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Research article

A study on the effect of biostimulant application on yield and quality of tomato under long-lasting water stress conditions

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ABSTRACT

Recently, the use of plant-derived biostimulants has been suggested as a sustainable way to improve the nutritional quality of tomato and mitigate the effects of environmental stresses. In this regard, a two-year experiment was conducted in open field on four cultivars of tomato (two commercial tomatoes and two local landraces of long shelf-life tomato), to assess the crop response, in terms of fruit yield and quality traits, to the foliar application of two plant-derived biostimulants based on protein hydrolysates (PH), under opposite water regimes (no irrigation and full irrigation), in a semi-arid environment of South Italy. Tomato plants in field were sprayed with a solution containing one of the two biostimulants approximately every 15 days. Full irrigation significantly promoted plant productivity, leading to yields the 22 % and 57 % higher than those produced under no irrigation. Biostimulants significantly promoted plant productivity (+57 % and +39 %, respectively under no and full irrigation, on the average of the two biostimulants), although in the first year only. Overall, fruit quality was better in fruits produced in plants exposed to prolonged soil water deficit. Biostimulants, across cultivars and water regimes, had no effect or even declined fruit quality in terms of total solids (TS), soluble solids (SS), titratable acidity (TA), reducing sugars (RS). The antioxidants were higher in fruits produced under prolonged soil water deficit. Except in the two commercial tomatoes, lycopene content was greater under full irrigation. Overall, the effects of biostimulants on the antioxidants were rather inconsistent. Significant interactions among the three experimental factors on fruit quality traits suggest that the application of biostimulant should be modulated according to water regime and cultivar, involving specific open-field experiments. Interesting correlations (positive or negative) among all the examined traits were described in the current study. A PCA analysis was conducted to reduce the dimensionality of dataset considering the large number of variables in combination. PCA analysis allowed to distribute cultivars and treatments in four distinct groups, according to quality traits. Fluctuating results between the two years of experiment indicated that the tomato response to the application of biostimulants is strictly season-dependent. Future multi-sites and multi-year research are needed to fine-tune the use of biostimulants and, ultimately, make the crop more economically and environmentally convenient than the cultivation of untreated plants.

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1. Introduction

In the last decades, the water availability in the Mediterranean region has been affecting by the unceasing climate changes, resulting in flooding and drought in certain areas [1].

The favourable conditions of light intensity and temperatures favour the cultivation of vegetables in hot and arid areas of the Mediterranean basin [2]. However, climatic changes have radically influenced agriculture, limiting crop productivity and leading to an extreme crop loss [3]. In this region, a sustainable use of the water in agriculture has become extremely important [4].

Recently, new strategies have been proposed in agriculture for a sustainable improvement of production systems under adverse environmental conditions [5]. One promising is the use of plant-derived biostimulants, which are driving great attention as sustainable and eco-friendly tools for reducing the negative environmental impact on crops and improving yield and quality of productions [6,7]. As defined by the new Regulation (EU) 2019/1009, the function of biostimulants is to stimulate plant nutrition processes independently of the product's nutrient content, underlying their different use and origin [8]. Among the various ones, protein hydrolysates (PHs) from legumes are a well-known group of natural biostimulants that have been proven to prevent yield losses and improve quality in vegetables under stressing conditions [9]. Plant-derived PH biostimulants contain free amino-acid and soluble peptides, which stimulate the accumulation of protective compounds with antioxidant activities in plants, minimizing the impact of abiotic stresses on crops [10]. Since the effect of these biostimulants is not attributable to their content in macro and micro nutrients, they are commonly used at very low doses [11].

Plant-derived PH biostimulants are available in liquid, soluble powder, or granular form [9]. They are preferred to animal-derived PH biostimulants for their greater agronomic value [10]. Indeed, plant-derived biostimulants exert both direct and indirect beneficial effects on crops: they have been proven to stimulate auxin- and gibberellin-like activities involved in plant metabolism (direct effect) [11,12], and increase root surface area, thus enhancing nutrients and water uptake (indirect effect) [9,11].

Tomato ranks as the most produced vegetable in the world, with 186 million tons in 2022 [13]. Tomato fruits are rich in bioactive compounds such as polyphenols, flavonoids, and carotenoids, with beneficial effects on human health [14–16]. These aspects justify the growing interest of agronomists toward an increase of the content of these compounds in tomato, also through a modified crop agronomic management.

Tomato requires large amounts of irrigation water for its growth and production, especially in dry areas of Southern Italy, where its productivity may be adversely affected by prolonged drought [17]. In these cultivation areas, the agronomic management of tomato should be addressed toward a more sustainable production, reducing the agronomic input in terms of irrigation water, while minimizing the yield losses [18].

Water saving-irrigation strategies have been proposed to maximize the accumulation of polyphenols and carotenoids in tomato fruits, maintaining satisfactory yields [15,19]. More recently, the use of plant-derived biostimulants has been suggested as well, as a sustainable way to improve the nutritional quality of tomato and mitigate the effects of environmental stresses [20–22]. Enhanced growth parameters in transplants sprayed with plant-derived PH has been evidenced in tomato [12], which confirm the practical interest towards these compounds.

The effects of plant-derived PH biostimulants on tomato yield and quality have been mainly investigated in growth chamber [12] or in greenhouse under unrestricted soil water content conditions [20,21,23]. Still limited is the scientific literature on the effects of the application of these compounds and their interaction with either cultivar or water regime, on tomato grown in open-field in semi-arid environment.

In this view, a two-year experiment was conducted in open field on four cultivars of tomato (two commercial tomatoes and two local landraces), to assess the crop response, in terms of fruit yield and quality traits, to the foliar application of two plant-derived PH biostimulants, under opposite water regimes (no irrigation and full irrigation), in a semi-arid environment of South Italy. The final goal of this study was to identify useful associations among all traits examined, and verify the efficacy of biostimulant application in alleviating the stressing effects of prolonged drought on crop productivity and enhancing the nutritional aspects of tomato fruits according to the new challenges generated by climate change.

2. Materials and methods

2.1. Open-field experiment

The experiment was conducted in open field, during the spring-summer season of 2021 and 2022, in a site of Eastern coast of Sicily (South Italy, 10 m a.s.l., 37°24′35.8″ N Lat, 15°03′31.7″ E Long). The soil was a vertic xerochrepts soil, having the following characteristics: clay 28.3 %, sand 49.3 %, loam 22.4 %, organic matter 1.4 %, pH 8.6, total N 1.0 ‰, available P_2O_5 5 ppm, exchangeable K_2O 245 ppm, bulk density 1.1 g cm^{-3}, field capacity (−0.03 MPa) 27 %, wilting point (−1.5 MPa) 11 %.

The following three experimental factors were studied: i) water regime (*I*) (DRY, no irrigation and IRR, long-season full irrigation); ii) tomato genotype (*C*) (two local Sicilian landraces of long-shelf life tomato and two commercial hybrids); iii) biostimulant application (*T*) (two PH biostimulants and one untreated control). The experiment consisted of total 24 treatments, deriving from the combination of two water regimes, four tomato genotypes and three biostimulant treatments. Treatments were arranged in a 2 × 4 × 3 factorial split-split-plot design with three replicates. *Irrigation* was applied to the main plot, *genotype* to sub-plot, *biostimulant treatment* to sub-sub-plot. Water regimes were randomly distributed within each replicate, genotypes were randomly distributed within each water regime, biostimulant treatments were randomly distributed within each genotype.

The two local Sicilian landraces of long-shelf life tomato were 'Vulcano', from the homonym Eolian island, Sicily, and 'Custonaci', from the western coast of Sicily, both belonging to the germplasm collection at CNR-IBE (Catania, Italy). The two commercial tomato hybrids were 'Paskualeto', of Pachino tomato type, and 'Febo', of mini plum tomato type (Syngenta seeds, The Netherland).

Plants were transplanted in field on April 14 and 20, respectively in 2021 and 2022, at the 4-leaf stage. Single plot measured 10.8 m^2 (3.0 × 3.6 m). Plant spacing was 0.75 m between row and 0.40 m within row, leading to a 3.3 plants m^{-2} plant density. Before transplanting, 75, 100 and 100 kg ha^{-1} of N (as ammonium sulphate), P_2O_5 (as mineral perphosphate) and K_2O (as potassium sulphate), respectively, were distributed in field. Approximately 30 days after transplanting, a further 75 kg ha^{-1} of N (as ammonium nitrate) was supplied as top dressing.

Two opposite levels of irrigation (DRY and FULL, this last used as control) were considered in this study. The two extreme levels of irrigation were chosen to assess the effectiveness of biostimulants under extreme conditions, taking into account that local landraces are traditionally cultivated under no irrigation (only few waterings at the very early stages of growing season to allow plant establishment) [2]. Irrigation water was distributed in field by means of a drip-irrigation system. At transplant, irrigation was applied to all plots to restore field capacity (FC). To this end, the amount of water to distribute (~50 mm) was calculated, considering soil water content (measured gravimetrically on five soil samples collected randomly at 0.40 m depth within the experimental field and oven-dried at 105 °C). A further irrigation (~40 mm) was applied in all plots, for allowing full plant establishment. Afterward, irrigation was suspended in DRY and kept in IRR until fruits ripening (early July), restoring 100 % of evapotranspiration (ETc, ET_0 × kc) at each watering, for total 9 irrigations, in 2021, and 10 irrigations, in 2022. Reference ET (ET_0) was measured in a class-A evaporation pan, and crop coefficients (kc) were those reported for tomato by Patanè et al. [17]. The amount of water to apply with irrigation (V) was calculated on the basis of maximum available soil water content (ASWC) in the first 0.4 m of soil depth [24]. Irrigation was applied when cumulative ETc matched V (~42 mm), *i.e.* approximately every week, depending on ET_0. Total seasonal volume of water distributed by irrigation was 867 and 4400 m^3 ha^{-1}, in 2021, 920 and 4888 m^3 ha^{-1}, in 2022, respectively in DRY and IRR. Irrigation water was provided by a public water supplier.

Two biostimulant treatments, as compared to a non-treated control (plants with no biostimulant treatment), were considered. The two biostimulants used separately in the experiment where the commercial TRAINER® and AQUAMIN® (Hello Nature Italy SRL, Rivoli Veronese, Italy) (hereafter indicated respectively as BIO1 and BIO2), both plant-derived products obtained by the enzymatic hydrolysis of proteins from the seeds of legumes. As reported by the manufacturer, they contain, respectively, 310 and 620 g kg^{-1} of free amino acids and soluble peptides. The first biostimulant (TRAINER), powdered, has been reported as effective in tomato under greenhouse conditions [23]. The second one (AQUAMIN) is a newly developed biostimulant from the same company, having similar composition to TRAINER but in a different formulation (liquid). Throughout the experiment, tomato plants were uniformly sprayed with one of the two biostimulants using a 2 L plastic sprayer. The following concentrations were used: 5 mL L^{-1}, for BIO1, and 2 g L^{-1}, for BIO2. The concentrations used were those recommended by the manufacturer. The timing of biostimulant application for the two years is reported in Table 1. At each application, plants were sprayed until dripping.

No chemical herbicides were used for weed control. A hand weeding was performed once only, since the crop covered the soil and weeds could no longer grow.

2.2. Measurements in field

During the field experiment, the following meteorological variables were recorded in both years: air temperature (°C), rainfall (mm), class-A pan evaporation (mm), using a data logger (CR10, Campbell Scientific, Logan, UT, USA) located approximately 50 m from the experimental field. The meteorological course recorded during the two-year experiment in open field was that of a typically Mediterranean environment (Fig. 1).

Minimum temperature ranged between 6.7 and 24.8 °C, in 2021, between 8.7 and 24.2 °C, in 2022. Maximum temperature picked at 40.5 °C, in 2021, and 36.7 °C, in 2022, both values recorded in late June. Summer was very dry in both years. Indeed, total rainfall during the experiment was very scarce (25.8 and 15.4 mm, in 2021 and 2022, respectively, from transplant to harvest), mostly distributed in few rainy events just after transplant. A quite considerable rainy event (36 mm) just few days before transplant in 2022 allowed an irrigation water saving at transplant. However, an average 2 °C hotter maximum temperature and 1 mm higher ET_0 in the same year, from late May to late June, imposed greater water requirements, leading to a total volume of water ~10 % higher than that applied in 2021 in fully watered plots.

The crop was hand harvested at the full ripening stage (July). At harvest, total fruit yield (t ha^{-1} fresh weight-FW) and single fruit weight (g FW) were measured, and ripen fruits were randomly sampled (approx. 2 kg per treatment) for laboratory analyses. These last were conducted in triplicate.

Table 1
Timing of biostimulant application (days after transplant).

	2021					2022				
	I	II	III	IV	V	I	II	III	IV	V
Trainer® (BIO1)	16	28	43	58	69	21	36	50	62	72
AQUAMIN® (BIO2)	16	–	43	58	69	21	–	50	62	72

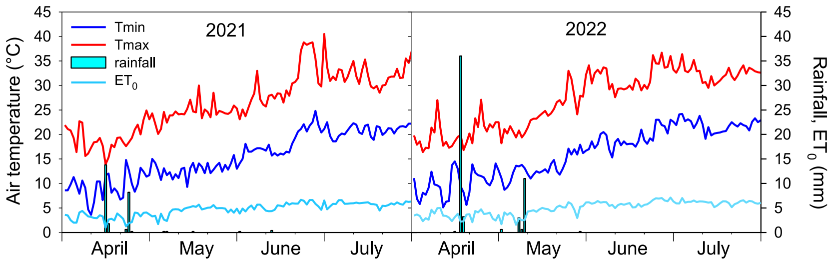

Fig. 1. Meteorological course (minimum-Tmin and maximum-Tmax air temperature, rainfall, reference evapotranspiration-ET_0) recorded in the experimental site from April to July in the two years.

2.3. Laboratory analyses for quality traits

For chemical analyses, fruit samples from each plot were washed with running water to remove dirt and dried thoroughly with absorbent paper, then they were homogenised in an Ultraturrax T25 (Janke & Kunkel, Staufen, Germany).

2.3.1. Total solids (TS) and soluble solids (SS)

Total solids (TS) were determined in homogenate samples dried at 65 °C in a thermo-ventilated oven until constant weight (72 h). TS were expresses as g 100 g^{-1} FW.

Total soluble solids (SS), expressed as °Brix, were read in a portable digital refractometer (Hanna Instruments Digital Brix Refractometer, HI96801) at 20 °C. Before use, the refractometer was standardized by adding few drops of distilled water (0 °Brix SS).

2.3.2. pH and titratable acidity (TA)

pH was measured in a portable read with a glass electrode pH-meter (InLab pH Level 1, WTW, Weilheim, Germany) after standardisation with pH 7 and 4 buffer solutions.

Titratable acidity (TA) was measured by titration [25]. A 50 mL sample of tomato homogenate was diluted with 50 mL distilled water and filtered, then 20 mL filtrate was titrated against 0.1 M NaOH, using phenolphthalein as an indicator. TA was expressed as g citric acid 100 g^{-1} FW.

2.3.3. Ascorbic acid (AscA)

Ascorbic acid (AA) was estimated by 2,6-dichlorophenolindophenol method [26]. Briefly, a 10 g sample of homogenate was diluted in 5 mL of a 3 % (w/v) metaphosphoric acid solution and 5 mL of a 8 % (v/v) acetic acid solution, then filtered. A 2.5 mL filtrate was titrated with a solution of 2,6-dichlorophenolindophenol standardized in a standard stock solution (1 mg mL^{-1}) of ascorbic acid with a known concentration. AA was expressed as mg ascorbic acid 100 g^{-1} FW.

2.3.4. Reducing sugars (RS)

Reducing sugars (RS) were determined spectrophotometrically according to the 3,5-dinitrosalicylic acid (DNS) method [27]. A 5 g homogenate sample was diluted in 5 mL distilled water, then centrifuged at 3500 *g* for 5'. An aliquot of diluted sample was mixed with 1 mL of DNS reagent, then incubated in a water bath at 90 °C for 15'. After a fast cooling in a cold water bath, the extract was mixed with 1 mL of 40 % sodium and potassium tartrate solution. Absorbance was read in a UV spectrophotometer (UV-30 Scan Spectrophotometer, Onda, Carpi, Italy) at 575 nm, using glucose (0–1 mg mL^{-1}) as standard curve ($R^2 = 0.99$). RS were expressed as g 100 g^{-1} FW.

2.3.5. Total phenols (TP)

Total phenols were assayed using the Folin-Ciocalteu reagent [28]. A 2 g homogenate sample was extracted in 10 mL of 80 % MeOH. The extract was vortexed and incubated for 15 h at room temperature, then centrifuged at 5000 *g* for 5'. After that, 125 μL of supernatant was diluted in 500 μL of distilled water, then mixed with 125 μL of the Folin-Ciocalteu reagent, and after few minutes, 1.5 mL of a 7 % (w/v) Na_2CO_3 solution and 1 mL of distilled water were added. All samples were incubated for 90' at room temperature. Absorbance was read spectrophotometrically at 760 nm, using gallic acid (0–250 μg mL^{-1}) as standard curve ($R^2 = 0.99$). Results were expressed as mg gallic acid equivalent (GAE) 100 g^{-1} FW.

2.3.6. Flavonoids

Flavonoids were measured according to the method proposed by Ilahy et al. [29] modified. A 2 g homogenate sample was extracted in 10 mL of 80 % MeOH. The extract was vortexed and incubated for 15 h at room temperature, then centrifuged at 5000 g for 5'. After that, 50 μL of the centrifuged methanolic extract were diluted in 450 μL distilled water, and 30 μL of 5 % (w/v) Na_2CO_3 were added. Samples were incubated for 6′ at room temperature, and 60 μL of 10 % (w/v) $AlCl_3$ were added. After 5' incubation, 200 μL of 1 M NaOH and 210 μL H_2O were added and the mixture was vortexed. Absorbance was read spectrophotometrically at 510 nm, using rutin (0–250 μg mL^{-1}) as standard curve ($R^2 = 0.99$). Flavonoids were expressed as mg rutin equivalent (RE) 100 g^{-1} FW.

2.3.7. Lycopene content

Lycopene content was measured according to the method proposed by Anton and Barrett [30]. A 0.1 g homogenate sample was extracted in 8 mL of a mixture of hexane/ethanol/acetone (2:1:1, v/v/v). The extract was vortexed and incubated at room temperature in the dark for 20′, then 1 mL of distilled water was added. The sample was vortexed again and incubated at room temperature in the dark for 10'. Absorbance was read spectrophotometrically at 503 and 444 nm. The lycopene content of tomato extract was calculated as follows:

$$Lycopene\ content\ \left(mg\ 100\ g^{-1}\ FW\right) = \left[\left(6.95 \times A_{503} - 1.59 \times A_{444}\right) \times 0.55 \times 537 \times V / W\right] / 10 \tag{1}$$

where V (mL) is the volume of mixed solvents added (8 mL) and W (g) is the weight of tomato sample.

2.3.8. Antioxidant activity (AA)

The antioxidant activity (AA) was measured using 2,2-Diphenyl-1-picrylhydrazyl-DPPH free radical-scavenging assay, according to the method proposed by Barbagallo et al. [31] modified. Two g of tomato homogenate were mixed with 2 mL of 80 % MeOH solution, vortexed and incubated at 4 °C for 30'. After centrifugation at 3500g for 5′, 50 μL of supernatant were mixed with 1 mL of freshly prepared 0.1 M DPPH, vortexed and incubated at room temperature in the dark for 30'. Absorbance was read spectrophotometrically at 517 nm. DPPH without tomato extract was used as blank. The scavenging activity (AA) of tomato extract was calculated as follows:

$$Scavenging\ activity\ (AA)\ (\%) = \left[1 - \left(\frac{A_{517sample}}{A_{517blank}}\right)\right] \times 100 \tag{2}$$

2.4. Statistical analyses

All data were subjected to four-way analysis of variance (ANOVA), using Minitab Statistical Software version 19, LLC. A mixed model ANOVA was applied considering 'year' as random factor and 'irrigation', 'genotype', 'biostimulant treatment' as fixed factors, and their interactions as sources of variation. The calculation of 'F' was conducted analysing each mean square on the relevant mean square of the interactions. Before conducting the ANOVA, the normality of residuals was checked by means of Shapiro Wilk test, and the homoscedasticity by means of Bartlett's test. The independence of data was assumed by random samplings. Means were separated by the Tukey's test at 95 % confidence level.

A Pearson's correlation test was conducted among all traits, separately per year (SigmaPlot11, Systat Software Inc., San Jose, CA, USA).

A principal component analysis (PCA) was performed on yield and quality traits, separately per year, using Minitab Statistical Software version 19, LLC. PCA analysis reduces the dimensionality of a dataset when a large number of variables in combination are considered, minimizing the loss of information. It is applicable when variables are intercorrelated and these correlations in some cases is high [32]. All data were normally distributed; therefore, they fulfilled statistical requirements for PCA.

3. Results

The significance of the variance of the mixed model ANOVA for all studied factors and the interactions are reported in Table 2. The analysis of the results is described in the following paragraphs.

Table 2

Table of significance for the studied factors: fresh fruit yield (FY), fruit weight (FW), total solids (TS), soluble solids (SS), pH, titratable acidity (TA), reducing sugars (RS) ascorbic acid (AscA), total phenols (TP), flavonoids (Flav.), lycopene (Lyc.), antioxidant activity (AA), in relation to *year* (Y), *irrigation* (I), *cultivar* (C), *biostimulant treatment* (T), and their interactions. Not significant (ns); significant at $p \leq 0.05$ (*).

	FY	FW	TS	SS	pH	TA	RS	AscA	TP	Flav.	Lyc.	AA
Y	*	ns	ns	ns	ns	ns	*	*	*	*	ns	ns
I	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*
T	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns
$Y \times I$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$Y \times C$	*	ns	*	ns	ns	ns	ns	*	*	ns	ns	*
$Y \times T$	*	*	*	ns	*	*	*	*	*	*	*	ns
$I \times C$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$I \times T$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$C \times T$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$I \times C \times T$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$Y \times I \times C$	ns	*	*	*	ns	*	ns	ns	ns	ns	*	*
$Y \times I \times T$	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
$Y \times C \times T$	ns	ns	*	ns	ns	ns	*	ns	ns	*	*	*
$Y \times I \times C \times T$	*	ns	ns	*	*	ns	*	*	*	*	ns	*

3.1. Fruit yield and single fruit weight

Fruit yield and single fruit weight were measured at harvest. As expected, across years, cultivars and biostimulant treatments, full irrigation significantly promoted plant productivity (Table 3).

Among cultivars, the most productive was 'Febo Hy.', with a final yield that exceeded 20 t ha^{-1}. Low yields (<14 t ha^{-1}) were measured in the two local tomatoes. Both cultivars reached higher yield levels in 2022 (14.5 t ha^{-1} in 'Vulcano' and 19.8 t ha^{-1} in 'Custonaci'). Biostimulant effects lack of significance across all factors. However, the significant interaction '$Y \times T$' showed that both biostimulants significantly promoted plant productivity (+50 % over the control), only in the first season.

Significant '$Y \times I \times$ C $\times T$' interaction ($p < 0.05$) on fruit yield indicated a different response of cultivars to irrigation, depending on bio-treatment (Fig. 2). In particular, the extent of the response of each cultivar to irrigation changed with bio-treatment, being significant BIO1 and BIO2 in 'Febo', in 2022.

According to fruit yield, a significantly greater weight was measured in fruits produced under full irrigation (up to +42 %). Fruit size changed with cultivar but not with bio-treatment, this last except in 2022 where a significant promoting effect ($p \leq 0.001$) of BIO1 on fruit size was recorded. Significant '$Y \times I \times C$' interaction revealed a different response of cultivars to irrigation. As an example, in 2021 fruit weight in 'Febo' and 'Custonaci' was similar under no irrigation, but significantly higher in 'Febo' than in 'Custonaci', under full irrigation (Fig. 3).

3.2. Fruit quality traits

Fruit quality of tomato was significantly affected by the experimental factors and their interactions. In the average of all factors TS, SS, TA and RS were higher in water-stressed fruits (those of DRY) (Table 4). Values of pH slightly but significantly lower were measured in fruits from well watered plants (≤4.01) (Table 5).

TS, SS, TA and RS were maximized in 'Paskualeto' ('Pachino' tomato type), which therefore revealed a greater fruit quality, at least for these aspects. Between the two local tomatoes, an overall better quality was ascertained in 'Custonaci', having higher TS and TA. pH was higher in 'Custonaci', although values overall below the maximum (4.30) allowed by the agro-industry to define the product as 'good' for processing tomato pulp, were observed in this experiment. Reducing sugars, which indicate the level of sweetness of a product, were lower in 'Febo' Hy' (≤2.60 g 100 g^{-1}) (Table 6).

The significant interaction '$Y \times C$' for TS is to be ascribed to the same content measured in the two local landraces in 2022. The significance of the interaction '$Y \times T$' for TS, pH, TA and RS, highlighted that between the two biostimulants, in 2021 BIO1 application led to an overall minor fruit quality.

The four experimental factors (year, water regime, cultivar, bio-treatment) differently interacted upon fruit quality, depending on the trait considered.

Total solids (TS) exhibited a different response to the experimental factors and their interaction. The significant interaction '$Y \times C \times T$' ($p < 0.05$), indicated that in 2021, cultivars and bio-treatments significantly interacted on TS across water regimes, being null the difference among bio-treatments in all tomatoes except 'Febo' where lower TS were measured in BIO1 (Fig. 4). In the same year TS was higher under long-lasting soil water deficit conditions (DRY), but significant '$Y \times I \times T$' interaction ($p < 0.05$) across cultivars also indicated that, while no effects were exerted by both biostimulants on TS under full irrigation, BIO1 slightly but significantly decreased TS under dry conditions (Fig. 5). Moreover, TS was affected by '$Y \times I \times C$' interaction ($p < 0.001$) (Fig. 6). In particular, TS did not change with cultivar in unirrigated plots (>10 g 100 g^{-1}); differently, in fruits produced under full irrigation, TS was overall reduced, but to a significantly greater extent in fruits of 'Febo' (7.10 g 100 g^{-1}).

'Year', 'Irrigation' and 'Cultivar' significantly interacted also upon soluble solids (SS). 'Febo' and 'Vulcano' in 2021 had same SS (5.5 °Brix) when wall-watered, but under dry conditions SS was significantly higher in 'Vulcano' (7 °Brix) than in 'Febo' (6.6 °Brix) ('$Y \times I \times$ C') (Fig. 7). In 2022, 'Febo' and 'Vulcano' were significantly but inversely different, being 'Febo' lower in IRR and higher in DRY, than 'Vulcano'. All the experimental factors significantly interacted on SS ('$Y \times I \times$ C $\times T$', $p < 0.05$), because lower SS were measured

Table 3
Fresh fruit yield (FY) and fruit weight (FW) in tomato in relation to *year* (*Y*), *irrigation* (*I*), *cultivar* (*C*) and *biostimulant application* (*T*). For the main effects, values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) (for FY, $LSD_{Y \times C} = 0.5$ and $LSD_{YxT} = 1.4$; for FW, $LSD_{Y \times T} = 1.8$).

Main effect		FY (t ha^{-1})			FW (g)		
		2021	2022	Mean	2021	2022	Mean
Irrigation (*I*)	Dry	10.6 ± 0.65	15.2 ± 0.86	12.9 b	13.5 ± 0.92	14.5 ± 0.96	14.0 b
	Irrigated	13.0 ± 0.78	23.9 ± 1.09	18.4 a	18.5 ± 1.22	21.8 ± 1.54	20.2 a
Cultivar (*C*)	Febo (Hy.)	14.9 ± 0.81	26.4 ± 1.40	20.7 a	23.4 ± 1.23	22.4 ± 1.29	22.9 a
	Paskualeto (Hy.)	15.2 ± 0.84	17.3 ± 1.78	16.3 b	7.1 ± 0.27	7.4 ± 0.29	7.3 c
	Vulcano (Local)	9.7 ± 0.48	14.5 ± 0.96	12.1 c	14.3 ± 0.73	16.0 ± 0.71	15.2 b
	Custonaci (Local)	7.5 ± 0.68	19.8 ± 1.41	13.7 c	19.3 ± 0.57	26.9 ± 0.47	23.1 a
Biostimulant (*T*)	BIO1	13.6 ± 0.83	19.6 ± 1.43	16.6 a	16.3 ± 1.46	19.4 ± 1.83	17.9 a
	BIO2	13.4 ± 0.88	18.4 ± 1.24	15.9 a	15.7 ± 1.45	17.7 ± 1.71	16.7 a
	Control	8.4 ± 0.58	20.6 ± 1.75	14.5 a	16.1 ± 1.40	17.4 ± 1.68	16.8 a
	Mean	11.8 b	19.8 a		16.0 a	18.1 a	

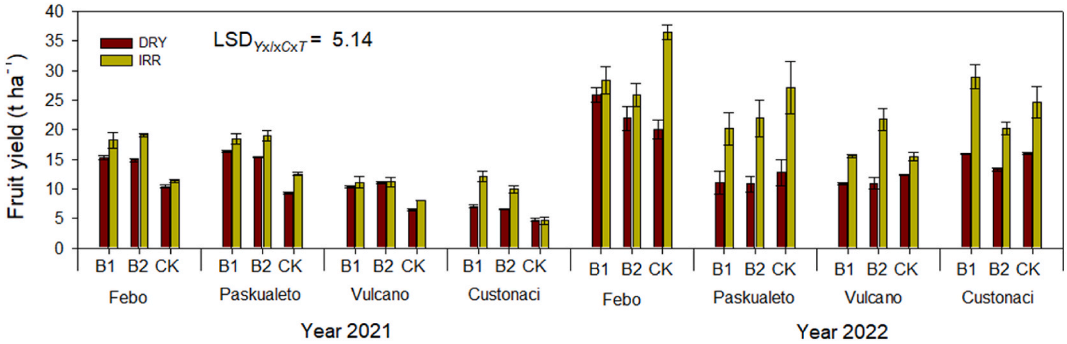

Fig. 2. Effect of interaction '*year* × *irrigation* × *cultivar*'× *biostimulant treatment*' on fruit yield (t ha^{-1}) in tomato (DRY: no irrigation; IRR: full irrigation; B1: BIO1; B2: BIO2, CK: control, no application). Small black vertical bars indicate the standard error ($n = 3$).

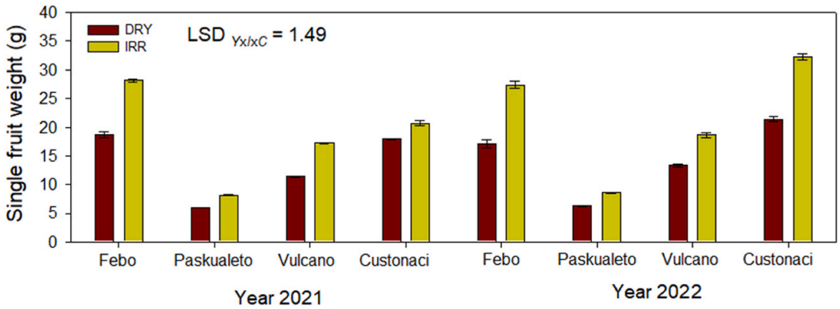

Fig. 3. Effect of interaction '*year* × *irrigation* × *cultivar*' on fruit weight (g) in tomato (DRY: no irrigation; IRR: full irrigation) in the two years of experiment. Small black vertical bars indicate the standard error ($n = 9$).

Table 4
Total solids (TS) and soluble solids (SS) in tomato in relation to *year*, *irrigation* (*I*), *cultivar* (*C*) and *biostimulant application* (*T*) (on a fresh weight basis). For the main effects, values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) (for TS, $LSD_{Y \times C} = 0.58$ and $LSD_{Y \times T} = 0.11$).

		TS (g 100 g^{-1})			SS (°Brix)		
		2021	2022	Mean	2021	2022	Mean
Irrigation (*I*)	Dry	10.2 ± 0.22	10.4 ± 0.12	10.3 a	7.2 ± 0.14	7.1 ± 0.10	7.1 a
	Irrigated	8.5 ± 0.22	8.2 ± 0.18	8.3 b	6.1 ± 0.14	5.8 ± 0.16	5.9 b
Cultivar (*C*)	Febo (Hy.)	8.1 ± 0.30	8.8 ± 0.41	8.4 c	6.1 ± 0.19	6.2 ± 0.25	6.1 b
	Paskualeto (Hy.)	11.2 ± 0.21	10.3 ± 0.15	10.8 a	7.9 ± 0.14	7.5 ± 0.09	7.7 a
	Vulcano (Local)	8.5 ± 0.23	9.0 ± 0.32	8.7 c	6.3 ± 0.18	6.3 ± 0.16	6.3 b
	Custonaci (Local)	9.6 ± 0.21	9.0 ± 0.29	9.3 b	6.3 ± 0.11	5.7 ± 0.21	6.0 b
Biostimulant (*T*)	BIO1	9.2 ± 0.35	9.4 ± 0.29	9.3 a	6.6 ± 0.20	6.4 ± 0.22	6.5 a
	BIO2	9.4 ± 0.30	9.3 ± 0.32	9.4 a	6.7 ± 0.20	6.4 ± 0.22	6.5 a
	Control	9.5 ± 0.32	9.3 ± 0.27	9.3 a	6.6 ± 0.22	6.5 ± 0.21	6.5 a
	Mean	9.4 a	9.3 a		6.6 a	6.4 a	

in 2022 in well-watered fruits, in all bio-treatments, in 'Febo' and 'Custonaci', but not in 'Paskualeto' and 'Vulcano' (Fig. 8).

Significant interaction '$Y \times I \times C \times T$' ($p < 0.05$) on fruit pH highlighted a different effect of biostimulants depending on the type of biostimulant and water regime. In particular, pH differed between the two water regimes, in 'Custonaci' in 2021 in BIO1, and in 'Febo' in 2022, in the control (Fig. 9).

Full irrigation reduced titratable acidity of tomato fruits, across bio-treatments, in all cultivars except 'Custonaci', where TA did not differ between DRY and IRR in 2021. TA in 'Custonaci' irrigated did not differ from TA of 'Febo' irrigated (significant '$Y \times I \times C$') (Fig. 10).

As expected, less reducing sugars (RS) were measured in fruits from well-watered plants. However, the experimental factors differently interacted on the amount of RS. The strongest interactive effect was '$Y \times C \times T$' ($p < 0.05$) (Fig. 11). In particular, RS were reduced after the application of both biostimulants, in 'Febo' and 'Paskualeto' but not in the two local landraces of tomato in 2021. The interaction '$Y \times I \times C \times T$' ($p < 0.01$) revealed that in 2022, RS were significantly lower in fruits from fully irrigated plots, in almost all cultivars and bio-treatments (Fig. 12).

Table 5
pH and Titratable acidity (TA) in tomato in relation to *year*, *irrigation* (*I*), *cultivar* (*C*) and *biostimulant application* (*T*) (on a fresh weigh basis). For the main effects, values (mean ± se) followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) (for pH, $LSD_{Y \times T} = 0.49$; for TA, $LSD_{Y \times T} = 0.015$).

		pH			TA (g 100 g^{-1})		
		2021	2022	Mean	2021	2022	Mean
Irrigation (*I*)	Dry	4.09 ± 0.02	4.02 ± 0.01	4.05 a	0.27 ± 0.01	0.29 ± 0.01	0.28 a
	Irrigated	4.06 ± 0.01	3.97 ± 0.02	4.01 a	0.25 ± 0.01	0.25 ± 0.01	0.25 b
Cultivar (*C*)	Febo (Hy.)	4.11 ± 0.01	3.95 ± 0.03	4.03 b	0.24 ± 0.01	0.28 ± 0.01	0.26 b
	Paskualeto (Hy.)	3.95 ± 0.01	3.90 ± 0.01	3.92 c	0.34 ± 0.01	0.34 ± 0.01	0.34 a
	Vulcano (Local)	4.07 ± 0.01	4.00 ± 0.01	4.03 b	0.22 ± 0.01	0.23 ± 0.01	0.22 c
	Custonaci (Local)	4.17 ± 0.03	4.20 ± 0.01	4.19 a	0.25 ± 0.01	0.24 ± 0.01	0.25 b
Biostimulant (*T*)	BIO1	4.12 ± 0.03	3.99 ± 0.02	4.05 a	0.25 ± 0.01	0.27 ± 0.01	0.26 a
	BIO2	4.05 ± 0.02	4.00 ± 0.02	4.02 a	0.27 ± 0.01	0.27 ± 0.01	0.27 a
	Control	4.05 ± 0.02	3.98 ± 0.02	4.01 a	0.26 ± 0.01	0.28 ± 0.01	0.27 a
	Mean	4.07 a	4.00 a		0.26 a	0.27 a	

Table 6
Reduced sugars (RS) in tomato in relation to year, irrigation (*I*), *cultivar* (*C*) and *biostimulant application* (*T*) (on a fresh weigh basis). For the main effects, values (mean ± se) followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) ($LSD_{Y \times T} = 0.19$).

		RS (g 100 g^{-1})		
		2021	2022	Mean
Water regime (*I*)	Dry	4.4 ± 0.17	3.0 ± 0.05	3.7 a
	Irrigated	3.7 ± 0.14	2.5 ± 0.06	3.1 b
	Febo (Hy.)	2.6 ± 0.09	2.5 ± 0.09	2.6 b
	Paskualeto (Hy.)	4.7 ± 0.15	3.3 ± 0.05	4.0 a
	Vulcano (Local)	4.4 ± 0.19	2.7 ± 0.06	3.6 b
	Custonaci (Local)	4.4 ± 0.11	2.6 ± 0.08	3.5 b
Biostimulant (*T*)	BIO1	3.9 ± 0.18	2.7 ± 0.09	3.3 a
	BIO2	4.0 ± 0.23	2.8 ± 0.09	3.4 a
	Control	4.2 ± 0.20	2.8 ± 0.08	3.5 a
	Mean	4.0 a	2.8 b	

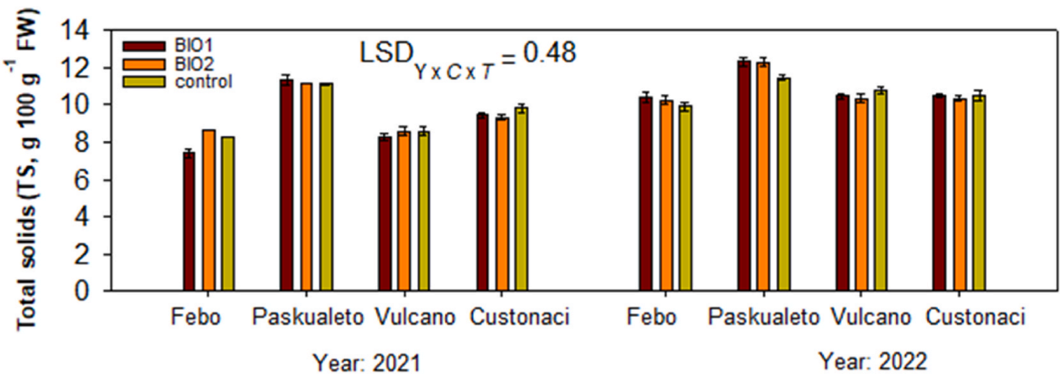

Fig. 4. Effect of interaction '*year × cultivar × biostimulant treatment*' on soluble solids (TS, g 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

3.3. Antioxidants and antioxidant activity

The antioxidants content was measured in the four tomatoes in the two years of experiment.

The content in vitamin C, expressed as ascorbic acid, significantly varied with year, irrigation, cultivar, bio-treatment and their interactions. It was maximized in fruits of DRY plots (+10–12 % higher than in IRR) (Table 7). Among cultivars, the greatest contents were measured in 'Paskualeto' (28.1 mg 100 g^{-1}, ($p < 0.05$). The significant interaction '$Y \times C$' depended on the high content (29.1 mg 100 g^{-1}) measured in 'Vulcano' in 2021, that, however, was not maintained in 2022. Significant interaction was observed also for the application of biostimulants in the two years ('$Y \times T$'), with a positive effect in 2021 but not in 2022 ($p < 0.05$).

Total phenols (TP) were greatly influenced by all the experimental factors. They were 16–17 % higher under no irrigation (80.8 and 69.5 mg GAE 100 g^{-1} FW, respectively for DRY and IRR) (Table 8). As for vitamin C, also TP were maximized in 'Paskualeto' (85.9 mg

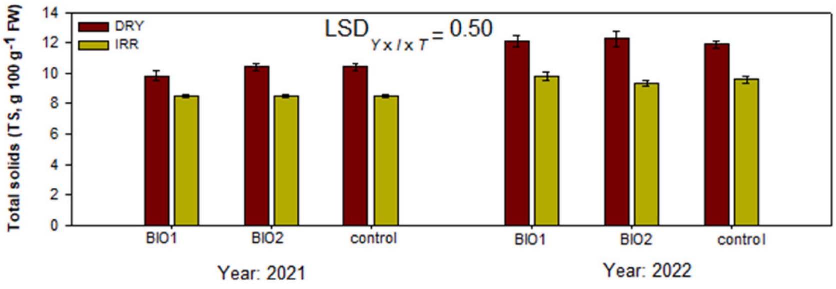

Fig. 5. Effect of interaction '*year* × *irrigation* × *biostimulant treatment*' on total solids (TS, g 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

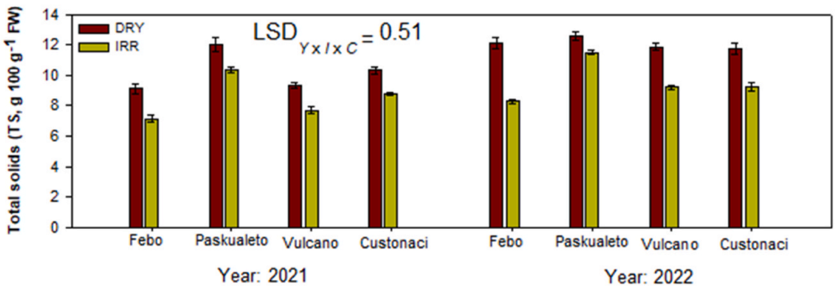

Fig. 6. Effect of interaction '*year* × *irrigation* × *cultivar*' on soluble solids (TS, g 100 g^{-1} FW)) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

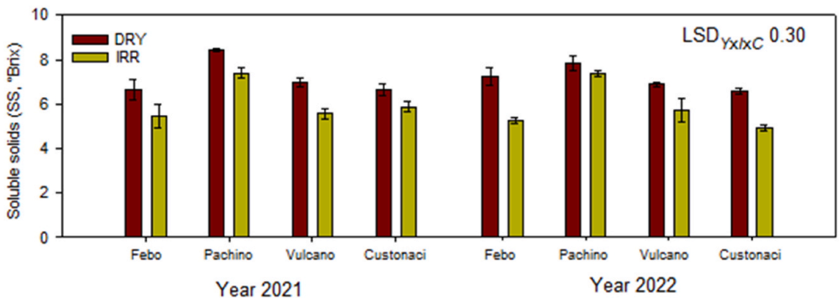

Fig. 7. Effect of interaction '*year* × *irrigation* × *cultivar*' on soluble solids (SS, °Brix) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

100 g^{-1} in the average). Great contents (>65 mg 100 g^{-1}) were also measured in the two local landraces. Moreover, the significant interaction '$Y \times C$' depended on the TP content of 'Custonaci' in 2021 (90.2 mg 100 g^{-1}), as high as that of 'Paskualeto'. Opposite effect (positive in 2021, negative in 2022) was exerted by the two biostimulants on TP '$Y \times T$', indicating that their effect was not consistent.

Flavonoids followed the same trend as TP. They were significantly higher in DRY plots (up to +15 %) and were maximized in 'Paskualeto' (45.5 mg RE 100 g^{-1} FW) ($p < 0.05$). High contents, significantly greater than that of the commercial miniplum 'Febo', were also measured in the two local tomatoes. The significant interaction '$Y \times T$' accounted for a significant different effects of the two biostimulants in the two years.

Lycopene was slightly but significantly higher ($p < 0.05$), under good soil water conditions. Among cultivars, the greatest contents were measured in the two local tomatoes (6.2 mg 100 g^{-1}, in Vulcano, and 6.8 mg 100 g^{-1}, in Custonaci) (Table 9). Differently than what observed for other nutraceuticals, low lycopene contents were found in 'Paskualeto' (3.5 mg 100 g^{-1}). Plants sprayed with BIO2 had fruits richer in lycopene than those sprayed with BIO1 or not sprayed. The significant interaction '$Y \times T$' showed that the results obtained in 2021 were not confirmed in 2022.

The antioxidant activity was affected by water regime and cultivar ('I', 'C', $p < 0.05$) but not by biostimulants ('T', $p > 0.05$). As expected, across cultivars and bio-treatments, a higher scavenging activity corresponded to fruits produced by plants exposed to severe water deficit (85.3 %, against 75.7 % in IRR). Among cultivars, 'Paskualeto' and the local "Vulcano' had respectively the highest (87.9 %) and the lowest (75.2 %) AA. The significant '$Y \times C$' depended on the different behaviour of 'Custonaci' in the two years.

As reported for fruit quality traits, the three experimental factors (water regime, cultivar, bio-treatment) differently interacted upon

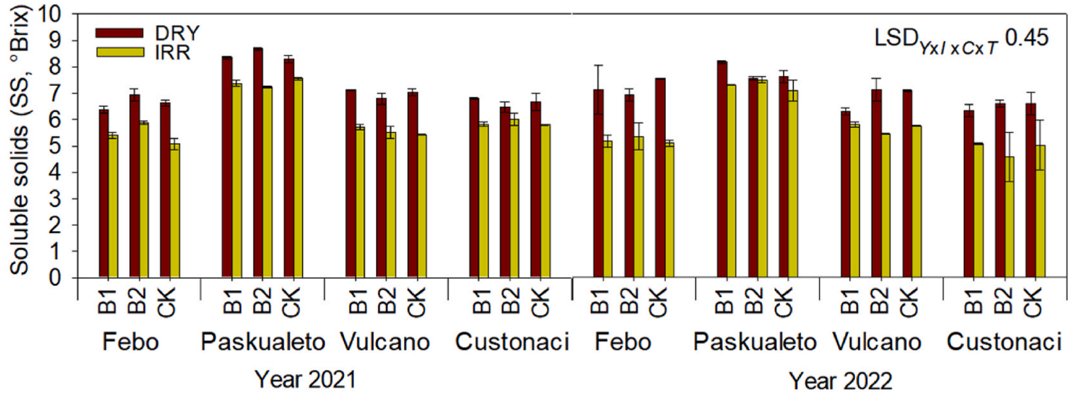

Fig. 8. Effect of interaction '*year* × *irrigation* × *cultivar* × *biostimulant treatment*' on soluble solids (SS, °Brix) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

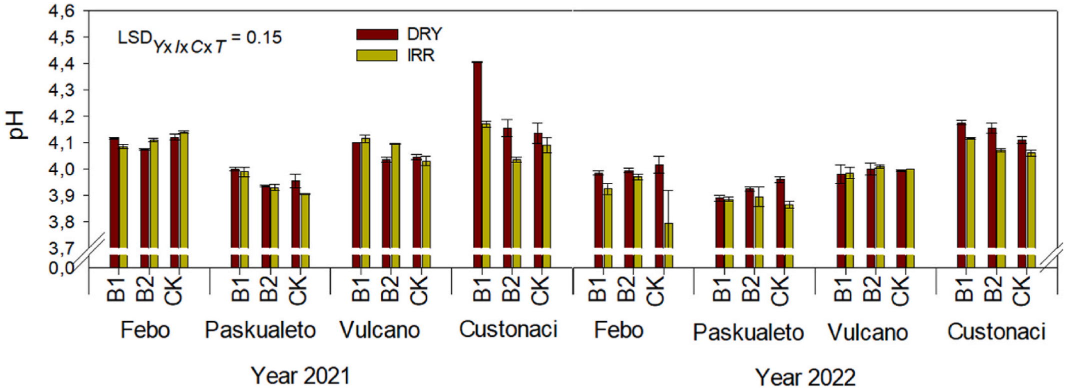

Fig. 9. Effect of interaction '*year* × *irrigation* × *cultivar* × *biostimulant treatment*' on pH in tomato (DRY: no irrigation; IRR: full irrigation; B1: BIO1; B2: BIO2, CK: control, no application). Small black vertical bars indicate the standard error.

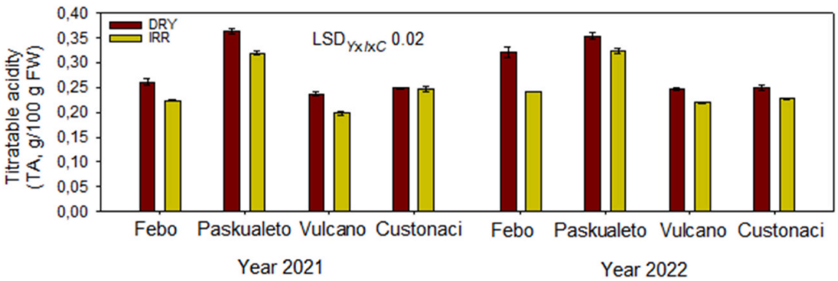

Fig. 10. Effect of interaction '*year* × *irrigation* × *cultivar*' on titratable acidity (TA, g 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

antioxidants and antioxidant activity.

Vitamin C, expressed as ascorbic acid, was affected by '$Y \times I \times C \times T$' interaction, basically because irrigation changed in its effect depending on cultivar and bio-treatment. Indeed, 'Custonaci' in both seasons, and 'Vulcano ', in 2022, were not affected by water regime, in all bio-treatments, whilst 'Febo' and 'Paskualeto' differently responded to irrigation, depending on bio-treatment (Fig. 13).

Significant '$Y \times I \times C \times T$' interaction was also observed on TP content (Fig. 14). In 2021, both biostimulants exerted a positive effect in 'Paskualeto', when not irrigated, and in both local landraces, when well-watered. However, this trend was not confirmed in 2022.

The content in flavonoids significantly varied with '$Y \times C \times T$' ($p < 0.05$), across water regimes (Fig. 15). In 2021 in 'Febo', the application of biostimulants had no effect, whilst in the other cultivars the effect was inconsistent (positive in some cases, negative in some others). The interaction '$Y \times I \times C \times T$' showed that all the experimental factors interacted on flavonoids content ($p < 0.01$),

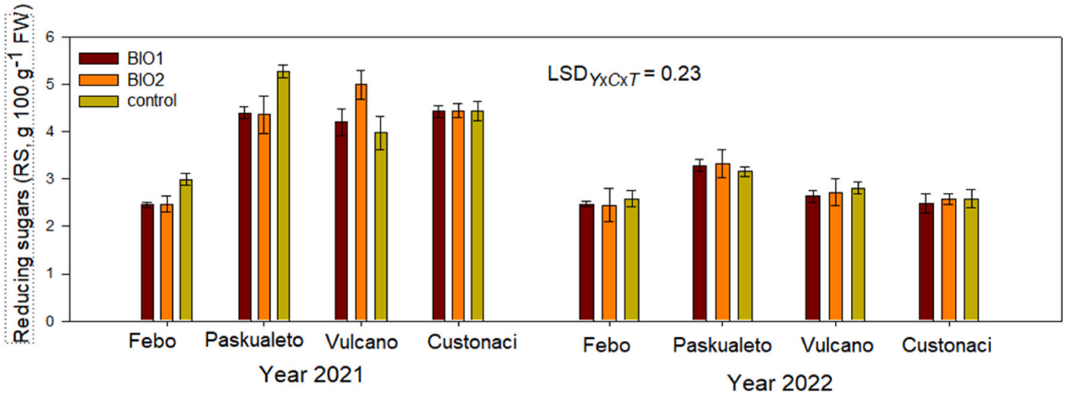

Fig. 11. Effect of interactions '*year* × *cultivar*' × *biostimulant treatment*' on reducing sugars (RS, g 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

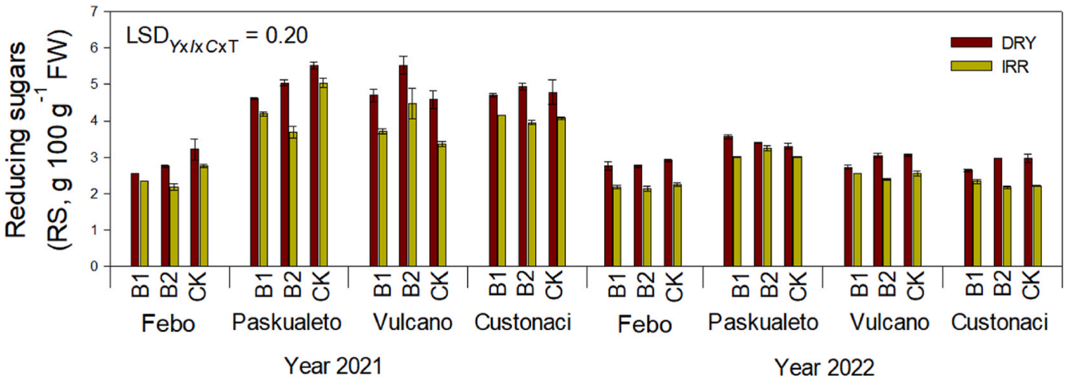

Fig. 12. Effect of interactions '*year* × *irrigation* × *cultivar*' × *biostimulant treatment*' on reducing sugars (RS, g 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation; F: Febo; P: Paskualeto; V: Vulcano; C: Custonaci; B1: BIO1; B2: BIO2, CK: control, no application). Small black vertical bars indicate the standard error.

Table 7
Vitamin C (AscA) in tomato in relation to year, irrigation (*I*), *cultivar* (*C*) and *biostimulant application* (*T*) (on a fresh weigh basis). For the main effects, values (mean ± se) followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) ($LSD_{Y \times C}$ = 2,09; $LSD_{Y \times T}$ = 1.71).

		AscA (mg 100 g^{-1})		
		2021	2022	Mean
Water regime (*I*)	Dry	25.9 ± 1.52	17.1 ± 0.83	21.5 a
	Irrigated	23.1 ± 1.25	15.7 ± 0.70	19.4 b
	Febo (Hy.)	17.4 ± 0.67	18.2 ± 0.58	17.8 c
	Paskualeto (Hy.)	34.1 ± 1.54	22.0 ± 0.64	28.1 a
	Vulcano (Local)	29.1 ± 1.00	13.1 ± 0.45	21.1 b
	Custonaci (Local)	17.5 ± 0.24	12.2 ± 0.56	14.8 c
Biostimulant (*T*)	BIO1	26.2 ± 2.07	16.0 ± 0.91	21.1 a
	BIO2	24.7 ± 1.81	16.1 ± 1.09	20.4 a
	Control	22.7 ± 1.12	17.0 ± 0.85	19.8 a
	Mean	24.5 a	16.4 b	

because in 2022 the depressive effect of irrigation on flavonoids was not evident in all cultivars and bio-treatments (Fig. 16).

Lycopene content was affected by the interactions among the experimental factors in the two years of experiment. In 2021, across bio-treatments, fruits of 'Custonaci' had a greater content under no irrigation, differently than the other cultivars where fruit lycopene content did not change with water regime, but in 2022 'Paskualeto' showed a higher content in irrigated fruits ('$Y \times I \times C$', $p < 0.05$) (Fig. 17). A significant '$Y \times C \times T$' interaction ($p < 0.05$) indicated that, in 2021 across water regimes, the application of BIO2 led to greater lycopene content than control in 'Paskualeto' and 'Vulcano' but not in 'Febo' and 'Custonaci' (Fig. 18).

The antioxidant activity (AA) was significantly affected by the three-way interactions '$Y \times I \times C$' ($p < 0.01$) and '$Y \times C \times T$' ($p <$

Table 8
Total phenols (TP) and flavonoids in tomato in relation *to year (Y), irrigation (I), cultivar (C) and biostimulant application (T)* (on a fresh weigh basis). For the main effects, values (mean ± se) followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) (for TP, $LSD_{Y\times C} = 5.1$ and $LSD_{Y\times T} = 2.9$; for Flavonoids, $LSD_{Y\times T} = 1.9$).

		TP (mg GAE 100 g^{-1})			Flavonoids (mg RE 100 g^{-1})		
		2021	2022	Mean	2021	2022	Mean
Irrigation (*I*)	Dry	86.2 ± 1.98	75.3 ± 1.69	80.8 a	47.4 ± 1.67	30.1 ± 1.33	38.8 a
	Irrigated	73.7 ± 2.34	65.3 ± 2.23	69.5 b	43.0 ± 1.48	24.4 ± 0.95	33.7 b
Cultivar (*C*)	Febo (Hy.)	59.7 ± 2.05	57.5 ± 2.45	58.6 d	35.8 ± 0.97	20.4 ± 0.82	28.1 c
	Paskualeto (Hy.)	87.1 ± 2.05	84.6 ± 1.73	85.9 a	58.5 ± 1.33	32.4 ± 1.35	45.5 a
	Vulcano (Local)	62.8 ± 1.80	71.5 ± 1.66	67.2 c	42.0 ± 1.13	32.6 ± 1.17	37.3 b
	Custonaci (Local)	90.2 ± 1.65	67.6 ± 2.06	78.9 b	44.4 ± 1.18	23.6 ± 1.50	34.0 b
Biostimulant (*T*)	BIO1	82.0 ± 3.08	68.8 ± 2.69	75.4 a	43.9 ± 2.20 b	25.4 ± 1.53	34.7 b
	BIO2	80.5 ± 2.86	69.0 ± 2.49	74.8 a	45.4 ± 1.65 a	27.1 ± 1.69 b	36.3 a
	Control	77.3 ± 2.90	73.0 ± 2.70	75.2 a	46.2 ± 2.07 a	29.3 ± 1.27 a	37.8 a
	Mean	77.7 a	70.3 b		45.2 a	27.3 b	

Table 9
Lycopene and Antioxidant activity (AA) in tomato in relation to *year* , irrigation (*I*), cultivar (*C*) and *biostimulant application* (*T*) (on a fresh weigh basis). For the main effects, values (mean ± se) followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test) (for lycopene, $LSD_{Y\times T} = 0.35$; for AA, $LSD_{Y\times C} = 5.11$).

		Lycopene (mg 100 g^{-1})			AA (DPPH, % scavenging activity)		
		2021	2022	Mean	2021	2022	Mean
Irrigation (*I*)	Dry	4.9 ± 0.36	4.9 ± 0.32	4.9 b	87.9 ± 1.20	82.7 ± 0.32	85.3 a
	Irrigated	5.3 ± 0.24	5.6 ± 0.27	5.5 a	79.8 ± 2.11	71.5 ± 0.28	75.7 b
Cultivar (*C*)	Febo (Hy.)	4.4 ± 0.26	3.9 ± 0.16	4.2 b	80.6 ± 2.95	77.0 ± 1.71	78.8 c
	Paskualeto (Hy.)	3.2 ± 0.14	3.7 ± 0.23	3.5 c	90.0 ± 0.96	85.7 ± 1.74	87.9 a
	Vulcano (Local)	5.9 ± 0.36	6.4 ± 0.36	6.2 a	72.4 ± 0.78	78.0 ± 1.77	75.2 c
	Custonaci (Local)	6.8 ± 0.35	6.8 ± 0.27	6.8 a	92.3 ± 0.61	67.8 ± 1.66	80.1 b
Biostimulant (*T*)	BIO1	4.6 ± 0.35	5.3 ± 0.40	4.9 b	83.9 ± 2.39	76.5 ± 2.10	80.2 a
	BIO2	5.8 ± 0.39	5.1 ± 0.37	5.4 a	85.0 ± 2.37	76.1 ± 2.16	80.6 a
	Control	4.9 ± 0.36	5.4 ± 0.33	5.1 b	82.6 ± 2.04	78.7 ± 1.59	80.7 a
	Mean	5.1 a	5.2 a		83.8 a	77.1 a	

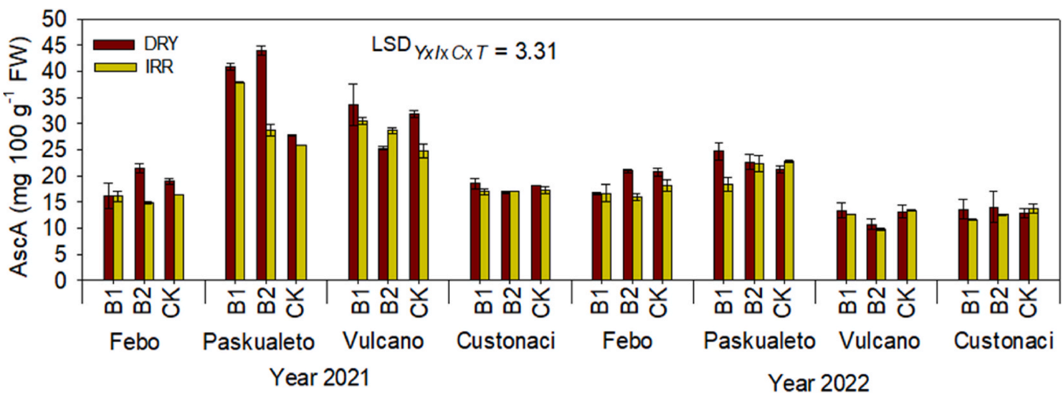

Fig. 13. Effect of interaction '*year* × *irrigation* × *cultivar* × *biostimulant treatment*' on ascorbic acid content (AscA, mg 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation; B1: BIO1; B2: BIO2, CK: control, no application) in the two years of experiment. Small black vertical bars indicate the standard error.

0.05). In particular, in 2021 all tomatoes had same AA when produced under dry or full irrigation conditions, except the commercial 'Febo', whose AA was significantly higher under no irrigation (Fig. 19). Furthermore, 'Febo' and the local 'Vulcano' had same AA when both not irrigated, but when irrigated, the scavenging activity was significantly higher in 'Vulcano' than in 'Febo'. Significant '$Y \times C \times T$' interaction also indicated that, across water regimes, the cultivar response to bio-treatments was not the same, with fruits of 'Paskualeto' (but not those of the other cultivars) having higher AA after biostimulant application (Fig. 20). Significant '$Y \times I \times C \times T$' on AA revealed a different cultivar response to biostimulants in the two years, depending on water regime (Fig. 21). In particular, no

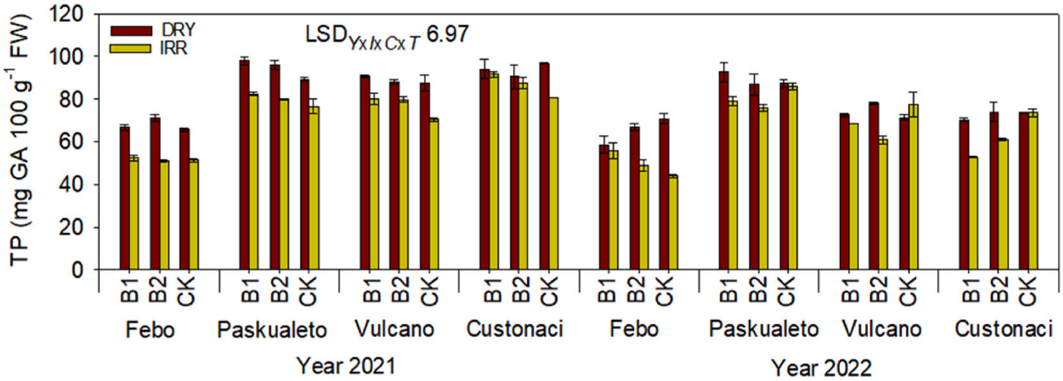

Fig. 14. Effect of interaction '*year* × *irrigation* × *cultivar* × *biostimulant treatment*' on total phenols content (TP, mg GAE 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation; B1: BIO1; B2: BIO2, CK: control, no application) in the two years of experiment. Small black vertical bars indicate the standard error.

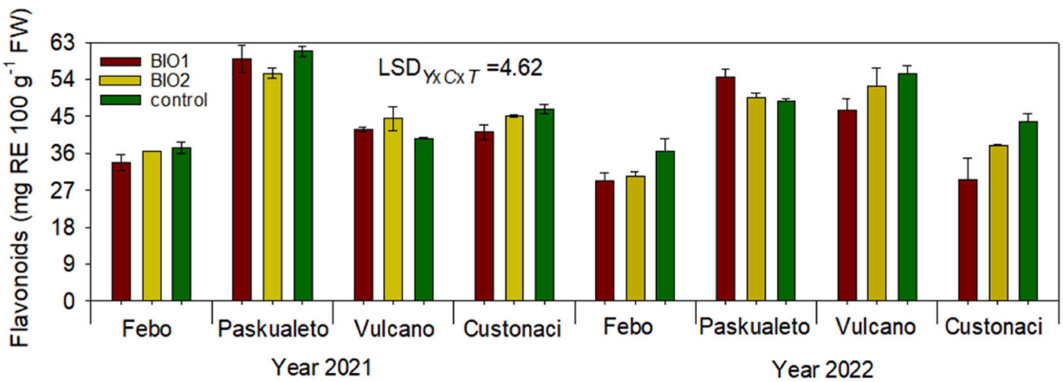

Fig. 15. Effect of interaction '*year* × *cultivar* × *biostimulant treatment*' on flavonoids content (mg RE 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

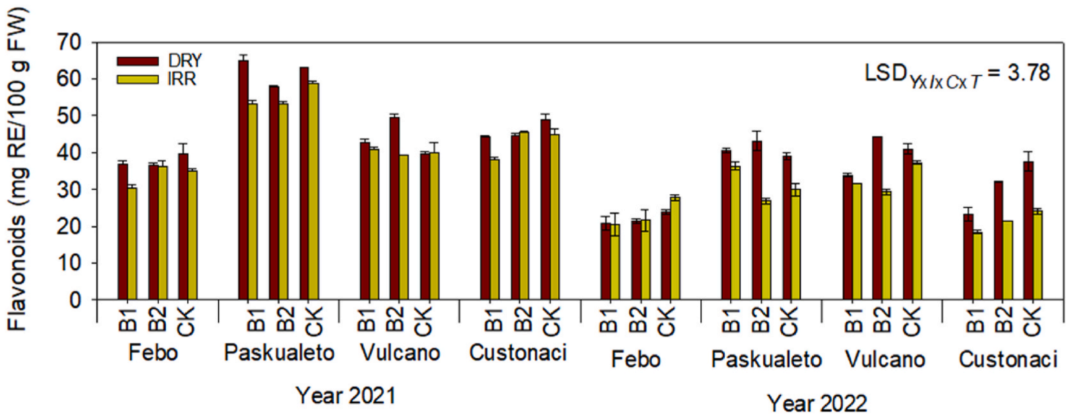

Fig. 16. Effect of interaction '*year* × *irrigation* × *cultivar* × *biostimulant treatment*' on flavonoids content (mg RE 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation; B1: BIO1; B2: BIO2, CK: control, no application). Small black vertical bars indicate the standard error.

response to biostimulants was also found under full irrigation in 2022, in 'Febo' and 'Custonaci' but not in 'Paskualeto' and 'Vulcano', where the application on biostimulants even depressed the antioxidant activity. In 2021 the AA in 'Paskualeto' was not significantly different in the two treatment BIO1 and BIO2.

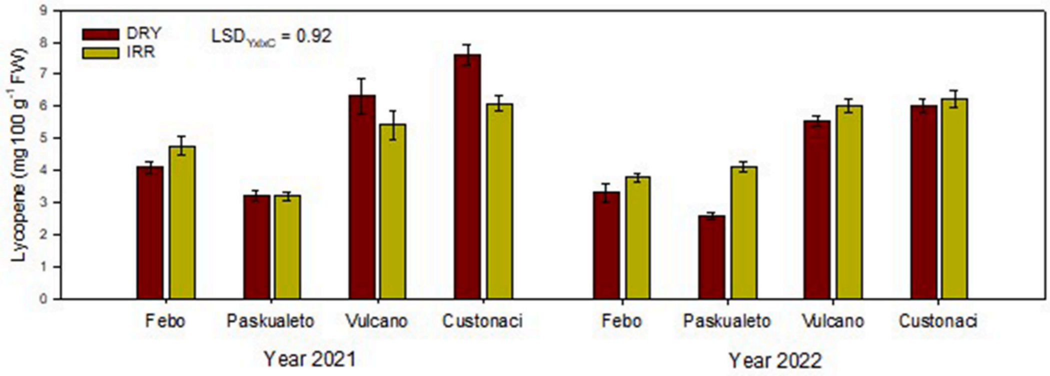

Fig. 17. Effect of interactions '*year* × *irrigation* × *cultivar*' on lycopene content (mg 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

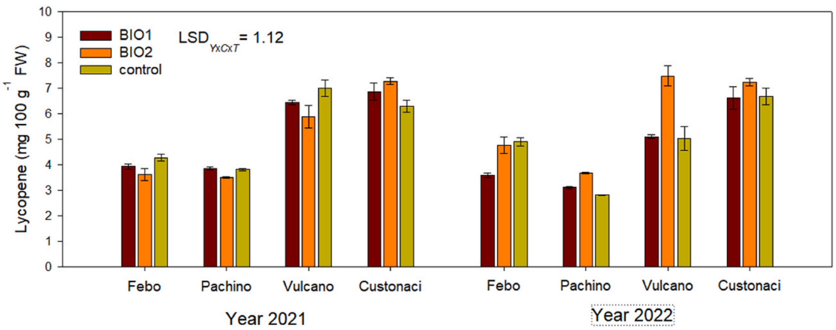

Fig. 18. Effect of interactions '*year* × *cultivar* × *biostimulant treatment*' on lycopene content (mg 100 g^{-1} FW) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

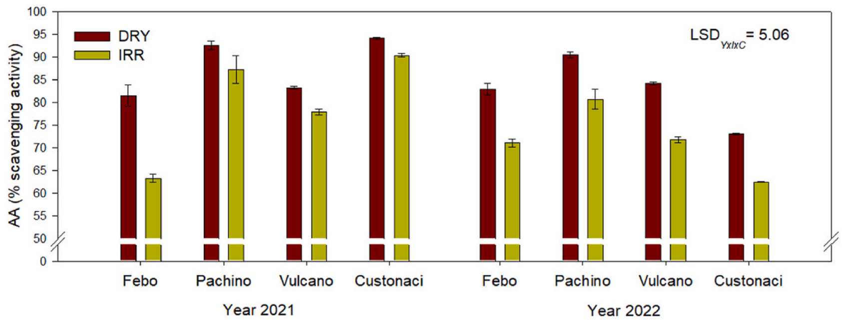

Fig. 19. Effect of interactions '*year* × *irrigation* × *cultivar*' on the antioxidant activity (% scavenging activity) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

3.4. Pearson's correlation among traits

A Pearson's correlation test was carried out to study the correlations among yield, quality and nutraceutical traits in tomato (Fig. 22). Fruit yield appeared to be positively correlated to fruit weight only in 2022, whilst in both seasons, it was negatively correlated to RS. Similar negative correlations were also observed between fruit weight (FW) and most of sensory (TS, SS, TA, RS) and nutraceutical traits (AscA, TP, flavonoids), including AA. Contrastingly, a positive correlation was found between FW and pH in both years, which indicates that fruit with greater size as those produced under full irrigation tend to overripe.

Both TS and SS positively correlated with TA and RS, and with all nutritional traits except the lycopene content. This last was found to correlate positively with FW (only in 2022) and pH (in both years), indicating that lycopene tends to accumulate to a greater extent in fruits produced under good soil water availability (those also having higher pH).

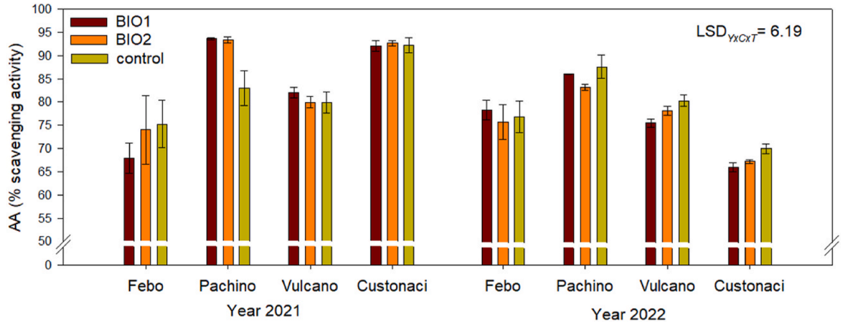

Fig. 20. Effect of interactions '*year* × *cultivar*'× *biostimulant treatment*' on the antioxidant activity (% scavenging activity) in tomato (DRY: no irrigation; IRR: full irrigation). Small black vertical bars indicate the standard error.

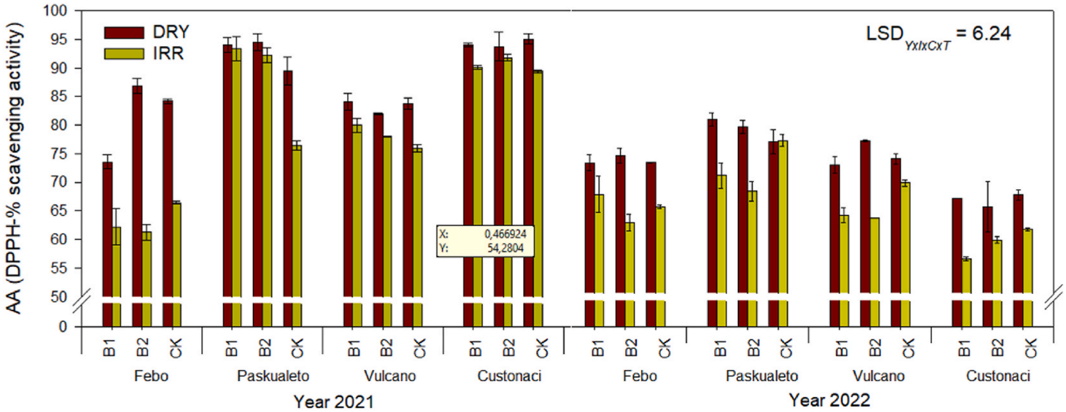

Fig. 21. Effect of interactions '*year* × *irrigation* × *cultivar* × *biostimulant treatment*' on the antioxidant activity (% scavenging activity) in tomato (DRY: no irrigation; IRR: full irrigation; B1: BIO1; B2: BIO2, CK: control, no application). Small black vertical bars indicate the standard error.

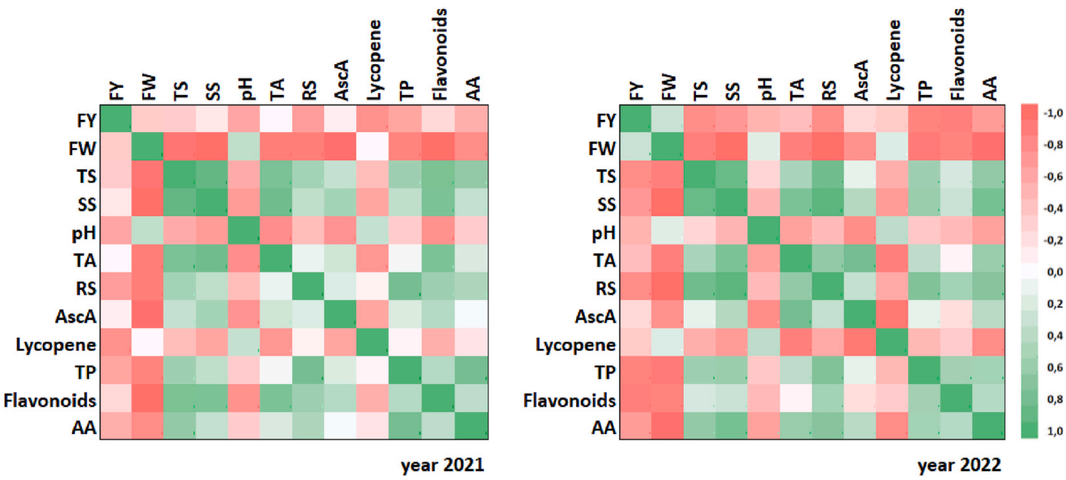

Fig. 22. Heatmap of Pearson's correlation matrix for the studied traits in tomato in the two years of experiment. Different abbreviations used in the figures are as follows: FY, fresh yield; FW, fruit weight; TS, totals solids; SS, soluble solids; TA, titratable acidity; RS, reducing sugars; AscA, ascorbic acid; TP, total phenols; AA, antioxidant activity.

Interestingly, in both years the antioxidant activity (AA) was strongly ($p < 0.01$) correlated positively to all antioxidants (except AscA in 2022, $p > 0.05$), but it negatively correlated, in 2022, or did not correlate at all, in 2021, with lycopene content. These results indicate that in tomato, the antioxidant activity is largely influenced by TP and flavonoids, but it is not associated with the lycopene content.

3.5. Principal component analysis of (PCA)

A Principal Component Analysis (PCA) was carried out to evaluate the effects of the experimental factors on the studied traits and identify any possible cluster within cultivars and treatments. In both seasons, PCA identified two factors with eigenvalue >1.0 (PC1 and PC2). These two factors accounted for 80.3 and 82.5 %, respectively in 2021 and 2022, of total variance, and were used to score plots. Loading plots reported in Fig. 23 illustrate the correlations among traits. Two vectors having an angle < than 90 °C are correlated positively, whilst two vectors having an angle >90 °C are correlated negatively. The amplitude of the angle between vectors also indicates the strength of correlation between traits (the closer the vectors the higher the correlation). In this regard, in both seasons, changes in TP are strongly correlated positively to changes in RS, and changes in TA are closely correlated positively to AscA, whilst changes in AscA content are strongly correlated negatively to lycopene and pH, indicating that fruit over-ripening as indicated by higher pH induced an increase in lycopene content but a decrease in ascorbic acid content.

The score plot analysis may provide information about the changes in quality and nutritional traits in tomato in relation to cultivar, water regime and the application of biostimulant. In 2021, PCA highlighted two distinct clustered groups: 1) the 'Febo' group in the lower quadrant on the left, together with the control of 'Vulcano' IRR, which included none of quality traits considered, thus indicating low quality traits; 2) the 'Paskualeto' group in the lower quadrant on the right, which included FY, SS, TA, AscA, and flavonoids. All 'Vulcano' and 'Custonaci' (except BIO1 for this last genotype) DRY treatments clustered into a third group in the upper quadrant on the right, which included TS, RS, TP, and AA. All IRR treatments of 'Custonaci', together with 'Custonaci' DRY BIO1 and 'Vulcano' IRR BIO1 and BIO2, clustered into a fourth group in the upper quadrant on the left, which included FW, pH and lycopene. In 2022, all 'Febo' IRR clustered in the same group, as for 2021 in the lower quadrant on the left, which included only FY and FW. All treatments of 'Paskualeto' and those DRY of 'Febo', clustered in the lower quadrant on the right, which included TA, AscA, and AA. A third group, of all DRY treatments of 'Vulcano', was identified in the upper quadrant on the right, which included TS, SS, RS, TP, flavonoids. The group of all treatments of 'Custonaci' and those IRR of 'Vulcano' clustered in the upper quadrant on the left, which included pH and lycopene.

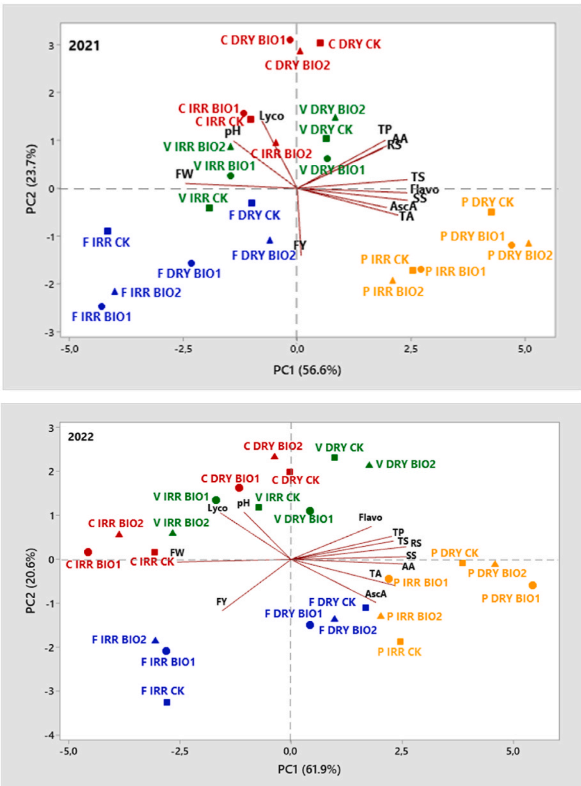

Fig. 23. Principal component biplot and scores of PCA for yield and quality traits in tomato in the two experimental years (FY: fresh yield; FW: fresh fruit weight; TS: totals solids; SS: soluble solids; TA: titratable acidity; RS: reducing sugars; AscA: ascorbic acid; TP: total phenols; Flavo: flavonoids; Lyco: lycopene; AA: antioxidant activity) as modulates by water regime (DRY: no irrigation; IRR: full irrigation), cultivar (F: Febo; P: Paskualeto; V: Vulcano; C: Custonaci), biostimulant treatment (B1: BIO1; B2: BIO2, CK: control, no application).

4. Discussion

The impact of abiotic stresses, such as drought, on crop productivity has been deeply studied, and remains one of the main environmental stresses influencing agriculture. Plants act different mechanisms to maintain their water balance during drought conditions, by increasing the root water uptake from the soil, closing the stomata, and adjusting the osmotic processes [33,34].

In this context, agriculture and research have to focus on different ways to both reduce the use of water and improve the crop sustainability. In this study, the effects of the application of two different biostimulants, on fruit yield and some quality and nutritional traits, were examined in four tomatoes cultivated under long-lasting drought conditions (DRY), in a two-season open-field experiment conducted in a semi-arid environment. The four tomatoes were: two local landraces of long shelf-life tomato, one commercial cultivar of 'Pachino' tomato type and one commercial mini plum tomato. Biostimulants based on plant-derived protein hydrolysate were applied to tomato plants during the growing season, and drought conditions were imposed via no irrigation following plant establishment. A fully irrigated control (IRR) was also considered for all tomatoes.

As expected, full irrigation significantly promoted plant productivity, leading to yields the 22 % and 57 % higher than those produced under no irrigation. As a summer-season crop, in the dry cultivation areas of South Italy tomato requires large amounts of irrigation water during the growth period [17]. However, the results from the present study indicate that tomato plants may survive to prolonged soil water deficit conditions (DRY), as revealed by adequate fruit yields produced in DRY. Greater yield levels were achieved in the second year. Perhaps, rainfall (approx. 35 mm) just before transplant, together with a greater total volume of water supplied by irrigation in 2022, may have positively affected plant productivity in the second year. Moreover, wider yield gap between water regimes in 2022 also indicate that the overall plant productivity and the yield response to irrigation in tomato are strongly cropping season-dependant. Indeed, experiments conducted on tomato cultivated under climatic conditions similar to those of the present study, demonstrated that the atmospheric conditions experienced by tomato plants during the growing season may limit the plant-water flux to the atmosphere, strongly influencing their physiological response to irrigation, and, ultimately, their productivity, even when fully irrigated [17]. Moderate water-sensitivity of tomato also depends on cultivar [35]. According to what observed in previous studies [2], overall, local landraces were less productive than commercial tomatoes, irrespective of irrigation, mainly since these local populations have never been included into breeding programs for yield improvement [36]. Nevertheless, much greater yield levels measured in 'Custonaci' than in 'Vulcano' in the second year of experiment, confirmed a genotypic-specific response to irrigation in long shelf-life tomato reported in previous studies [35].

As mentioned, abiotic stresses, such drought and salt stress, affect negatively morphological, physiological, biochemical and metabolomic mechanisms, influencing the plant growth, development and production [37]. On this basis, biostimulants improve the plant's response to stress, through an increase in antioxidant activity and the accumulation of polyphenols and osmolytes [38]. In particular, abiotic stresses induce the production of ROS (reactive oxygen species), which have the dual function of regulating the crop growth under stress, but in the other hand leads to an overproduction of oxidative stress. In this sense, it has been studied that biostimulants may prevent metals from auto-oxidizing, thus reducing the availability of electrons and the consequential reduction of ROS [37]. Biostimulants significantly promoted plant productivity, either under no and full irrigation. Colla et al. [12] reported a similar promoting effect of plant-derived protein hydrolysate on tomato, demonstrating that the application of this biostimulant stimulated flowering, fruit setting and final yield through an elicited endogenous phytohormonal biosynthesis. However, in the present study, the beneficial effect of biostimulant ascertained in the first season was not maintained in the second year of experiment. Indeed, the indications on the efficacy of biostimulants on plant productivity in horticulture are still controversial. Rouphael et al. [23], working on greenhouse tomato cultivars, reported a promoting effect of the same biostimulant (BIO1) used in the current study, at a 5.0 g L^{-1} dose (the same used in the present experiment) but not at lower rate (2.5 g L^{-1}). According to our results, the extent of the stimulating effect of biostimulant on fruit yield also varied with cultivar, ranging from 18 to 23 %.

As expected, overall fruit quality was better in fruits produced in plants exposed to prolonged soil water deficit. Several studies conducted in open-field cultivated tomato under Mediterranean climate confirmed that, although regular irrigation is preferred to achieve satisfactory yields, water shortage induces an overall quality improvement, with positive implications for consumer acceptance and processing industry [31,39]. Cultivar 'Paskualeto' confirmed the high sensory quality (sweetness in particular) typical of 'Pachino' type of tomato, as reported in literature [40]. Differences ascertained between 'Custonaci' and 'Vulcano', confirm the great variability in fruit quality within wide pools of long shelf-life landraces reported in previous studies [41–43]. Biostimulants, across cultivars and water regimes, had no effect or even declined fruit quality in terms of TS, SS, TA, RS. Rouphael et al. [23], studying the effects of the application of BIO1 at two doses (2.5 and 5.0 g L^{-1}) in tomato under green-house conditions, observed no effects of biostimulant on TS and pH, corroborating the results of Colla et al. [20]. However, the same authors reported an improved content in soluble solids (SS) following the protein-derived biostimulant application, that, conversely, was not found in the current study. We may assume that, differently than what occurring under controlled conditions in greenhouse, the efficacy of biostimulant may be minimized under unfavourable weather conditions in open-field, as those of the current study, and that probably greater doses may be required. However, promoting effects of the same biostimulant (BIO1) used in this study, on most of quality traits, were reported by Caruso et al. [22] in fully irrigated local landraces of tomato, under both organic and conventional management in open-field, indicating that our assumption could be not consistent. Nevertheless, significant interactions among the three experimental factors suggest that the application of biostimulant should be modulate according to water regime and cultivar, involving specific open-field experiments in this regard. Indeed, if we consider single significant interactions, some enhancing effects of biostimulants (BIO1 or BIO2, or both), on SS or RS, were observed under full irrigation, that, however, were not extended to all cultivars.

As well known, tomato is an excellent source of natural antioxidants and secondary metabolites, including ascorbic acid, phenols, carotenoids, thus playing an important role in human nutrition [44]. As observed for fruit quality traits, the antioxidants were higher in fruits produced under prolonged soil water deficit. Only lycopene content exhibited an opposite trend in relation to water regime, being unaffected (in the first season) or even greater (in the season) in fruits grown under favourable soil water conditions. Similar depressing effects of severe water shortage upon fruit lycopene content were reported by Barbagallo et al. [31] in processing tomato. A reasonable explanation of this result is associated with the carotenoid biosynthetic pathway under water stress, which is more oriented towards β-carotene than towards lycopene, being β-carotene the precursor of the abscisic acid formation in plant response to stressing conditions [45]. Similarly, Riggi et al. [46] reported a negative influence of water stress on lycopene accumulation in processing tomato.

According to a high fruit quality, the 'Pachino' tomato type exhibited the greatest levels of AscA, TP, flavonoids, but the lowest content in lycopene. In turn, the highest lycopene content was measured in fruits of 'Vulcano' and 'Custonaci'. Tomato fruit skin has been found to contribute for the 50–60 % of total lycopene content [47]. According to previous findings [41], a thick skin typical of fruits of long shelf-life tomato may account for their high lycopene content. Differently than what observed in the two commercial tomatoes, lycopene content in the two local tomatoes was higher under drought. Together with high lycopene, great TP contents in these tomatoes confirm the great nutraceutical value of these local landraces of long shelf-life tomato [15,41,48]. Although many studies indicate that the use of biostimulants leads to a general increase in fruit quality in tomato [20,49], in the current study, as for quality traits, overall, the effects of biostimulants on the antioxidants were rather inconsistent. Caruso et al. [22] reported promoting effects of a plant-derived biostimulant on TP, AscA, lycopene content, in greenhouse tomato. Conversely, Colla et al. [20] observed no influence of the same biostimulant, on TP and AscA in greenhouse tomato, but positive effects on lycopene content. Rouphael et al. [23], as well, found no effects of BIO1 on TP, but significant positive influence of biostimulant on AscA and lycopene. In our study, in the first season, the application of protein-derived biostimulant resulted in higher AscA, TP and lycopene, but not flavonoids. Moreover, differently than what occurred in the two local landraces, in the two commercial tomatoes biostimulants somehow improved TP content in fruits produced under prolonged drought stress. The lack of response to biostimulant application in terms of TP content in the two local landraces may be ascribable to the fact that long-storage tomatoes, traditionally cultivated under no water supply, have been subjected to an environmental pressure selection towards those tomatoes higher in phenol biosynthesis [50]. Indeed, the role of TP as defence mechanism against water stress damages has been widely demonstrated [50,51]. In turn, the increased TP content in the two commercial tomatoes sprayed with biostimulants under prolonged drought may be considered as a strategy to counteract such stressing conditions. However, in the second year, biostimulants had no or even negative effect on these compounds, indicating that the stimulating effect is not constant but may greatly vary with the season. Our findings are consistent with those of according to Erge and Karadenix [52], who working on field-tomato cultivars, asserted that differences in bioactive compounds found between two years of experiment were probably caused by seasonal conditions.

Interesting correlations (positive or negative) among all the examined traits were described in the current study. Lycopene was found to correlate positively with fruit fresh weight and pH, indicating that the carotenoid tends to accumulate to a greater extent in fruits produced under good soil water availability (heavier and with higher pH). Positive correlations of the antioxidant activity (AA) *vs*. TP and flavonoids, and negative (in 2022), or null (in 2021) correlation vs. lycopene, indicate that in tomato, the antioxidant activity is largely influenced by TP and flavonoids, and is not associated with the lycopene content. Positive correlation found between sugars TSS and total ascorbic acid could be predictable since soluble hexoses are reported as precursors of the synthesis of ascorbate [23,53].

PCA analysis allowed to distribute cultivars and treatments in four distinct groups, according to quality traits. One group, with high sensory (high TS, SS, TA, RS) and nutritional (high AscA, TP, flavonoids) quality, includes the commercial 'Pachino' tomato type. A second group, with high lycopene content, includes the two long shelf-life tomatoes. A third group, having good sensory (high RS) and high nutritional (high TP and flavonoids) quality, includes the long shelf-life 'Vulcano' DRY. A fourth group, of low sensory and nutritional quality, includes the commercial 'Febo'. Score plot does not make a clear separation of biostimulant treatments along PCs, being all (including the untreated control) distributed in the positive or the negative part of both PC1 and PC2, depending on cultivar.

5. Conclusions

In conclusion, the present study revealed that negative changes in fruit yield (down to −36 %) and positive changes in fruit sensory (up to +20 % in reducing sugars) and nutritional quality (up to +23 % in flavonoids), occurred in tomato in response to long-lasting drought conditions. However, the results also indicated that effect of severe soil water deficit is strictly cultivar-dependent.

Plant-derived biostimulant application did not provide the expected clear positive amelioration in fruit yield and quality. Fluctuating results (overall positive in the first season, negative in the second year) between the two years of experiment also indicated that the tomato response to the application of biostimulants is strictly season-dependent.

Increased production costs involved by the application of biostimulants suggest the need of a better knowledge of their management, to make their use more appropriate and sustainable. In this regard, future multi-sites and multi-year research should be carried out in open field tomato under restricted environmental conditions (e.g., high salinity, low levels of soil water availability or soil nutrients) to fine-tune the use of biostimulants and, ultimately, make the crop more economically and environmentally convenient than the cultivation of untreated plants.

CRediT authorship contribution statement

Cristina Patanè: Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alessandra Pellegrino:** Software, Methodology, Data curation. **Alessandro Saita:** Investigation, Data curation.

Silvio Calcagno: Investigation, Data curation. **Salvatore L. Cosentino:** Writing – review & editing, Conceptualization. **Alessio Scandurra:** Visualization, Investigation. **Valeria Cafaro:** Investigation, Data curation.

Data availability statement

All data are contained within the article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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