



## RESEARCH ARTICLE OPEN ACCESS

# A Two-Year Evaluation of Biostimulant Effects on Yield and Quality Parameters of Tomato Landrace 'Pizzutello Delle Valli Ericine' Cultivated Without Irrigation

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## ABSTRACT

The use of biostimulants in agriculture provides a sustainable and efficient technology to improve resource-use efficiency. Biostimulants may boost vegetative growth, enhancing plant tolerance to biotic and abiotic stress. The tomato (*Solanum lycopersicum* L.) is sensitive to drought stress, particularly during fruit setting and fruit development stages. In Italy, long-storage tomato genotypes characterised by drought resistance were selected. In this 2-year study, the foliar application of different biostimulants (betaine, seaweed extracts, vegetal protein hydrolysate and animal protein hydrolysate) was evaluated to determine effects on yield and quality of a local tomato landrace (Pizzutello) cultivated in Sicily without irrigation. The highest dry matter (9.9%) and solid soluble content (6.9° Brix) were observed in plants treated with betaine. Plants treated with *A. nodosum* or animal protein hydrolysate showed the highest potassium concentrations, whereas those supplied with vegetal protein hydrolysate had the highest calcium concentrations. Tomato treated with betaine were found to have the highest nitrate concentrations. The highest marketable yield (13.8 t ha<sup>-1</sup>) was recorded in plants treated with vegetal protein hydrolysate, with an increase of 17.4% compared to the control plants. The highest unmarketable yield was observed in control plants and in those treated with betaine (1.1 t ha<sup>-1</sup>). In conclusion, we can say that each biostimulant had a different effect on the different parameters analysed. Overall, the application of biostimulants has improved tomato growth, productivity and quality in limited water conditions. Our results highlight the potential of biostimulant applications to optimise both the yield and fruit quality of renowned local varieties. This study demonstrated the improvement in the agronomic performance of the Pizzutello tomato, which is particularly significant not only in response to the growing consumer demand for high-quality traditional tomatoes, but also for the enhancement of the technological traits valued by the food industry.

## 1 | Introduction

In arid and semi-arid regions, drought is considered the most significant abiotic stress factor limiting crop productivity (Zhang et al. 2015). Many countries around the world are

affected by this stress condition with subsequent losses in crop yields and negative impacts on the agricultural sector (Boyer et al. 2013; Dwivedi, Goldman, and Ortiz 2019). Originating in South America, the tomato (*Solanum lycopersicum* L.) is the second most cultivated vegetable in the world after the

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potato, with approx. 189 million tons grown on an estimated 5 Mha (Faostat et al. 2019). Over the decades, demand for tomatoes has increased due to the development of numerous processed products, such as soups, juices, purees and sauces. It is estimated that approx. 75% of tomatoes are consumed in a processed form, of which 35% as sauces, 18% as tomato concentrate, 17% as canned tomatoes, 15% as juices and 15% as ketchup (Del Giudice et al. 2017). Over the years, farmers have carefully and scrupulously selected ecotypes that produce adequate yields and which are suited to adverse and extreme environmental conditions. The Mediterranean basin is thus considered a secondary centre of diversification for tomato cultivation (Bai and Lindhout 2007; Panno et al. 2021). Sicily is the Italian region with the greatest number of tomato landraces (Conesa et al. 2020). These varieties are called 'Pizzutello' or 'Locale' followed by the name of the area of origin. A long shelf life is an important characteristic on which farmers base their selection criterion. Adaptability to difficult environmental conditions and tolerance to drought and high temperatures also play an important role in identifying tomato genotypes more suited to local growing conditions (Fullana-Pericàs et al. 2019). In a world scenario of limited water availability, there is an urgent need to ensure crop productivity through improved water use efficiency and through the introduction of new agronomic practices. The use of biostimulants has recently become an innovative solution to address climate change challenges and to obtain more sustainable agricultural systems, reducing the use of chemical fertilisers (Povero et al. 2016; Di Stasio et al. 2018) and minimising the negative effects caused by abiotic stress (Niu et al. 2022). The beneficial effects of biostimulants on crops may be due to a range of processes, such as adaptation of the metabolic processes involved in stress response (Zhang and White 2021), improved nutrient uptake, more efficient water use and increased tolerance to biotic and abiotic stress (Bulgari, Franzoni, and Ferrante 2019). In tomato cultivation, biostimulants based on algae extracts and protein hydrolysates, applied under drought stress and deficit irrigation conditions, have been associated with improved plant growth, yield and fruit quality (Villa e Vila et al. 2023; Jiménez-Arias et al. 2022; Paul et al. 2019). In addition, it has been suggested that animal-derived biostimulants may alleviate the negative effects of water stress in tomato plants through both the regulation of photosynthetic processes and osmoregulation, and the activation of antioxidant mechanisms (Wang et al. 2022). Foliar application of a plant biostimulants (containing flavonoids and organic acids) in tomato plants subjected to water stress conditions triggered protection effects in the photosynthetic apparatus (Sudiro et al. 2022). Francesca et al (Francesca et al. 2022). reported that protein hydrolysates promoted hormonal biosynthesis in tomato plants cultivated under high temperatures and severe water stress. Subramaniyan et al (Subramaniyan et al. 2023). reported that the application of *Ascophyllum nodosum*-based biostimulants can positively influence morphological, physiological, yield, and quality parameters in tomato. Therefore, the application of biostimulants could help to reduce the negative impacts of heat stress and water stress and improve the nutraceutical profile of fruits. This study aims to fill the research gap regarding the effects of applying different classes of biostimulants on long-storage tomato genotypes grown without

irrigation. Based on the above considerations, this study was conducted using five foliar biostimulants (vital and animal protein hydrolysates, seaweed extracts, and beet extract) to evaluate the morphological, physiological, productive, and qualitative performance of the 'Pizzutello delle Valli Ericine' tomato landrace, known for its long storage. The results of this study could enhance the characteristics of this genotype and increase the technical resources available for local crops with high traditional, economic, and qualitative value.

## 2 | Materials and Methods

### 2.1 | Experimental Site

Trials were carried out for 2 years (10 April–10 September 2021 and 10 April–10 September 2022) on a farm located in Marsala (Sicily, Italy, 37°81'36" N, 12°55'20" E Google Earth, 125 m s.l.m.). The experimental site had been a vineyard in the years previous to the test. After grubbing up the vineyard, the plot was ploughed down to 25–30 cm depth in August. In March of both years, soil tillage was carried out to create the best conditions for the plants. Soil characteristics are given in Table S1.

### 2.2 | Weather Data

Data on rainfall and temperature were collected from a meteorological station belonging to the Agro-Meteorological Information Service of the Sicilian Government (Servizio Informativo Agrometeorologico Siciliano 2024) the station was situated close to the experimental field. The station was equipped with an MTX datalogger (model WST1800) and various climate sensors. More specifically, a temperature sensor MTX (model TAM platinum PT100 thermo-resistance with anti-radiation screen) and a rainfall sensor MTX (model PPR with a tipping bucket rain gauge) provided data on average daily air maximum and minimum temperatures (°C), and total daily rainfall frequency (mm).

### 2.3 | Plant Material

During the 2-year experiment, tomato landrace 'Pizzutello delle Valli Ericine' was sown in February at a local nursery in 84-hole alveolar containers. The seedlings were transplanted at third-leaf stage during the last ten days of April in both years. Plant density adopted was 0.33 plants m<sup>-2</sup> (3 m × 1 m). The landrace is a determined growth tomato.

### 2.4 | Agronomic Management

Tamping was carried out 2 weeks after transplantation and tillage every 2 weeks using a harrow to disrupt capillarity and preserve water in the soil. Copper-based foliar treatments to prevent the onset of fungal diseases and *Bacillus thuringiensis* treatments against lepidoptera were carried out in July of both years.

## 2.5 | Biostimulants Treatments

For the tests, five commercial biostimulants formulations were used: B1 provided by the company 'Hydro Fert', and B2-B5 provided by the company 'Mugavero Fertilizzanti'.

During the 2-year study, foliar biostimulants were applied every 15 days after transplantation, with a total of six applications per crop cycle. For each application 4 hL ha<sup>-1</sup> of water was used. A portable sprayer with an operating pressure of 250 kPa was adopted. The following foliar treatments were compared:

- B0: control, only water.
- B1: amino acids obtained from beet extracts (1.0 mL L<sup>-1</sup>).
- B2: liquid extract of *Ecklonia maxima* (2.5 mL L<sup>-1</sup>).
- B3: liquid extract of *Ascophyllum nodosum* (2.5 mL L<sup>-1</sup>).
- B4: animal protein hydrolysate (3.0 mL L<sup>-1</sup>).
- B5: vegetal protein hydrolysate (5.0 mL L<sup>-1</sup>).

The dosage of each biostimulant, reported in Table S2, was standardised according to nitrogen content. A randomised complete block design with three replicates was used for the tests. Each plot was 30 m<sup>2</sup> with 10 plants.

## 2.6 | Morphological and Productive Parameters

70 days after transplantation (70 DAT), the following parameters were measured on 15 tomato plants for each plot: plant height (cm); relative water content (RWC) of leaf (percentage); as described by Alyemeni et al (Alyemeni et al. 2018), and chlorophyll content (SPAD units, using a Minolta SPAD-502 Plus chlorophyll metre). Subsequently, the following parameters were measured at harvest: total yield (t ha<sup>-1</sup>); marketable yield (t ha<sup>-1</sup>) and unmarketable yield (t ha<sup>-1</sup>) (fruit rotten, damaged, affected by diseases and insects).

## 2.7 | Qualitative Parameters

A number of quality parameters were measured on representative samples of fruit for each treatment and each replicate at harvest. Samples were weighed and then dried in an oven at 105°C until a constant weight was reached to determine the percentage of fruit dry matter. Soluble solids content (SSC) was determined using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd. Taiwan) and results were expressed in °Brix. Calcium (Ca) and potassium (K) contents were determined using a wet mineralisation technique followed by atomic absorption spectroscopy (SavantAA, 200 ERRECI, Milan, Italy), as described by Morand and Gullo (Morand and Gullo 1970) and the values were expressed in grams per kilogram of dry weight (g kg<sup>-1</sup> d.w.).

Nitrate content was determined using a Foss FIAstar 5000 continuous-flow analyser (Sah 1994) and values were expressed in grams per kilogram of dry weight (g kg<sup>-1</sup> d.w.). Fruit firmness was measured on 15 tomatoes with uniform ripening for

each plot using a digital penetrometer (Trsnc, Forli, Italy) with a 4 mm tip and values expressed in Newtons (N). Colour parameters were established on a sample of ten marketable fruits for each treatment and replicate. CIElab colour parameters (L\*: brightness, between 0 (black) no reflection and 100 (white); a\*, chromatic parameter between -60 (green) and +60 (red); and b\*, chromatic parameter between -60 (blue) and +60 (yellow)) were determined using a Chromameter CR-400 (Minolta Corporation Ltd. Osaka Japan). Coloration (C\*) and hue angle (H°) were also calculated as follows: C\* = (a\*<sup>2</sup> + b\*<sup>2</sup>)<sup>1/2</sup>; HUE = (arctan (b\*/a\*)).

## 2.8 | Statistical Analysis

Year (Y) and foliar biostimulant (B) were used as fixed effects in the linear model/analysis of variance (ANOVA). All collected data were subjected to the ANOVA using the package MINITAB 19 (State College, PA, USA) for Windows. The difference between means was analysed using Tukey's test ( $p \leq 0.05$ ). The graphs were created using the Excel application of the package Office 365 for Windows.

## 3 | Results

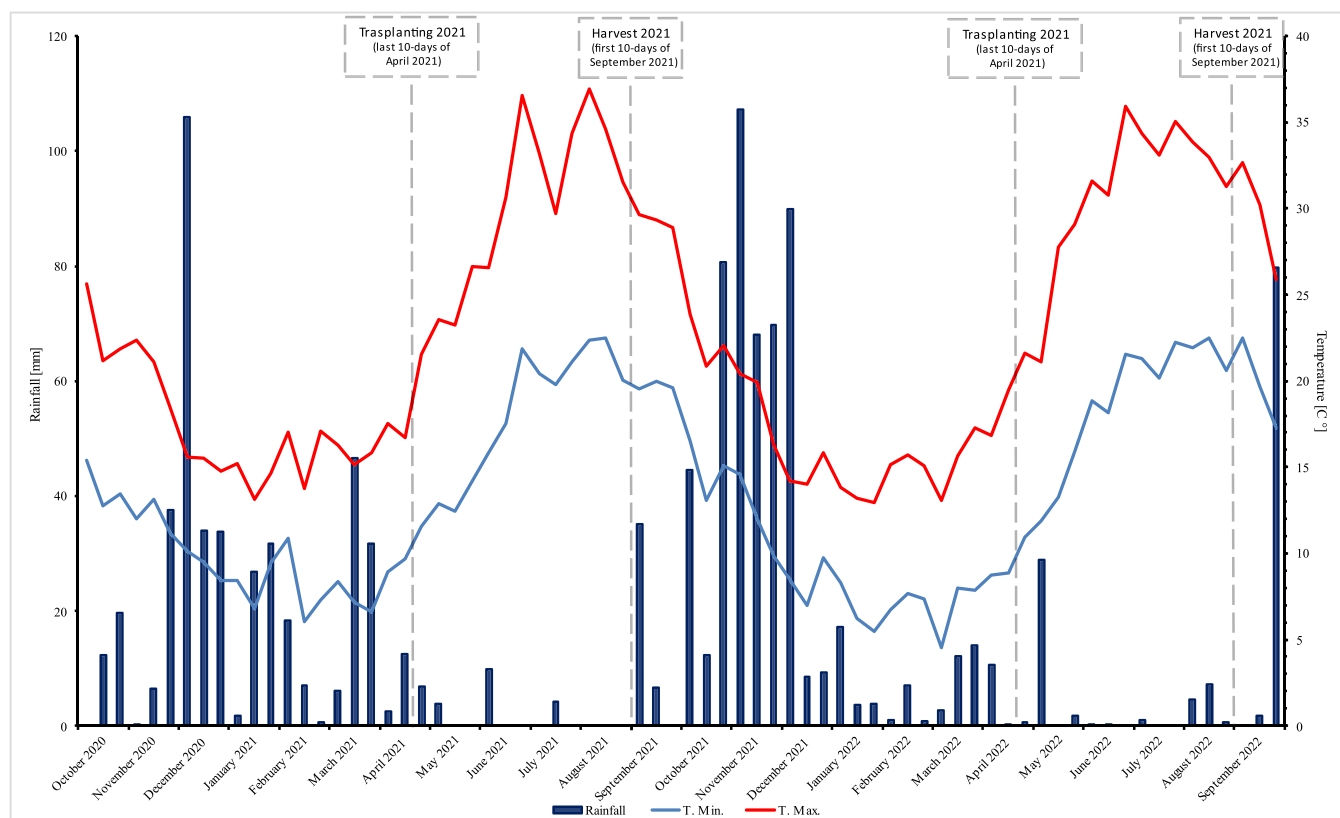
### 3.1 | Rainfall and Air Temperature Trends During the 2-Year Study

In the first growing season (October 2020–September 2021), 501 mm of total rainfall were observed. Rainfall was mainly distributed from October to April, except for the first 10-day period of December, during which a peak of 106 mm was observed. From May to the end of the cycle, 14 mm of rainfall were recorded (Figure 1).

In the second year (October 2021–September 2022), 689 mm of total rainfall were observed. Almost 70% of the rainfall was concentrated in autumn. From January to the end of the cycle, 102 mm of rainfall were observed. Greatest precipitation occurred during the first 10-day period of May (28.8 mm) (Figure 1).

### 3.2 | Plant Height, RWC, and Chlorophyll Content

Analysis of variance revealed that the factors year (Y), biostimulants (B) and their interaction significantly influenced plant height, RWC and chlorophyll content. Highest values were observed in these parameters during the first year of research (Table 1). Considering the biostimulant application, plant height values ranged between 65.5 and 74.9 cm. Greatest plant heights were recorded in B2- and B5-treated plants followed by those treated with B1 and B4. These latter results were statistically similar to those observed in control plants (B0). The lowest plant height was obtained in B3-treated plants (Table 1). Highest RWC values (from 82.7% to 84.7%) were recorded in B1-, B3- and B4-treated plants. Control plants (B0) showed a decrease in RWC of approx. 30 percentage points compared to most high-performance plants. B1-, B2-, B4-, and B5-treated plants produced the highest chlorophyll content (from 44.4 to 44.7 SPAD Unit). The lowest chlorophyll content was recorded in



**FIGURE 1** | Temperature and rainfalls trends at the experimental site during the two growing seasons (2020–2021 and 2021–2022).

**TABLE 1** | Effect of year (Y) factor and biostimulants (B) factor on plant height, relative water content (RWC) and chlorophyll content.

Factor	Plant height [cm]	RWC [%]	Chlorophyll Content [SPAD Unit]
Year (Y)			
2021	72.6 ± 1.05 a	79.3 ± 3.40 a	45.8 ± 0.47 a
2022	70.5 ± 0.85 b	75.6 ± 1.50 b	42.0 ± 0.08 b
Biostimulant (B)			
B0	69.6 ± 1.58 b	56.6 ± 3.13 c	42.3 ± 0.25 b
B1	73.0 ± 0.95 ab	84.7 ± 1.48 a	44.7 ± 1.00 a
B2	74.6 ± 1.11 a	79.2 ± 2.28 b	44.4 ± 1.25 a
B3	65.5 ± 0.96 c	83.2 ± 2.30 a	42.8 ± 0.50 b
B4	71.8 ± 0.83 ab	82.7 ± 3.32 a	44.7 ± 1.09 a
B5	74.9 ± 1.09 a	78.5 ± 1.02 b	44.5 ± 1.22 a
p value			
Y	0.000	0.000	0.000
B	0.003	0.000	0.000
Y × B	0.003	0.000	0.000

Note: Means and standard errors are shown. B0: Control; B1: amino acids obtained from beet extracts; B2: liquid extract of *Ecklonia maxima*; B3: liquid extract of *Ascophyllum nodosum*; B4: animal protein hydrolysate; B5: vegetal protein hydrolysate. Values followed by different letters are significantly different at  $p \leq 0.05$ , in accordance with Tukey's test.

B3- and B0-treated plant, with 42.77 and 42.35 SPAD units, respectively (Table 1).

Considering the interaction  $Y \times B$ , greatest plants heights were measured during 2021 in B2- and B5-treated plants (76.9 and 76.7 cm, respectively), while the lowest by the interaction 2021-B3, 2022-B0 and 2022-B3 (Figure S3).

RWC showed highly variable values, ranging from 49.8% to 90% observed in control plants (B0) and in B4-treated plants, respectively, in 2021. During 2021, RWC values were higher than those observed during 2022, except for B0- and B5-treated plants (Figure S4).

Highest chlorophyll content (between 47.2 and 46.9 SPAD Units) was observed during the first year in B1-, B2-, B4- and B5-treated plants, followed by B3-treated plants, not statistically different from control plants (B0) (Figure S5).

### 3.3 | Yield Parameters

Foliar biostimulant application (B) and the interaction  $Y \times B$  significantly ( $p \leq 0.05$ ) affected total yield. Factors year (Y), biostimulant (B) and their interaction ( $Y \times B$ ) significantly ( $p \leq 0.05$ ) influenced marketable yield and unmarketable yield (Table 2)

During both years, total yield was approx.  $13.5 \text{ t ha}^{-1}$ . During the first year, a higher marketable yield and lower unmarketable yield

was observed compared to the second year (Table 2). Considering the effect of the biostimulant factor, the foliar application of B5 produced the highest total yield ( $14.7 \text{ t ha}^{-1}$ ) and marketable yield ( $13.8 \text{ t ha}^{-1}$ ), with an increase of approx.  $2 \text{ t ha}^{-1}$  compared to untreated plants, in which the lowest values were observed (Table 2).

As reported in Figure 2, total yields ranging from 12.5 to  $14.8 \text{ t ha}^{-1}$  were recorded over the 2 years of research. Highest total yield and marketable yield were observed in B5-treated plants during both years. Unmarketable yield ranged from 0.7 to  $1.2 \text{ t ha}^{-1}$  over the test period. The highest values were obtained by B0- and B5-treated plants during the 2021. The lowest values were obtained by the interaction  $2021 \times B3$  and  $2022 \times B5$  (Figure 2).

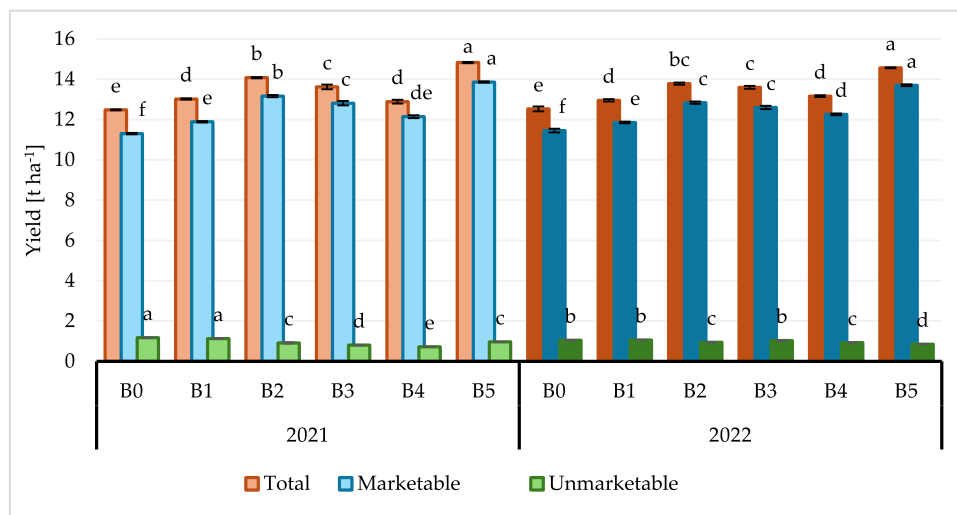
### 3.4 | Qualitative Parameters

Statistical analysis revealed that dry matter, SSC, potassium, calcium, nitrate ( $\text{N-NO}_3$ ) and firmness were significantly influenced ( $p \leq 0.05$ ) by the factors year and biostimulant (Table 3). All parameters showed the highest values during the second year, except nitrate content, which was higher during 2021. Greatest dry matter (9.9%) and SSC ( $6.9^\circ \text{Brix}$ ) were observed in B1-treated plants, while the lowest in B2-treated plants (Table 3). Potassium, calcium and nitrate levels were different in biostimulated plants. The application of B3 and B4 generated higher potassium concentration, the application of B5 produced higher calcium concentration and the application of B1 created higher nitrate concentration.

**TABLE 2** | Effect of year (Y) factor and biostimulants (B) factor on total yield, marketable yield and unmarketable yield.

Factor	Total Yield [ $\text{t ha}^{-1}$ ]	Marketable Yield [ $\text{t ha}^{-1}$ ]	Unmarketable Yield [ $\text{t ha}^{-1}$ ]
Year (Y)			
2021	$13.5 \pm 0.19$ a	$12.5 \pm 0.21$ a	$0.9 \pm 0.04$ b
2022	$13.4 \pm 0.16$ a	$12.4 \pm 0.18$ b	$1.0 \pm 0.02$ a
Biostimulant (B)			
B0	$12.5 \pm 0.06$ e	$11.4 \pm 0.06$ f	$1.1 \pm 0.03$ a
B1	$13.0 \pm 0.03$ d	$11.9 \pm 0.02$ e	$1.1 \pm 0.02$ a
B2	$13.9 \pm 0.07$ b	$13.0 \pm 0.08$ b	$0.9 \pm 0.02$ b
B3	$13.6 \pm 0.06$ c	$12.7 \pm 0.08$ c	$0.9 \pm 0.05$ b
B4	$13.0 \pm 0.08$ d	$12.2 \pm 0.05$ d	$0.8 \pm 0.05$ c
B5	$14.7 \pm 0.06$ a	$13.8 \pm 0.04$ a	$0.9 \pm 0.03$ b
<i>p</i> value			
Y	0.173	0.000	0.002
B	0.000	0.039	0.000
Y × B	0.003	0.005	0.000

Note: Means and standard errors are shown. B0: Control; B1: amino acids obtained from beet extracts; B2: liquid extract of *Ecklonia maxima*; B3: liquid extract of *Ascophyllum nodosum*; B4: animal protein hydrolysate; B5: vegetal protein hydrolysate. Values followed by a different letter are significantly different at  $p \leq 0.05$ , in accordance with Tukey's test.



**FIGURE 2** | Influence of the interaction  $Y \times B$  on total yield, marketable yield and unmarketable yield. Means and standard errors are shown. B0: Control; B1: amino acids obtained from beet extracts; B2: liquid extract of *Ecklonia maxima*; B3: liquid extract of *Ascophyllum nodosum*; B4: animal protein hydrolysate; B5: vegetal protein hydrolysate. Values indicated by different letters are significantly different at  $p \leq 0.05$ , in accordance with Tukey's test.

**TABLE 3** | Effect of year (Y) factor and biostimulants (B) factor on dry matter, soluble solids content (SSC), potassium, calcium, nitrate and firmness of tomato fruits.

Factor	Dry Matter [%]	SSC [°Brix]	Potassium [g kg <sup>-1</sup> ]	Calcium [g kg <sup>-1</sup> ]	N-NO <sub>3</sub> [g kg <sup>-1</sup> ]	Firmness [N]
Year (Y)						
2021	8.7 ± 0.08 b	6.2 ± 0.06 b	8.2 ± 0.20 b	2.7 ± 0.20 b	0.76 ± 0.12 a	20.5 ± 0.47 b
2022	10.1 ± 0.10 a	6.7 ± 0.11 a	10.6 ± 0.22 a	4.0 ± 0.17 a	0.50 ± 0.02 b	22.2 ± 0.26 a
Biostimulant (B)						
B0	9.4 ± 0.45 abc	6.7 ± 0.18 ab	8.6 ± 0.74 c	3.0 ± 0.26 c	0.51 ± 0.03 cd	23.9 ± 0.39 a
B1	9.9 ± 0.27 a	6.8 ± 0.26 a	8.5 ± 0.53 c	3.1 ± 0.79 c	0.96 ± 0.27 a	20.9 ± 0.46 b
B2	9.0 ± 0.19 c	6.0 ± 0.04 c	9.3 ± 0.28 b	3.5 ± 0.31 b	0.43 ± 0.05 cd	21.5 ± 0.54 b
B3	9.2 ± 0.29 bc	6.4 ± 0.14 bc	10.3 ± 0.60 a	3.0 ± 0.06 c	0.86 ± 0.16 ab	21.1 ± 0.74 b
B4	9.3 ± 0.41 bc	6.3 ± 0.09 bc	10.5 ± 0.58 a	3.1 ± 0.23 c	0.36 ± 0.02 d	20.4 ± 0.44 b
B5	9.5 ± 0.38 ab	6.4 ± 0.09 b	9.1 ± 0.44 b	4.5 ± 0.21 a	0.64 ± 0.04 bc	20.0 ± 0.71 b
p value						
Y	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000
Y × B	0.001	0.006	0.000	0.000	0.000	0.051

Note: Means and standard errors are shown. B0: Control; B1: amino acids obtained from beet extracts; B2: liquid extract of *Ecklonia maxima*; B3: liquid extract of *Ascophyllum nodosum*; B4: animal protein hydrolysate; B5: vegetal protein hydrolysate. Values followed by a different letter are significantly different at  $p \leq 0.05$ , in accordance with Tukey's test.

Greatest fruit firmness was observed in control plants, while the lowest in all biostimulated plants (Table 3).

As reported in Figure S6, greater dry matter content was observed during the 2022 compared to 2021. B0-, B1-, B4- and B5-treated plants produced greatest dry matter (from 10.1% to 10.5%). Lowest values were observed during 2021 in fruits obtained from B0- and B4-treated plants.

Higher SSC were observed in 2022 compared to those obtained in 2021, except for B2, which generated the lowest values in both years. The highest value was observed in B1-treated plants while the lowest in B2-treated plants in 2022 (Figure S7).

Highest potassium concentrations were recorded in 2022 in B3- and B4-treated plants, 11.7 and 11.8 g kg<sup>-1</sup>, respectively. The interactions 2021 × B0 and 2021 × B1 produced the lowest values (Figure S8). A similar trend was observed for calcium concentrations. Highest values were measured in 2022 in plants treated with B1 (4.8 g kg<sup>-1</sup>) and B5 (5.0 g kg<sup>-1</sup>). The lowest value was observed in B1-treated plants in 2021 (1.3 g kg<sup>-1</sup>). The highest nitrate concentrations were obtained in B1- and B3-treated plants in 2021, 1.5 g kg<sup>-1</sup> and 1.2 g kg<sup>-1</sup>, respectively (Figure S8). The other treatments produced similar values in both years.

### 3.5 | Colour Parameters

Concerning parameters that contribute to colour definition (HUE, Chroma, L) and based on the Munsel system, the factor year and the interaction Y × B significantly influenced ( $p \leq 0.05$ ) HUE parameters. Greatest HUE was detected in the

**TABLE 4** | Effect of year (Y) factor and biostimulant (B) factor on HUE angle, chroma and L of tomato fruits.

Factor	HUE [°]	Chroma	L
Year (Y)			
2021	0.93 ± 0.01 a	43.6 ± 0.38 a	43.8 ± 0.28 a
2022	0.89 ± 0.01 b	42.8 ± 0.32 a	43.4 ± 0.25 a
Biostimulant (B)			
B0	0.89 ± 0.01 a	43.1 ± 0.79 a	43.8 ± 0.54 a
B1	0.91 ± 0.01 a	43.0 ± 0.89 a	43.3 ± 0.32 a
B2	0.93 ± 0.01 a	43.5 ± 0.57 a	43.7 ± 0.52 a
B3	0.91 ± 0.02 a	43.6 ± 0.27 a	43.8 ± 0.52 a
B4	0.91 ± 0.01 a	43.0 ± 0.61 a	43.9 ± 0.40 a
B5	0.93 ± 0.01 a	43.0 ± 0.67 a	43.0 ± 0.52 a
p value			
Y	0.000	0.090	0.351
B	0.067	0.947	0.703
Y × B	0.003	0.140	0.251

Note: Means and standard errors are shown. B0: Control; B1: amino acids obtained from beet extracts; B2: liquid extract of *Ecklonia maxima*; B3: liquid extract of *Ascophyllum nodosum*; B4: animal protein hydrolysate; B5: vegetal protein hydrolysate. Values followed by a different letter are significantly different at  $p \leq 0.05$ , in accordance with Tukey's test.

first year (0.930). No statistical differences were observed for Chroma and brightness (L) (Table 4).

The highest HUE angle was observed in 2021 in B2- and B3-treated plants, 0.957 and 0.958, respectively (Figure S9).

The lowest values were recorded by the interaction 2022 × B0, 2022 × B3 and 2022 × B4.

## 4 | Discussion

### 4.1 | General Aspects

One of the main goals of 21st century agriculture is to increase yields while saving water, nutrients and genetic resources. In this scenario, the recovery and improvement of tomato landraces is an opportunity in areas where abiotic stresses affect both yield and quality. In this 2-year experiment, the agronomic response of the tomato landrace 'Pizzutello delle Valli Ericine' cultivated in open field without irrigation and treated with different foliar biostimulants was evaluated. The western area of Sicily is characterised by limited water availability for irrigation purposes. In this area, the local ecotype 'Pizzutello' is a widespread crop and one of the few herbaceous species able to be cropped. Harvest takes place in July and August and fruits are consumed until late winter and stored without refrigeration (Conesa et al. 2020). Due to its particular characteristics and strong link to the territory, the Slow Food Foundation has included the tomato landrace 'Pizzutello delle Valli Ericine' on its list of genetic resources to be preserved and protected (Slow Food Foundation 2024).

In this 2-year experiment, temperature trends were similar while rainfall trends differed. 180 mm more rainfall was observed in the second cycle compared to in the first. Precipitation was mainly concentrated in the winter period, thus encouraging the recovery of soil water reserves. These rains alleviated the need for irrigation, which is normally carried out during the transplant stage, as reported by Patanè et al. 2016. and Siracusa et al. 2018.

The growth and development of tomato plants are affected by various environmental factors, such as air temperature, relative humidity and solar radiation (Jiang et al. 2013; Silva et al. 2019; Cao et al. 2021; Flores-Velázquez et al. 2022).

### 4.2 | Morphological and Productive Parameters

Plant height observed at the end of the cycle showed highly significant differences over the 2 years and greater plant heights were found in 2021 in plants treated with *E. maxima* (B2) and vegetal protein hydrolysate (B5).

Other studies on tomato (Lefi et al. 2023; El Khattabi et al. 2023) and on several other species (Papenfus et al. 2013; Rouphael et al. 2017; Van Oosten et al. 2017) have shown that brown algae extracts are rich in plant growth regulators, such as auxins, cytokinins, gibberellins and abscisic acid. These phytohormones improve plant growth and support root development, cell division and stem elongation. Francesca et al (Francesca et al. 2020). achieved greater height in tomato plants grown under high temperature conditions and treated with a vegetal protein hydrolysate. The effects of protein hydrolysate on plant growth could be attributed to the high content of short-chain peptides which, in addition to having hormone-like activity, can perform

the function of nutrients in stress conditions (Colla et al. 2014; Polo and Mata, 2018).

RWC is a widely used index to identify water status in plants (Siddique, Hamid, and Aslam 2000). High values of this index indicate greater efficiency in preserving water contained in plant tissues and increased resistance to high temperatures and water shortage (Sánchez-Rodríguez et al. 2010). RWC varies greatly depending on the plant organ, stage of development and species (Rampino et al. 2006; Egert and Tevini 2002; Hojati et al. 2011; Chen et al. 2016; Aghaie et al. 2018; Farruggia et al. 2024). Highest RWC in our study was obtained in 2021 and in plants treated with animal protein hydrolysate (B4), *A. nodosum* (B3) and beet extract (B1). In tomato plants under water stress, Wang et al (Wang et al. 2022). observed higher RWC in the leaves of plants treated with animal protein hydrolysate. The positive effect may be due to the presence of betaine in these biostimulants. Li et al (Li et al. 2014). found that the exogenous application of betaine in tomato plants improved tolerance to high temperatures and increased levels of heat shock gene expression.

The highest chlorophyll content was recorded during the first year of study and in all biostimulated plants, except for those treated with *A. nodosum*. These results are consistent with other authors who have shown that applications of biostimulants are able to increase photosynthesis rates through increased efficiency in carbon assimilation (Cristiano et al. 2018; Xu and Leskovar 2015), limited chlorophyll degradation and foliar senescence (Di Mola et al. 2019). In an experiment on tomato cultivated under water deficit, the protein hydrolysate action was triggered by the antioxidant defence system, as demonstrated by reduced ascorbic acid and total ascorbic acid content in treated leaves (Francesca et al. 2020). Although the reasons for increases in chlorophyll content are not well-known, it is widely recognised that components in biostimulants can promote activity of specific enzymes linked to photosynthetic processes (Rouphael et al. 2017; Drobek, Fraç, and Cybulska 2019).

Tomato yield is a polygenic characteristic controlled by many internal and external factors that affect growth and the morphological, biochemical and physiological aspects of the plant (Subramaniyan et al. 2023). In both years of this study, higher total and marketable yields were found in biostimulated plants compared to control plants. The number and weight of fruits contributed more to yields following the application of biostimulants, as reported by Caruso et al (Caruso et al. 2019). It should be noted that the highest total and commercial yields were obtained in both years as a result of the application of vegetal protein hydrolysate. Many studies (Francesca et al. 2022; Rouphael et al. 2017; Francesca et al. 2020; Rouphael et al. 2021) have reported increases in tomato yields grown under high temperatures or severe water stress when treated with vegetal protein hydrolysate. This result could be due to a combination of effects. In addition to cytokinin-like activity, the high level of proline in protein hydrolysate biostimulants may have played an important role (Francesca et al. 2022). The positive effects of vegetal protein hydrolysate on growth and yield are related to their amino acid content, which stimulates plant defence, participates in the synthesis of organic compounds (such as amines, purines, pyrimidines, vitamins) and influences the absorption of macro and micronutrients (Colla et al. 2017).

### 4.3 | Qualitative Parameters

Fruit dry matter is influenced by both intrinsic (genotype) and extrinsic factors (climate and agronomic practices) (Kyriacou and Rouphael 2018). Greatest dry matter content, an important parameter for tomato concentrates and paste production (Young, Juvik, and Sullivan 1993), was detected in fruits obtained from control plants and from those treated with amino acid extracted from beet, animal protein hydrolysate and vegetal protein hydrolysate, during the second year of experimentation. In other studies (Caruso et al. 2019; Rouphael et al. 2021) on tomato landraces cultivated under stress conditions and treated with protein hydrolysates, dry matter of biostimulated fruits was not found to be statistically different from those observed in control fruits. Higher dry matter recorded in the second year is likely to be related to lower environmental temperatures recorded during flowering/ripening stage compared to the first year and confirmed by leaf RWC values.

Total soluble solids (expressed as °Brix) are the soluble fraction (sugars, acids and mineral salts) of the tomato fruit dry matter and one of the most important quality characteristics sought by the tomato processing industry and consumers (Nemeskéri et al. 2019; Cozzolino et al. 2021). In both test years, soluble solids content was always above the minimum grade (4.9 °Brix) indicated by the Italian processing industries. In particular, the highest value (7.4 °Brix) was recorded during 2022, with the application of amino acids extracted from beet. From the literature review (Sidhu Murmu and Purnendu Sekhar Bera 2017), it appears clear that the application of betaine-based products not only increases plant tolerance to stress by maintaining cellular turgor, but also seems to facilitate starch, protein, pectin and hemicellulose conversion to soluble simple sugars, resulting in an increase in total soluble solids (Islam et al. 2023).

Although mineral elements are a small fraction of dry matter in vegetables, they are part of the nutritional profile and final quality of these products. This study highlights important information on the mineral profile of the chosen tomato landrace in combination with different climatic conditions and the foliar application of various biostimulants. Among the components of the mineral profile of tomato fruits, potassium was the most abundant element followed by calcium and nitrate. Potassium content was positively influenced by temperature and rainfall in the second year and by the application of *A. nodosum* (B3) and animal protein hydrolysate (B4). As noted by other authors (Caruso et al. 2019; Sidhu Murmu and Purnendu Sekhar Bera 2017; Ali et al. 2016), biostimulants made from seaweed extracts and protein hydrolysates increased potassium concentrations, improving the nutritional value of tomato fruits. Calcium content in this study was positively influenced by the application of biostimulants and, in particular, by beet extract and vegetal protein hydrolysate. The nitrate content was higher in tomatoes during the first year and in fruits obtained from plants treated with beet extract and *A. nodosum*. In accordance with Rouphael et al (Rouphael et al. 2021), the effect of biostimulation was mineral-specific since increasing the concentration of potassium in the fruit decreased that of the anion NO<sub>3</sub> and vice versa.

Greater mineral content in tomato fruit may be associated with the presence in biostimulants of bioactive compounds (amino acids, peptides and carbohydrates), which improve both the

absorption and translocation of these minerals within the plant (Calvo, Nelson, and Kloepper 2014). In addition, Kossak and Dyki (Kossak and Dyki 2008) showed that tomato plants treated with biostimulants had a greater number and larger xylem cells and phlegmatic vascular bundles than untreated plants. We can assume, therefore, greater efficiency of translocation of minerals and photosynthesis products to sites with higher demand (sink). The increased mineral concentration in tomato fruits obtained from biostimulated plants could result from greater mineral absorption capacity due to stimulation of root growth and nutrient transport in cell membranes (Colla et al. 2014; Billard et al. 2014).

### 4.4 | Colour Parameters

Colour is one of the most important physical parameters for fresh tomatoes and processed products. Colour in tomato is mainly related to the biosynthesis of carotenoids, which, in turn, depends on genetic factors, temperature, water status and nitrogen fertilisation (Brandt et al. 2006; Nangare et al. 2016; Ronga et al. 2020). In our study, the HUE angle was found to be highest in 2021 in plants treated with seaweed-based biostimulants (B2 and B3). This could be explained by increased concentration of chlorophyll in the leaves of plants treated with seaweed-based biostimulants. Ali et al (Ali et al. 2024), claimed greater accumulation of chlorophyll in the leaves could lead to an increased accumulation of lycopene and β-carotene in tomato fruits.

Further investigations are needed to assess the effects of foliar treatments using biostimulants on other tomato landraces grown under the same conditions and to investigate possible effects on the nutraceutical profile of tomato fruits.

## 5 | Conclusion

The experiment confirms the enhancement of the species' hardiness and the improvement in fruit quality with the use of biostimulants. The results obtained, although preliminary, encourage further research to optimise the use of biostimulants, as they enable higher yields and superior-quality produce with low cultivation inputs and, most importantly, without irrigation. Observations made over the 2-year evaluation period suggest that animal protein hydrolysate and *A. nodosum* extract promote greater potassium accumulation in the fruit, while beet-root extract is effective in increasing yields and calcium concentration in the fruit. It is recommended that future research test the most promising biostimulants in combination to explore potential synergistic effects in terms of yield and quality, which could have a positive impact on areas of cultivation where climatic conditions are becoming increasingly unpredictable, such as in the Mediterranean basin.

Furthermore, this study, which represents an initial contribution to the literature on the topic, not only reinforces the potential for cultivating the tested ecotype in challenging, nonirrigated environments such as the experimental site, but also helps preserve local biodiversity. It also fosters the development of artisanal and industrial initiatives in the region,

leading to the production of a wide range of derived products, while promoting tourism and spreading awareness of this rich cultural heritage tied to local traditions and culinary practices.

### Author Contributions

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors on request.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section.