0.0	a	A	0.0	a	A	1.1	bc	B	1.1	a	B	0.3	a	A
Treatment Mean			1.5	BC		0.5	A		0.7	A		2.2	C		0.9	AB		0.3	A	
Total Sugars (g kg^{-1}fw)																			
BGV13004	53.8	A	54.2	a	A	51.5	a	A	56.0	ab	A	54.6	abc	A	56.5	ab	A	50.2	a	A
Najerano	58.3	B	55.5	a	A	60.1	bc	A	56.8	abc	A	56.7	bc	A	61.3	b	A	59.3	bc	A
Piquillo	59.9	B	60.2	a	AB	63.7	bc	B	61.6	c	B	54.3	ab	A	60.6	ab	B	59.0	bc	AB
H1	58.0	B	53.3	a	A	65.4	c	B	61.2	bc	AB	57.7	bc	AB	52.7	ab	A	57.8	bc	AB
H2	59.4	B	57.1	a	AB	64.3	bc	B	61.1	bc	AB	60.1	bc	AB	49.8	ab	A	63.9	c	B
Isabel F1	57.4	B	56.0	a	AB	58.4	b	AB	57.2	abc	AB	61.8	c	B	57.7	ab	AB	53.4	ab	A
Cabañeros F1	50.6	A	55.1	a	A	50.5	a	A	54.0	a	A	47.3	a	A	46.8	a	A	49.9	a	A
Treatment Mean			55.9	A		59.1	A		58.3	A		56.1	A		55.0	A		56.2	A	

[1] Significant differences between overall treatment means and overall genotype means are indicated by capital letters; [2] significant differences between genotypes within the same treatment are indicated by lowercase letters; and [3] significant differences between treatments within the same genotype are indicated by capital letters. The statistical tests were performed using Duncan's test at the 5% level.

At the green-ripe stage, treatment means differed significantly for all sugars (Table 6), consistent with the ANOVA (Table 3). Glucose content was the highest under the control treatment (100F + 100I), with 12.4 g kg^{-1}, although not significantly different from the stress treatments (50F + 100I, 100F + 75I and 50F + 75I) (Table 6). By contrast, a significant increase in glucose was observed under the combined fertilizer and irrigation reduction with PGPR (50F + 75I + PGPR), with 13.5 g kg^{-1}, representing an 11.6% rise compared to 50F + 75I, with 12.1 g kg^{-1}. Fructose followed a similar trend, with non-significant reductions under 100F + 75I and 50F + 75I compared to control (Table 6). In addition, a significant 13.9% increase in fructose was observed under 50F + 75I + PGPR compared to 50F + 75I (Table 6). In contrast, sucrose showed a different behavior. Slightly higher concentrations were recorded under 100F + 75I and 50F + 75I, with 6.2 and 5.6 g kg^{-1}, respectively, compared to 100F + 100I, with 5.4 g kg^{-1}, but no significant differences were observed under stress. A significant reduction in sucrose concentration was determined in both treatments involving PGPR inoculation (50F + 100I + PGPR and 50F + 75I + PGPR), with 2.5 and 3.2 g kg^{-1}, respectively, compared to their corresponding stress treatments without PGPR and the control. Accordingly, reductions of 53.7% and 46.8% were quantified under 50F + 100I +

PGPR compared to 100F + 100I and 50F + 100I, respectively, while reductions of 40.7% and 42.9% were quantified under 50F + 75I + PGPR compared to 100F + 100I and 50F + 75I (Table 6). Total sugars exhibited limited variability across treatments, as no significant differences were found between 100F + 100I and the stress treatments (50F + 100I, 100F + 75I and 50F + 75I). However, 50F + 100I + PGPR, with 25.7 g kg^{-1}, was significantly lower compared to 100F + 100I, with 28.7 g kg^{-1}, quantifying a reduction of 10.5% (Table 6).

In fully ripe fruits, treatment effects were less pronounced, particularly for fructose and total sugars, as shown by the ANOVA (Table 3). Regarding glucose concentration, the treatment mean corresponding to fertilizer reduction (50F + 100I), with 27.3 g kg^{-1}, was higher than the control (100F + 100I), with 25.7 g kg^{-1}, whereas lower levels were observed under irrigation reduction (100F + 75I) and combined fertilizer and irrigation reduction (50F + 75I), with concentrations of 24.8 and 24.6 g kg^{-1}, respectively. However, none of these differences were statistically significant. Similarly, PGPR treatments (50F + 100I + PGPR and 50F + 75I + PGPR) did not significantly affect glucose content compared to their corresponding stress treatments or the control (Table 7). Fructose also did not vary significantly among treatment means at the ripe stage (Table 7). For sucrose, a significant reduction was observed under 50F + 100I, with 0.5 g kg^{-1}, compared to 100F + 100I, which showed 1.5 g kg^{-1}. Treatments 100F + 75I and 50F + 75I did not differ significantly from the control. Regarding PGPR application, no significant differences were found between PGPR treatments and their corresponding stress conditions (Table 7). Given the particularly low sucrose concentrations quantified in fully ripe fruits, the significant differences observed among treatments and genotypes should be interpreted with caution. Finally, no significant differences were detected among treatment means for total sugars, as also observed for fructose (Table 7). Despite some significant treatment effects, most notably for glucose and sucrose, overall variability in fructose and total sugars was limited. This suggests that sugar accumulation in response to treatments is genotype-dependent, as also indicated by the significant G × T interactions described in the ANOVA (Table 3), which highlight the need for deeper genotype-specific interpretation.

Regarding fertilizer reduction (50F + 100I), in green-ripe fruits, no significant differences in sugar concentrations were observed between 50F + 100I and 100F + 100I for most genotypes, with the exception of a 13.5% reduction in fructose in Isabel F1, and a 55% reduction in sucrose in H2 (Table 6). Similarly, fertilizer reduction had little impact in ripe fruits, except for H1, which was the only genotype showing increased glucose, fructose and total sugars concentrations under 50F + 100I compared to 100F + 100I (22.4%, 28.3% and 22.7% increases, respectively). Piquillo also showed an increase in sucrose under 50F + 100I, while BGV13004 and Cabañeros F1 exhibited marked reductions compared to 100F + 100I (Table 7).

For water reduction (100F + 75I), no significant differences were found among most genotypes at the green-ripe stage between 100F + 100I and 100F + 75I, except for BGV13004, which exhibited a 271.4% increase in sucrose under 100F + 75I (Table 6). However, Najerano showed a significant 16.5% reduction in glucose content, and H1 an 11.2% reduction in fructose, under 100F + 75I compared to 100F + 100I (Table 6). Similarly, at the fully ripe stage, most genotypes did not show differences in sugar concentrations between 100F + 100I and 100F + 75I (Table 7). However, Piquillo exhibited significant reductions of 10.8% in glucose and 11.1% in fructose under 100F + 75I compared to 100F + 100I, and BGV13004 accumulated less fructose under 100F + 75I, with a 15.8% decrease (Table 7). In contrast, Isabel F1 showed a significant 16% increase in fructose under 100F + 75I compared to 100F + 100I, as was also observed in BGV13004 and Piquillo for sucrose (Table 7).

Under combined fertilizer and irrigation reduction (50F + 75I) at the green-ripe stage, H1 showed a significant 10.2% increase in glucose concentration, reaching 14.0 g kg^{-1}

under the combined stress compared to 12.7 g kg^{-1} under control conditions. In contrast, Isabel F1 exhibited a 19.4% reduction in glucose content under 50F + 75I, with 10.0 g kg^{-1}, relative to the control, with 12.4 g kg^{-1}. Fructose was significantly affected only in Isabel F1, decreasing from 10.4 g kg^{-1} under 100F + 100I to 7.9 g kg^{-1} under 50F + 75I, representing a 24.0% reduction. Similarly, total sugar content in Isabel F1 declined by 14.9%, from 32.8 g kg^{-1} under control to 27.9 g kg^{-1} under 50F + 75I (Table 6). At the fully ripe stage, no significant differences were observed under combined stress, except in BGV13004, which showed a reduction in sucrose compared to 100F + 100I (Table 7).

PGPR application significantly influenced glucose content only at the green-ripe stage in Isabel F1 and Piquillo, where the combined fertilizer and irrigation reduction with PGPR (50F + 75I + PGPR) resulted in increases of 23% and 42.2%, respectively, compared to their corresponding stress treatment (50F + 75I). Fructose was also higher in H1 under fertilizer reduction with PGPR (50F + 100I + PGPR) compared to 50F + 100I, with an increase of 10.9%. Similarly, fructose increases of 27.9%, 17.5% and 47.8% were observed in Isabel F1, Najerano and Piquillo under 50F + 75I + PGPR compared to 50F + 75I. Sucrose content remained largely unaffected by PGPR, except in Piquillo, where notable reductions of 63.4% under 50F + 100I + PGPR compared to 50F + 100I, and 62.8% under 50F + 75I + PGPR compared to 50F + 75I, were observed (Table 6). Similarly, total sugar content decreased by 22.4% in BGV13004 under 50F + 100I + PGPR compared to 50F + 100I (Table 6). At the fully ripe stage, PGPR inoculation had limited impact on glucose and fructose concentrations. The only exception was H2, which showed a 35% increase in fructose under 50F + 75I + PGPR compared to 50F + 75I, while BGV13004 exhibited a 12.8% reduction in glucose under the same conditions. For sucrose, Cabañeros F1 showed a reduction under 50F + 75I + PGPR compared to 50F + 75I, while Piquillo exhibited a reduction, but under 50F + 100I + PGPR compared to 50F + 100I (Table 7). Total sugars were generally unaffected by PGPR inoculation, with the exception of H2, which showed a 28.3% increase under 50F + 75I + PGPR compared to 50F + 75I (Table 7).

On the whole, a significant effect of genotype was observed on the sugar profile, along with some specific genotype-treatment combinations, particularly at the green-ripe stage (Table 6).

4. Discussion

Most pepper varieties cultivated in the Mediterranean basin are high-demand cultivars [27], well-adapted to greenhouse production systems [4], and optimized for high yields [28]. However, the urgent need to transition to more sustainable crop management is promoting the optimization of agronomic inputs [29] and the identification of genotypes better adapted to low-input conditions, which includes local ecotypes [30]. This shift also reflects the priorities set by the Common Agriculture Policy (CAP 2023–2027) [31]. In this context, our study evaluated yield and fruit weight, three selected rhizosphere enzymatic activities, and the fruit sugar profile at green-ripe and red maturity stages in seven sweet pepper genotypes. Six treatments were tested, combining two fertilizer and irrigation regimes, with PGPR inoculation. Identifying well-performing genotypes under reduced inputs and in response to PGPR is a key step toward sustainable pepper cultivation [32].

Yield is a complex trait influenced by environmental conditions as well as genotype-related factors, such as vegetative development, fruit weight and fruit number [33]. Optimizing input requirements is essential to balance productivity with sustainability [34]. In our study, fertilizer reduction did not significantly compromise yield or fruit weight, suggesting tolerance to lower nutrient inputs. These data are consistent with Vadillo et al. [35] for commercial and total yields in green peppers, although other authors reported increased pepper yields with higher nitrogen availability [36]. By contrast, irrigation reduction in our

study produced genotype-dependent responses, with some genotypes maintaining yield, others declining, and even increasing productivity. Significant reductions under water stress were also reported by Çolak et al. [37] and Mostafa et al. [38]. Combined stress of reduced fertilization and irrigation further decreased yield and fruit weight in some of our genotypes. This negative effect was supported by Zhang et al. [39] and Xiang et al. [40] in different sweet peppers, whereas Zamljen et al. [41] reported variable responses in pepper yield and fruit dry weight under multiple fertilizer and irrigation combinations. PGPR inoculation has been reported to enhance agronomic traits in pepper seedlings and other crops under stress conditions [42,43]. In contrast, PGPR did not increase yield or fruit weight in our study. This finding aligned with authors that reported limited effects of bacterial biostimulants under nutrient or water reduction [44,45]. This lack of response may be explained by the restricted adaptability of most PGPR strains, whose efficacy is highly dependent on host genotype, cultivation practices and soil type [46]. It is also influenced by competition with endogenous microbial communities [47] and by strains' ability to adopt survival strategies under water deficit, such as siderophore and microbial exopolysaccharide production [48], or under reduced fertilization, which may involve nitrogen fixation, linked to the presence of *nifH* genes [49]. Overall, the limited changes observed in yield and fruit weight across treatments in our collection were in agreement with the non-significant G × T interaction for yield, and the low significance of G × T interaction detected for fruit weight, indicating that the differences observed were mostly attributed to the main effects, i. e., genotype and treatment.

In our collection, although the commercial hybrid Cabañeros F1 had the highest yield and fruit weight under control conditions, its performance was declined under stress. In contrast, the experimental hybrid H2 achieved the highest average yield across treatments. In particular, Najerano, Piquillo, H2 and Isabel F1 were the best-performing genotypes under low-input treatments due to their stability, highlighting the potential of traditional cultivars for sustainable farming [50,51]. Previous studies suggest that part of this genotype-dependent tolerance to certain abiotic stresses is linked to root architecture [52] and their specific interactions with soil and microbial communities [21]. These results are particularly relevant in Mediterranean horticulture, where water scarcity and salinization are major challenges [53]. They also align with the European Green Deal and the Farm to Fork strategy, which set ambitious targets for reducing fertilizers, among other chemicals, by 2030 in EU Member States [54].

At the soil level, microorganisms are key endogenous biological components, driving essential biochemical processes [55]. Plant roots influence soil properties by realizing metabolites into the rhizosphere, stimulating microbial activity, and creating enzyme hotspots that foster plant-microbe interactions [56]. Among these microorganisms, PGPR are of particular interest because they cannot only promote plant growth, but also improve rhizosphere environment through their influence on biochemical processes and microbial dynamics [57].

ALP activity differed among our genotypes under fertilizer reduction. High variability in ALP activity has been reported in other studies, with increases in fertilized soils compared to unfertilized ones, sometimes linked to nitrogen availability, which can promote microbial growth and greater enzyme production [58]. Conversely, other studies observed higher ALP activity in unfertilized plots [59], possibly due to the inhibition of the *phoD* gene by orthophosphate from mineral fertilizers, as *phoD* encodes alkaline phosphatase enzyme [60]. However, some authors found stable ALP levels regardless of fertilization [61]. Under irrigation reduction, ALP activity tended to decrease due to reduced microbial activity and phosphatase production [62,63]. In our study, only a subset of genotypes showed a significant reduction in ALP activity under water deficit, while several genotypes

maintained stable ALP levels. This genotype-dependent variation in ALP activity has been documented previously [64,65]. Additionally, in our study, PGPR inoculation generally had little effect compared to the corresponding stress treatments, in line with observations reported by Jing et al. [66]. By contrast, positive effects have been reported in other studies, such as in yellow lupine with *Bacillus subtilis* under unfertilized conditions at the emergence stage [67], and in *Poa pratensis* with *Morchella* inoculation under drought [68].

CAT activity remained largely stable in most of our genotypes under reduced fertilization, which was consistent with Yuan et al. [61]. Other studies observed marked increases in CAT activity with fertilization [69], reporting that organic fertilizers can produce a higher impact on this enzyme compared to chemical ones, since fertilizer type can influence microbial communities and organic matter dynamics [70]. Irrigation reduction produced variable responses in our study, since some genotypes increased CAT activity under water deficit, while others experienced a reduction compared to control. Similar contrasting outcomes have been reported in the literature [65,71], indicating that CAT activity depends on soil conditions, genotype-specific responses of plant host and shifts in microbial communities [72]. Again, PGPR inoculation did not generally modify CAT activity in our genotypes compared to their corresponding stress treatments. These results were consistent with observations in PGPR-inoculated poplar seedlings under drought compared to non-inoculated plants under stress [66]. Conversely, positive effects have been described in other studies, such as in yellow lupin inoculated with *B. subtilis* under unfertilized conditions compared to non-inoculated plants under stress [67].

For DH activity, reduced fertilization did not produce major differences among our genotypes. However, the literature reports variable trends, as some studies observed increases in DH levels under both mineral and organic fertilization [73], while others reported no significant changes under mineral fertilization but increases with certain organic amendments [74]. Some studies also indicated reductions at higher mineral fertilizer doses [75]. Regarding water reduction, DH activity is frequently associated with declines [76,77]; however, our findings showed no significant differences under irrigation reduction, remaining stable under stress. Similar results were partially reported by Atika et al. [78] in some of the evaluated species. Regarding PGPR inoculation, its effect was generally limited in our genotypes. In contrast, variable responses have been reported by authors, such as Sood et al. [79], who observed variable tendencies in DH values in wheat soil inoculated with PGPR under various reduced fertilization doses. By contrast, Nader et al. [80] showed that *Bradyrhizobium japonicum* enhanced DH activity in soybean under reduced fertilization and under combined drought and low fertilization at the early pod stage, compared to non-inoculated plants at full doses. Similarly, Atika et al., [78] reported increased DH activity under severe and moderate drought in PGPR-inoculated *Atriplex halimus* and *Peganum harmala*, respectively, although no effect was detected in *Pennisetum setaceus*, compared to non-inoculated plants under the same drought levels.

In general, rhizosphere enzymatic activities showed limited responses to treatments in most genotypes. Only a few genotype-specific patterns, particularly for ALP and CAT, were in agreement with the highly significant G × T interaction reported by the ANOVA. The stability of DH was consistent with its non-significant G × T interaction. This limited response could be attributed to environmental conditions, soil characteristics, or limited microbial interactions within the rhizosphere of our pepper cultivars [46,65]. Nevertheless, this consistency under stress conditions suggested a degree of resilience in maintaining soil functionality under low-input farming, which aligned with the stability observed in yield and fruit weight. Notably, BGV13004, H1 and Isabel F1 showed the strongest increases in rhizosphere enzymatic activities under stress or PGPR inoculation, suggesting

the establishment of particular relationships with microbial communities at the rhizosphere level [81], and highlighting their potential for sustainable agriculture.

Free sugars are important contributors to pepper fruit flavor, nutritional value and consumer acceptance [82]. They also play a key role in plant growth, flowering, and in the responses to different abiotic stresses [83]. Because sugar concentrations in pepper fruits typically increase during ripening [84], our analysis were performed separately for green-ripe and fully ripe stages.

The effects of fertilization on sugar accumulation in horticultural crops are complex, and the literature reports no consistent trends [85]. In our study, sugar concentrations remained mostly stable across genotypes and ripening stages, indicating limited sensitivity to fertilizer reduction. These results agreed with previous studies in ripe peppers, where glucose content was unaffected by varying nitrogen doses [33]. Similar stability was observed for soluble and reducing sugars under moderate fertilizer reductions, although severe nutrient limitation was associated with declines [86]. Conversely, Urrea-López et al. [87] reported a significant increase in fructose under low nitrogen in Habanero pepper. Water reduction is commonly associated with sugar accumulation, since soluble sugars contribute to osmotic potential, maintain cell turgor, and mitigate changes in water potential [88]. Consistent with this, Haris et al. [89] observed increases in fructose, glucose, and sucrose contents in peppers under severe irrigation stress. Similar results for fructose and glucose were reported in tomato by Lu et al. [90]. In contrast, Zamljen et al. [91] found higher sugar contents under full irrigation in peppers. In our collection, most genotypes showed no significant changes in sugar content, being particularly stable at green-ripe stage. This finding was consistent with the study of Poomkokrak et al. [92], who reported stable glucose concentration in cherry tomato under water stress. The general stability in sugars between control and reduced irrigation in our study suggested that sugars played only a minor role in the response to water deficit, or that stress intensity was insufficient to induce marked sugar accumulation in fruits. Moreover, the limited impact of water reduction on yield and fruit weight indicated that the observed sugar stability was not merely a consequence of concentration or dilution effects related to fruit weight, as commonly reported in other studies [93,94]. PGPR application has been widely explored as a strategy to improve not only crop health and productivity, but also fruit quality [95]. In our study, PGPR inoculation positively influenced the sugar profiles of certain genotypes at the green-ripe stage, including Piquillo, Najerano, H1 and Isabel F1. This suggests that plant-microbe interactions in the rhizosphere can modulate plant host physiology and metabolites related to fruit composition [96]. The rest of the collection generally did not show significant effects in the presence of PGPR, as was reported by Del Amor et al. [97], who observed no significant changes in sugar content in green peppers under low nitrogen with PGPR compared to full fertilization. At the fully ripe stage, PGPR effects were limited. The complex metabolic changes that occur during ripening may partially explain the lack of clearer PGPR effects at full ripeness [84]. Similar observations were reported by Del Amor et al. [97], who found no significant differences in glucose and fructose contents between low fertilizer and low fertilizer with PGPR in ripe peppers. Conversely, Kim et al. [96] reported reduced total soluble sugar content in pumpkin inoculated with *Kushneria konosiri*, under drought compared to non-inoculated stressed plants.

Although commercial hybrids generally exhibited higher sugar contents at the green-ripe stage, several traditional genotypes and experimental hybrids displayed interesting sugar profiles at full ripeness, highlighting their potential for breeding programs targeting fruit quality [27]. These differences in sugar accumulation were reflected in the variable responses to treatments depending on genotype and ripening stage, which corroborates the significant G × R interaction and, to a lesser extent, in some G × T interactions.

Additionally, BGV13004 and H1 improved their sugar profiles under specific stress conditions in green-ripe peppers, while H1 and Isabel F1 showed the highest performance at fully ripe stage. PGPR inoculation enhanced sugar content in Najerano, Piquillo, H1 and Isabel F1 at the green-ripe stage, whereas H2 was the only genotype to benefit from PGPR at full ripeness. Although PGPR did not improve yield or fruit weight in our collection, increases in specific sugars in some genotypes highlight improvements in fruit quality under low-input conditions. Moreover, the effect of PGPR was influenced by genotype and ripening stage, suggesting that these microorganisms can be strategically applied to enhance fruit quality in sustainable agriculture. To improve the identification of PGPR strains with beneficial effects adapted to specific cultivars and environmental conditions, several authors recommend implementing molecular tools. These approaches include the analysis of specific plant stress-related genes responsive to PGPR, as well as broader studies of transcriptional responses of cultivars under stress combined with PGPR [49,98].

Identifying genotypes that combine stable yields and rhizosphere enzymatic activities with enhanced sugar contents under stress, alongside complementary strategies such as PGPR inoculation, represents a promising step toward sustainable pepper breeding adapted to the new Mediterranean conditions. Future multi-year and multi-location studies could further validate the broader applicability of these findings on other different pedoclimatic conditions.

5. Conclusions

The genotype factor had a significant effect on all the evaluated traits, highlighting the importance of genotype selection for the development of sustainable pepper production systems. Independent fertilizer and irrigation reductions generally did not significantly reduce yield in most genotypes. However, the combined reduction in both inputs caused significant decreases in some cases. In particular, Najerano, Piquillo, H2 and Isabel F1 maintained stable yields and fruit weights across all stress treatments compared to the control, making them promising candidates for further evaluation under low-input management.

Rhizosphere enzymatic activities exhibited genotype-dependent patterns and were only moderately affected by the treatments, particularly ALP and CAT. BGV13004 and Isabel F1 showed the most pronounced increases in enzyme activity under stress, highlighting their potential to enhance rhizosphere functioning under the evaluated conditions.

Sugar accumulation in fruits was strongly dependent on ripening stage. Under stress conditions, BGV13004 and H1 showed increased sugar contents in green fruits, while H1 and Isabel F1 exhibited higher sugar contents at full ripeness. These results indicate genotype-specific resilience in fruit quality.

PGPR inoculation had limited effects on yield, fruit weight and rhizosphere enzymatic activities under the tested conditions. Nevertheless, beneficial effects were observed in specific cases. For instance, PGPR promoted ALP activity in H1, and improved sugar profiles at the green-ripe stage in genotypes such as Najerano, Piquillo, H1 and Isabel F1, whereas H2 benefited at full ripeness. These findings support the potential of PGPR and justify further research to promote the adaptation of peppers to sustainable agriculture.

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