



Article

Seaweed Extract Improves *Lagenaria siceraria* Young Shoot Production, Mineral Profile and Functional Quality

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Abstract: Vegetable landraces represent the main source of biodiversity in Sicily. *Lagenaria siceraria* is appreciated by Southern Mediterranean consumers for its immature fruits and young shoots. Plant-based biostimulants supply, such as seaweed extract (SwE), is a contemporary and green agricultural practice applied to ameliorate the yield and quality of vegetables. However, there are no studies concerning the effects of SwE on *L. siceraria*. The current study evaluated the effects of SwE foliar application (0 or 3 mL L⁻¹) on five *L. siceraria* landraces (G1, G2, G3, G4 and G5) grown in greenhouses. Growth traits, first female flower emission, fruit yield, young shoot yield, fruit firmness, young shoot nitrogen use efficiency (NUE_{ys}) and specific young shoot quality parameters, such as soluble solids content (SSC), mineral profile, ascorbic acid, and polyphenols, were appraised. Plant height and number of leaves at 10, 20 and 30 days after transplant (DAT) were significantly higher in plants treated with SwE as compared with untreated plants. Treating plants with SwE increased marketable fruit yield, fruit mean mass, young shoot yield and number of young shoots by 14.4%, 15.0%, 22.2%, 32.4%, and 32.0%, respectively as compared with untreated plants. Relevant increments were also recorded for NUE_{ys}, P, K, Ca, Mg, ascorbic acid and polyphenols concentration. SwE application did not significantly affect total yield and SSC. Furthermore, SwE treated plants produced a lower number of marketable fruits than non-treated plants. The present study showed that SwE at 3 mL L⁻¹ can fruitfully enhance crop performance, young shoot yield and quality of *L. siceraria*.

Keywords: plant-based biostimulants; foliar application; bottle gourd landraces; greenhouse cultivation; crop production; NUE



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1. Introduction

Lagenaria siceraria (Mol.) Stand., generally known as the bottle gourd or white-flowered, is a climbing annual monoecious plant belonging to the cucurbitaceous family native to Africa (Zimbabwe). *L. siceraria* species comprises two different subspecies: *L. siceraria* ssp. *siceraria* and *L. siceraria* ssp. *asiatica*. Tribal groups sited in the Northern Telangana area use its dry fruit shells as bottles, pots, music instruments or as fishing tools [1]. Bottle gourd is also used in traditional Indian medicine as cardioprotective, aphrodisiac, hepatoprotective, analgesic, anti-inflammatory and diuretic [2–4]. Nowadays, the bottle gourd is grown in India and the Mediterranean area—mostly in Sicily—for its immature fruits, young leaves and shoots, these last being consumed as green leafy vegetables.

In Sicily, over an area of 26,000 km², Raimondo et al. [5] estimated 3252 taxa. Consequently, Sicily is an essential centre of origin and differentiation of a number of vegetables [6–11] cultivated both in the open field and in a protected environment. Although

Sicily is not the area of origin for *L. siceraria*, bottle gourd landraces cultivated in Sicily show significant diversity [8]. Herbaceous grafting is considered a toolbox to face biotic and abiotic plant distresses related to monocropping in intensive protected vegetable cultivation systems [12–14]. In this regard, *L. siceraria* is also used as a rootstock for watermelon to improve growth, yield, fruit quality, biotic and/or abiotic stresses tolerance [15,16].

Bottle gourd yield and quality depend on diverse factors such as climatic conditions, soil fertility, agronomical practices and diseases [17]. Currently, to enhance crop production, modern agriculture usually adopts high quantities of fertilizers which, however, have a deleterious environmental impact [18]. Thus, there are considerable research efforts to find new green cultivation technologies to boost the yield and quality of vegetables. In regard to these considerations, biostimulant applications is a valuable and eco-friendly technology to improve vegetable quality traits [18–24]. Among different classes of biostimulants, seaweed extracts (SwEs) are very appreciated. They are composed of different types of seaweeds, although the most used in agriculture are brown algae (e.g., *Ecklonia maxima* and *Ascophyllum nodosum*). These algae are appreciated for their content of polysaccharides, betaines, micro- and macronutrients and hormones, which improve plant production and overall quality [22,25,26]. Their positive effects on plants under optimal, sub-optimal or unfavourable conditions are related to several biochemical and physiological mechanisms such as the elicitation of enzymes involved in carbon and nitrogen metabolic paths, the stimulation of phytohormones synthesis and the improvement in mineral uptake and accumulation through the increase of the root system size [27–29]. However, the application of SwEs on *Lagenaria siceraria* has not been examined yet. The SwEs supply might affect immature fruits, young shoot yield and quality.

Taking into account all the abovesaid and considering that: (i) bottle gourd is an under-utilised species; (ii) immature fruits, young leaves and shoots of bottle gourd are, however, very appreciated by Mediterranean consumers [30]; (iii) seaweed extracts may boost plant performance of vegetables, the purpose of the current work was to appraise the influence of seaweed extract on yield and quality of fruits and young shoots of five local landraces of *L. siceraria* grown in greenhouses.

2. Materials and Methods

2.1. Experimental Field and Treatments

The study was performed in Marsala, during the winter-spring period of 2019, in an experimental field of the Department of Agricultural, Food, and Forestry Sciences of the University of Palermo (SAAF) (latitude 12°26' N, longitude 37°47' E, altitude 37 m). Seeds of five *L. siceraria* landraces (coded G1, G2, G3, G4 and G5) [8]—from self-pollinated flowers—were sown on 10 December 2018 in plug trays (66 cells) filled with a peat moss-based substrate (FAP, Padova, Italy). Plug plants were transplanted on 15 January 2019 in an unheated greenhouse, 2 m between rows and 1 m intra-row, obtaining a plant density of 0.5 plant m⁻². The soil hosting the experiment was composed of sand (<78%) at a pH of 8.3 and high activity limestone at 9.0%. The exchangeable K₂O (655 mg kg⁻¹), P (70 mg kg⁻¹), total N (2.2%), and organic matter (8 t·ha⁻¹) were also determined [31–33].

Plants were fertigated through a drip irrigation system, with 80, 50 and 80 kg ha⁻¹ of N, in form of ammonium nitrate (Yara Italia S.p.A., Milan, Italy), P₂O₅, in form of superphosphate (Siriatic, Ragusa, Italy) and K₂O, in form of potassium sulphate (Fertilisud s.r.l., Barletta, Italy), respectively. During the whole experiment, the conventional bottle gourd cultivation technique was followed, and plant needs were satisfied as recommended [34]. Genotypes were separated by an insect-proof net. At the floral anthesis stage, all-female flowers were manually pollinated, and a clip insulator was applied to prevent cross-pollination among landraces.

The seaweed extract application was performed with an extract of *Ecklonia maxima* (Kelpstar[®], Mugavero fertilizers, Palermo, Italy). This seaweed extract was produced via a cold micronisation process to not alter the seaweed components. This product was composed of 1% of organic nitrogen, 10% of organic carbon, phytohormones (11 mg L⁻¹

of auxins and 0.03 mg L^{-1} of cytokinins) and 30% of organic components characterised by a nominal molecular weight $< 50 \text{ kDa}$. Treatments were administered weekly by foliar spray starting seven days after transplant. One L m^{-2} of the SwE-based solution was supplied for each application.

Two doses of seaweed extract (0 and 3 mL L^{-1}) were combined with five *L. siceraria* landraces (G1, G2, G3, G4 and G5) in a randomised blocks design. All treatments were replicated 3 times (15 plants per replication) obtaining 30 experimental plots (2 seaweed extract doses \times 5 genotypes \times 3 replicates), resulting in a total of 450 plants.

Maximum and minimum temperatures inside the greenhouse were collected by a data logger (Figure 1).

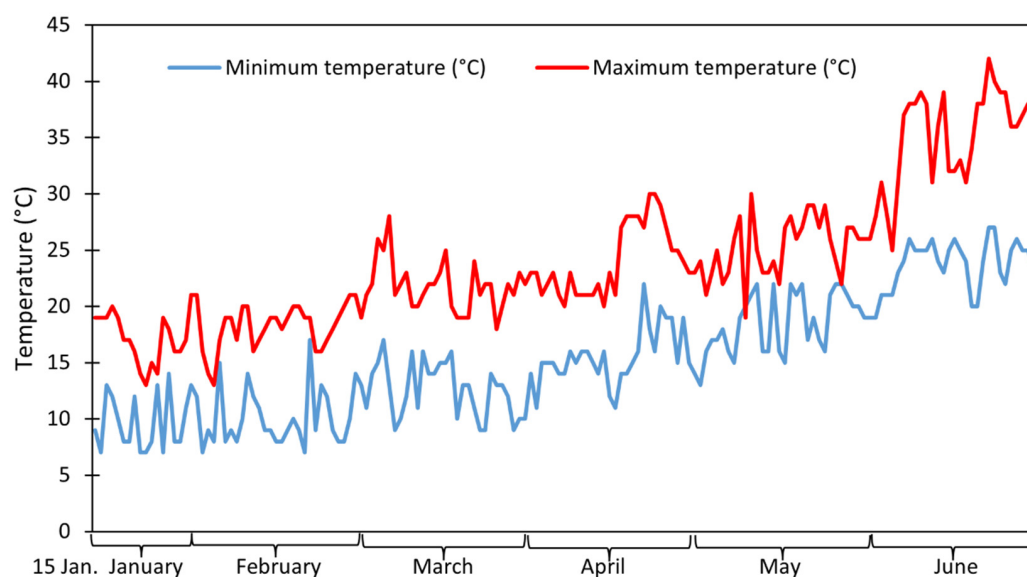


Figure 1. Daily maximum and minimum temperature recorded from 15 January 2019 to 30 June 2019 inside the experimental greenhouse.

2.2. Plant Growth, Fruit Yield and Firmness

Plant growth features, fruit yield and yield-related traits were recorded on all plants. Fruit firmness was collected on 15 randomly selected fruits per replicate. Plant height and number of leaves at 10, 20 and 30 days after transplant (DAT) were recorded. First female flower emission was recorded and expressed as DAT. Immediately after harvest, total yield (kg plant^{-1}), marketable yield (kg plant^{-1}), number of marketable fruits (No.) and fruit mean mass (kg) were collected. Fruit firmness was measured via a digital penetrometer (FR-5120, Lutron electronic enterprise Co., Ltd., Taiwan) and the values were expressed as Newton (N).

2.3. Young Shoot Yield, Nutritional and Functional Components and NUE_{ys}

All the shoots used for yield, nutritional and functional assessments were 30 cm in length.

After harvest, young shoot yield (kg plant^{-1}) and number of young shoots per plant (No. plant^{-1}) were recorded on all young shoots produced.

Five young shoots per plant, randomly selected from each replicate and collected from the 2nd and 3rd harvest, were washed with distilled water and used to determine nutritional and functional compounds. To appraise soluble solid content (SSC), 100 g of young shoot sample was juiced and clarified. Subsequently, SSC was appraised via a refractometer (MTD-045 nD, Three-In-One Enterprises Co., Ltd., New Taipei, Taiwan) and was expressed as $^{\circ}\text{Brix}$. The ascorbic acid concentration was evaluated by a reflectometer (Merck RQflex10 Reflectoquant[®], Sigma-Aldrich, Saint Louis, MO, USA) and ascorbic acid strips (Merck, Darmstadt, Germany) and the value was expressed as $\text{mg } 100 \text{ g}^{-1}$ fresh

weight (fw). Polyphenols' concentration was measured following the Folin–Ciocâlteu method [35] (absorbance at 750 nm). Polyphenols value was presented as gallic acid equivalent (GAE) 100 g⁻¹ dry weight (dw). Calcium (Ca), potassium (K) and magnesium (Mg) concentrations were assessed using the procedure reported by Morand and Gullo [36]. Phosphorous (P) concentration in shoots was appraised following the Fogg and Wilkinson method [37]. Young shoot nitrogen (N) concentration was measured using the Kjeldahl procedure. All the mineral concentrations were presented as g kg⁻¹ dw.

Nitrogen use efficiency (NUE_{ys}) was calculated as follow: young shoot yield (t)/N application rate (kg).

2.4. Statistics

All data were analysed via the SPSS software v.20 package (StatSoft, Inc., Chicago, IL, USA) using a two-way Analysis of Variance (ANOVA). Tuckey's HSD test ($p \leq 0.05$) was used for multiple comparisons of means. Data reported as percentage were subjected to an arcsin transformation prior to ANOVA as follow: $\emptyset = \arcsin(p/100)^{1/2}$. A heat map summarising all *L. siceraria* traits was performed via the online program clustvis (<https://biit.cs.ut.ee/clustvis/>, accessed on 14 September 2021) with a Euclidean distance as the similarity measure and hierarchical clustering with complete linkage.

3. Results

3.1. Plant Growth Traits, Production Features, NUE and Fruit Firmness

ANOVA for plant height at 10, 20, 30 DAT and for number of leaves at 10 and 20 DAT showed no significant interaction between SwE doses and genotype (Table 1).

Table 1. Effect of the seaweed extract treatments (SwE) and genotypes (G) on plant height at 10, 20 and 30 DAT and on the number of leaves at 10 and 20 DAT of *L. siceraria* plants.

Treatments	Plant Height 10 DAT (cm)		Plant Height 20 DAT (cm)		Plant Height 30 DAT (cm)		Number of Leaves 10 DAT (No.)		Number of Leaves 20 DAT (No.)	
<i>Seaweed extract dose (mL L⁻¹)</i>										
0	20.7	b	30.2	b	48.5	b	6.8	b	9.4	b
3	25.3	a	35.7	a	74.3	a	9.0	a	13.2	a
<i>Genotype</i>										
G1	22.9	b	31.1	b	60.4	b	7.5	b	10.8	b
G2	23.2	b	30.5	b	61.9	b	8.3	ab	9.8	b
G3	16.0	c	25.9	c	48.5	c	4.5	c	8.3	c
G4	26.1	a	38.0	a	69.0	a	9.7	a	13.3	a
G5	26.6	a	39.4	a	67.0	a	9.7	a	14.2	a
<i>Significance</i>										
SwE	***		***		***		***		***	
G	***		***		***		***		***	
SwE × G	NS		NS		NS		NS		NS	

Values present in a column and followed by different letters are significantly dissimilar at $p \leq 0.05$. NS, *** non-significant or significant at 0.001, respectively.

Regardless of the genotype, SwE meaningfully enhanced the aforesaid plant growth parameters. On the other hand, irrespective of the SwE application, G4 and G5 genotypes revealed the highest plant height at 10, 20 and 30 DAT and the highest number of leaves at 10 and 20 DAT, whereas, G3 landrace had the lowest values (Table 1).

Statistic on the number of leaves at 30 DAT displayed a significant interaction between SwE and genotype (Figure 2).

Overall, plants treated with SwE revealed a higher number of leaves at 30 DAT compared with the untreated ones. G4 and G5 landraces treated with SwE showed the highest values, followed by G1 and G2 landraces treated at 3 mL L⁻¹ SwE. G3 untreated plants had the lowest value (Figure 2).

ANOVA for first female flower emission did not have a significant interaction between SwE and genotype (Figure 3).

Regardless of the genotype, SwE application delayed the first female flower emission (Figure 3). Disregarding the SwE treatment, G3 landrace had the earliest female flower emission, followed by G2 landrace. G4 and G5 landraces revealed the latest female flower emission (Figure 3).

ANOVA for yield and yield-related traits did not reveal a significant influence of the interaction SwE \times G (Table 2).

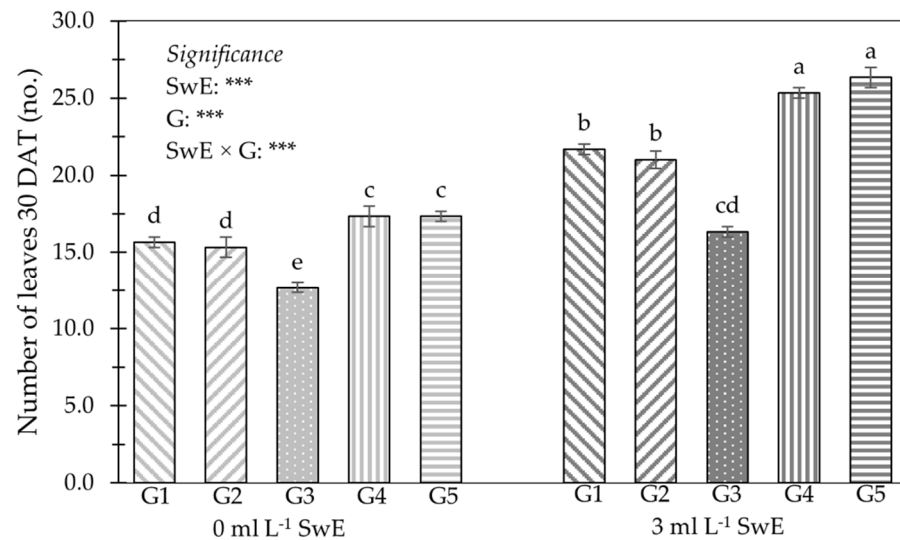


Figure 2. Effect of the seaweed extract treatments (SwE) and genotypes (G) on the number of leaves at 30 DAT of *L. siceraria*. Values with different letters indicate significant differences at $p \leq 0.05$. *** significant at 0.001. Bars represent the standard error.

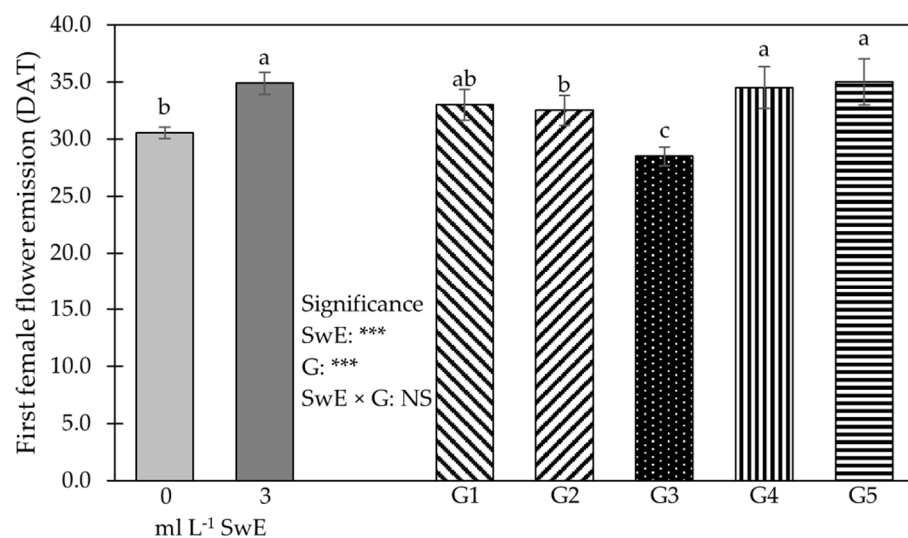


Figure 3. Effect of the seaweed extract treatments (SwE) and genotypes (G) on first flower emission of *L. siceraria*. Values with different letters indicate significant differences at $p \leq 0.05$. NS, *** non-significant or significant at 0.001, respectively. Bars represent the standard error.

Table 2. Effect of the seaweed extract treatments (SwE) and genotypes (G) on fruits total yield, fruits marketable yield, No. of marketable fruits, fruit mean mass, young shoot yield and number of *L. siceraria*.

Treatments	Fruits				Young Shoots							
	Total Yield (kg plant ⁻¹)		Marketable Yield (kg plant ⁻¹)		Marketable Fruits (No. plant ⁻¹)		Mean Mass (kg)		Yield (kg plant ⁻¹)		Number (No. plant ⁻¹)	
<i>Seaweed extract dose (mL L⁻¹)</i>												
0	4.86	a	4.0	b	4.5	a	0.9	b	2.04	b	51.0	b
3	4.89	a	4.6	a	4.0	b	1.1	a	2.70	a	67.3	a
<i>Genotype</i>												
G1	6.80	a	6.0	a	4.4	b	1.4	a	2.35	c	58.8	b
G2	5.30	c	4.6	c	4.9	ab	0.9	b	2.45	bc	61.0	b
G3	5.67	b	5.0	b	5.6	a	0.9	b	1.60	d	40.2	c
G4	3.12	e	2.8	e	3.0	c	1.0	b	2.73	a	68.3	a
G5	3.50	d	3.1	d	3.2	c	1.0	b	2.70	ab	67.3	b
<i>Significance</i>												
SwE	NS		***		**		***		***		***	
G	***		***		***		***		***		***	
SwE × G	NS		NS		NS		NS		NS		NS	

Values present in a column and followed by different letters are significantly dissimilar at $p \leq 0.05$. NS, **, *** non-significant or significant at 0.01 or 0.001, respectively.

SwE application did not affect total fruit yield (Table 2). Conversely, notwithstanding the biostimulant treatment, G1 had the highest total yield, followed by G3, which in turn had a higher total fruit yield than the G2 landrace. The lowest total fruit yield value was recorded in the G4 landrace.

When averaged over genotype, fruit marketable yield was increased by SwE treatment (Table 2). Regardless of the SwE application, data collected on fruit marketable yield followed the trend recognised for total fruit yield.

SwE non-treated plants showed a greater number of marketable fruits compared with the treated ones (Table 2). Averaged over the SwE application, G3 landrace showed the highest number of marketable fruits, followed by G1 landrace. Whereas G2 plants did not meaningfully diverge neither from G3 plants nor from G1 plants. G4 and G5 landraces had the lowest number of marketable fruits.

SwE treatment significantly increased fruit mean mass compared with the control (Table 2). Regardless of the SwE application, the G1 genotype gave the highest fruit mean mass compared with the other genotypes.

Young shoot yield in SwE treated plants was higher by 22.2% compared to untreated control (Table 2). Averaged over SwE application, the G4 genotype showed the highest young shoot yield, whereas the G3 landrace revealed the lowest one.

SwE application boosted the number of young shoots (Table 2). G4 landrace gave the highest number of young shoots, followed by G1, G2 and G5. The genotype G3 gave the lowest value.

Statistic for NUE_{ys} underlined no significant interaction SwE × G (Figure 4).

Regardless of the genotype, SwE treated plants displayed the highest NUE_{ys} value (Figure 4). Averaged over SwE treatment, G4 and G5 landraces gave the highest NUE_{ys} value. However, the G5 landrace did not significantly differ neither from the G4 landrace nor from the G2 landrace. The lowest values were observed in the G3 landrace (Figure 4).

Statistic on fruit firmness revealed no significant interaction SwE × G (Figure 5).

Fruits from plants treated with SwE revealed a higher firmness than fruits from untreated plants. When averaged over SwE treatment, G2 and G3 landraces displayed the highest firmness, followed by G1 landrace. G4 and G5 landraces had the lowest values (Figure 5).

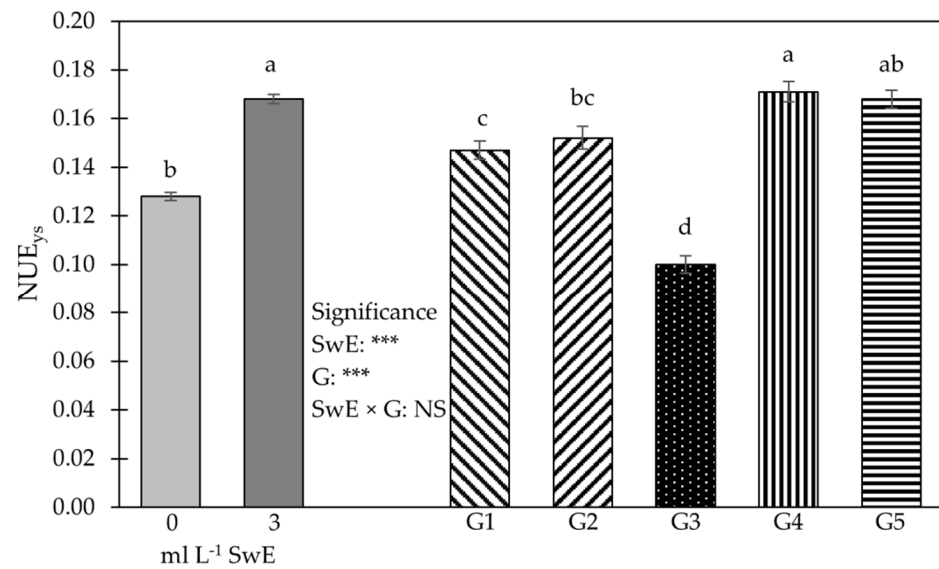


Figure 4. Effect of the seaweed extract treatments (SwE) and genotypes (G) on shoot nitrogen use efficiency of *L. siceraria*. Values with different letters indicate significant differences at $p \leq 0.05$. NS, *** non-significant or significant at 0.001, respectively. Bars represent the standard error.

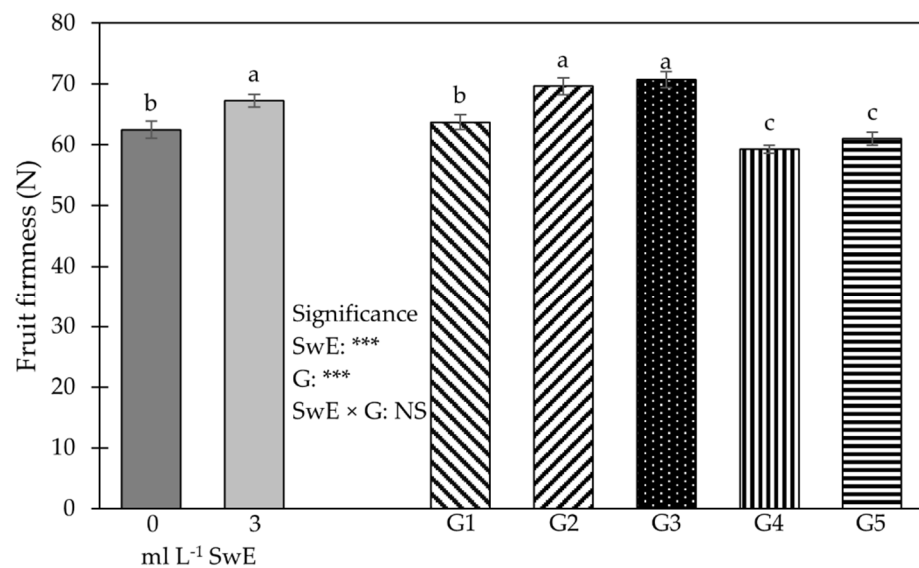


Figure 5. Effect of the seaweed extract treatments (SwE) and genotypes (G) firmness of *L. siceraria* fruits. Values with different letters indicate significant differences at $p \leq 0.05$. NS, *** non-significant or significant at 0.001, respectively. Bars represent the standard error.

3.2. Young Shoot Nutritional Properties, Mineral Profile and Functional Components

Statistical analysis for SSC did not show a significant interaction between SwE and G (Figure 6).

Averaged over genotype, SwE treatment did not affect young shoot SSC (Figure 6). Contrariwise, when averaged over SwE application, G2, G4 and G5 landraces had the highest SSC, followed by G1 genotype which in turn had a higher SSC value than G3 landrace (Figure 6).

The mineral profile was mainly influenced by SwE application and genotype. However, ANOVA for N, P, K, Ca, and Mg concentrations highlighted no significant interaction SwE \times G (Table 3).

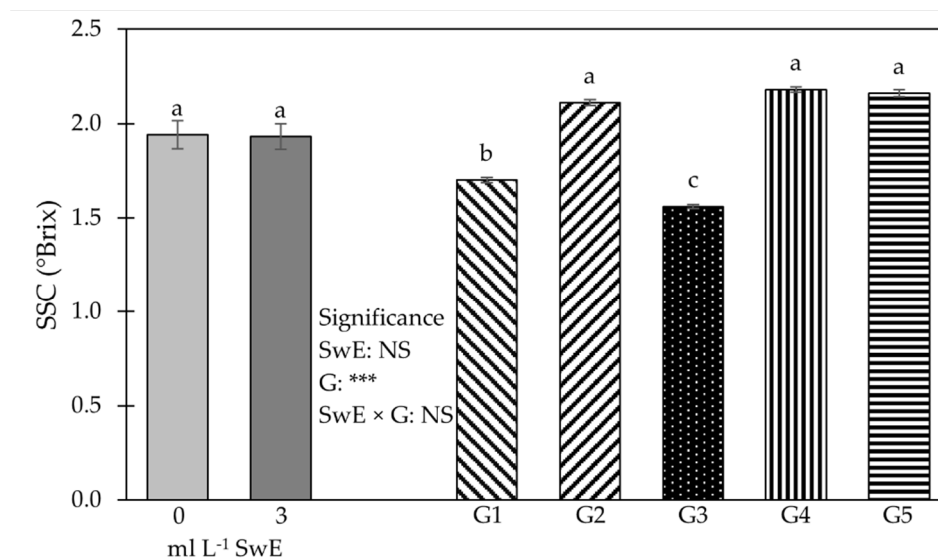


Figure 6. Effect of the seaweed extract treatments (SwE) and genotypes (G) on young shoot soluble solid content of *L. siceraria*. Values with different letters indicate significant differences at $p \leq 0.05$. NS, *** non-significant or significant at 0.001, respectively. Bars represent the standard error.

Table 3. Effect of the seaweed extract treatments (SwE) and genotypes (G) on nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) concentrations of *Lagenaria siceraria* young shoots.

Treatments	N (g kg ⁻¹ DW)		P (g kg ⁻¹ DW)		K (g kg ⁻¹ DW)		Ca (g kg ⁻¹ DW)		Mg (g kg ⁻¹ DW)	
<i>Seaweed extract dose (mL L⁻¹)</i>										
0	1.86	a	5.14	b	19.30	b	25.77	b	9.54	b
3	1.74	b	5.30	a	19.75	a	26.00	a	10.00	a
<i>Genotype</i>										
G1	1.80	c	5.30	b	19.80	a	26.10	a	9.62	b
G2	1.70	d	5.11	c	19.08	b	25.60	b	9.60	b
G3	1.60	e	4.99	d	18.72	b	25.00	c	9.44	b
G4	1.94	b	5.38	a	20.13	a	26.40	a	10.11	a
G5	2.00	a	5.33	ab	19.82	a	26.30	a	10.02	a
<i>Significance</i>										
SwE	***		***		***		***		***	
G	***		***		***		*		***	
SwE × G	NS		NS		NS		NS		NS	

Values present in a column and followed by different letters are significantly dissimilar at $p \leq 0.05$. NS, *, *** non-significant or significant at 0.05 or 0.001, respectively.

Averaged over genotype, the highest N concentration was observed in young shoots from untreated plants (Table 3). Disregarding the SwE treatment, genotype G5 revealed the highest N concentration, followed by G4 landrace which in turn displayed a higher N concentration than the G1 landrace. The lowest value was observed in young shoots from the G3 genotype.

P, K, Ca, and Mg contents in plants exposed to 3 mL L⁻¹ of SwE was higher by 3.1%, 2.3%, 0.9% and 4.8%, respectively compared with untreated plants (Table 3). Averaged over SwE application, G4 and G5 landraces revealed the highest P concentration, followed by G1 landrace. However, young shoots from the G5 landrace did not show a significant difference in terms of P concentration. The lowest P concentration was recorded in young shoots from the G3 genotype. The highest K concentration was found in young shoots from G1, G4 and G5 landraces (19.80, 20.13 and 19.82 g kg⁻¹ dw, respectively), whereas, G2 and G3 genotypes had the lowest values. Data on Ca concentration followed a similar trend to that described for K concentration. Regardless of SwE application, G4 and G5 landraces had a higher Mg concentration compared with the other genotypes.

ANOVA for shoot ascorbic acid concentration showed a significant interaction between SwE and G (Figure 7).

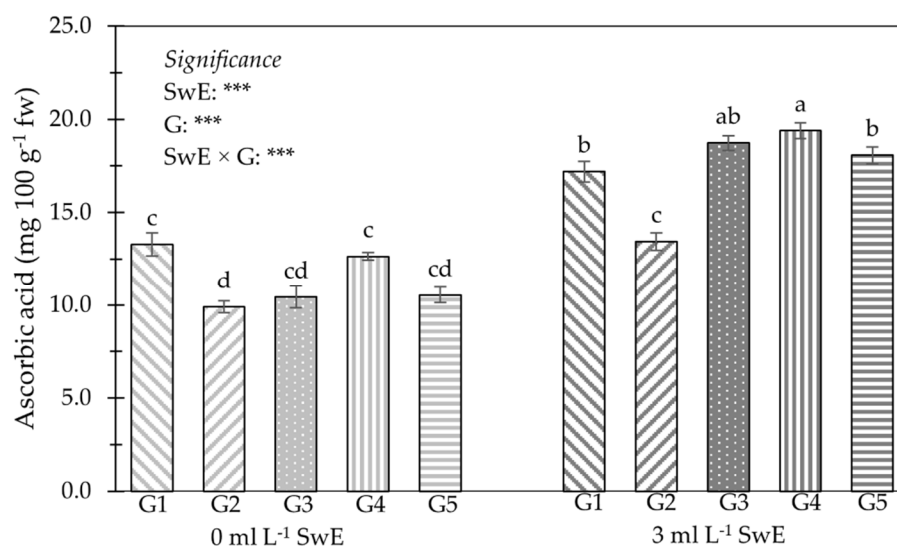


Figure 7. Effect of the seaweed extract treatments (SwE) and genotypes (G) on the ascorbic acid concentration of *L. siceraria* young shoots. Values with different letters indicate significant differences at $p \leq 0.05$. *** significant at 0.001. Bars represent the standard error.

Overall, SwE treatment enhanced ascorbic acid concentration in G1, G2, G3, G4 and G5 young shoots by 29.5%, 35.9%, 79.6%, 53.4% and 71.6%, respectively (Figure 7). The combinations 3 mL L⁻¹ SwE × G3 and 3 mL L⁻¹ SwE × G4 gave the highest ascorbic acid content. However, when G3 landraces were exposed to SwE did not significantly differ neither from G4 nor from G5 in terms of ascorbic acid concentration. The lowest ascorbic acid concentration was detected in young shoots from the 0 mL L⁻¹ SwE × G2 combination.

ANOVA for polyphenols concentration showed a significant interaction SwE × G (Figure 8).

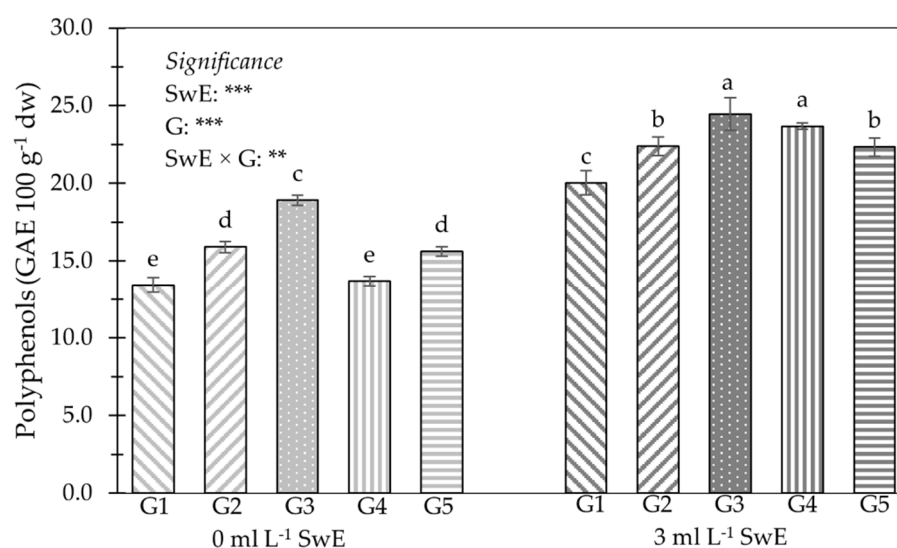


Figure 8. Effect of the seaweed extract treatments (SwE) and genotypes (G) on polyphenols concentration of *L. siceraria* young shoots. Values with different letters indicate significant differences at $p \leq 0.05$. ***, ** significant at 0.01 or 0.001, respectively. Bars represent the standard error.

As for ascorbic acid concentration in all tested genotypes, polyphenols were significantly enhanced by SwE treatment. The highest values were recorded in G3 and G4 landraces supplied with 3 mL L⁻¹ of SwE, followed by G2 and G5 landraces (Figure 7). The lowest values were observed in young shoots harvested from G1 and G4 untreated plants.

3.3. Heat Map Analysis of the Whole Data Set

A data heat-map analysis of all assessed features (agronomic, nutritional and functional) was performed to realise a graphical appraisal of the influences determined by the experimental factors on *L. siceraria* (Figure 9).

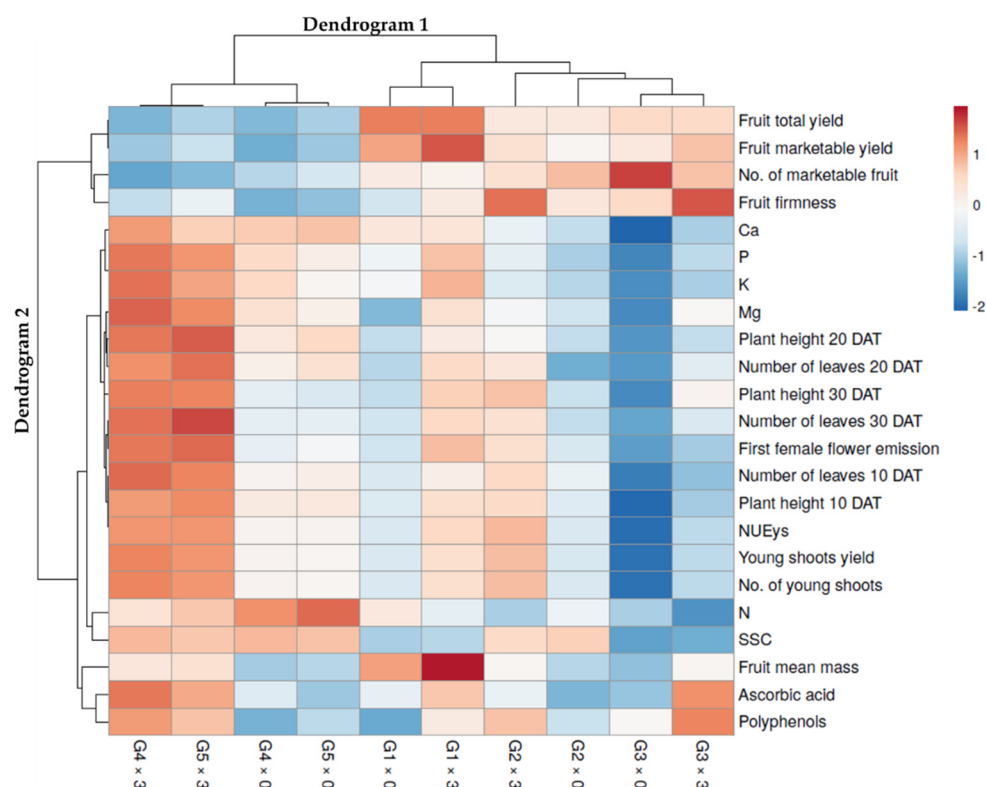


Figure 9. Heat map assessment including all *L. siceraria* plant traits in response to seaweed extract treatments (0 or 3 mL L⁻¹) and genotypes (G1, G2, G3, G4 or G5). The heat map picture was produced via <https://biit.cs.ut.ee/clustvis/> (accessed on 14 September 2021).

The heat-map output consisted of two dendrograms, Dendrogram 1 sited on the top containing all the combinations of SwE doses and genotypes and Dendrogram 2, located on the left side, comprising all traits that influenced this distribution. Dendrogram 1 presented two main clusters, the first on the left included the G4 and G5 landraces both treated and untreated with SwE. The other site on the right side contained G1, G2 and G3 landraces both treated and untreated with SwE. Expressly, in the left cluster of Dendrogram 1, the G4 and G5 landraces treated with SwE were parted from the other G4 and G5 untreated controls due to the higher P, K, Mg, plant height (at 10, 20 and 30 DAT), number of leaves (at 10, 20 and 30 DAT), first female flower emission, NUE_{ys}, young shoot yield, number of young shoots, fruit mean mass, ascorbic acid and polyphenols. The group on the left included G4 and G5 landraces treated with SwE. Within this group, the G4 × 3 combination was clearly separated by lower total yield, marketable yield, number of marketable fruits, plant height at 10 and 20 DAT, number of leaves at 20 and 30 DAT and N. While, the group on the right side included the G4 and G5 landraces untreated plants. Inside this cluster, the G4 × 0 combination was separated by higher P, K, Mg and ascorbic acid and lower total yield, marketable yield, fruit firmness, N, fruit mean mass and polyphenols.

On the right of the Dendrogram 1, two main groups were documented, the one on the left included the combinations $G1 \times 0$ and $G1 \times 3$, separated from the $G2$ and $G3$ landraces treated with 0 or 3 mL L⁻¹ of SwE, that had, in particular, lower fruit total yield, fruit marketable yield, Ca, P, K, N, Mg, plant height at 20 DAT and fruit mean mass, but the higher number of marketable fruits, firmness and polyphenols.

The group on the left side comprised $G1$ untreated control and $G1$ SwE treated plants; the $G1 \times 0$ combination was separated from $G1 \times 3$ by lower fruit marketable yield, fruit firmness, P, K, Mg, plant height (at 10, 20 and 30 DAT), number of leaves (at 10, 20 and 30 DAT), first female flower emission, NUE_{ys} , young shoot yield, number of young shoots, fruit mean mass, ascorbic acid and polyphenols.

The cluster on the right comprised $G2$ and $G3$ untreated and SwE treated combinations. In this group, the $G2 \times 3$ combination was evidently parted from $G2 \times 0$, $G3 \times 0$ and $G3 \times 3$ combinations by higher Ca, P, K, plant height (at 10, 20 and 30 DAT), number of leaves (at 10, 20 and 30 DAT), first female flower emission, NUE_{ys} , young shoot yield and number of young shoots. The right side of this cluster included $G2 \times 0$, $G3 \times 0$ and $G3 \times 3$ treatments; the $G2$ landrace untreated control was parted by higher Ca, P, K, Mg, plant height (at 10, 20 and 30 DAT), number of leaves (at 10 and 30 DAT), first female flower emission, NUE_{ys} , young shoot yield, number of young shoots, N and SSC. The right part of this group comprised $G3$ landraces SwE treated and untreated control. $G3 \times 0$ combination was separated from $G3 \times 3$ combination by lower fruit marketable yield, fruit firmness, Ca, P, K, Mg, plant height (at 10, 20 and 30 DAT), number of leaves (at 10, 20 and 30 DAT), first female flower emission, NUE_{ys} , young shoot yield, number of young shoots, fruit mean mass, ascorbic acid and polyphenols.

4. Discussion

In the present work, we investigated the effect of SwE application on yields and young shoot quality features of five *L. siceraria* landraces. Irrespective of the genotype, SwE supply improved plant height and the number of leaves. These results are coherent with those of Rouphael et al. [38] who, investigating the influence of *Ecklonia maxima* SwE on production, quality and physiological traits of zucchini squash cultivated under saline conditions, found that, regardless of the salinity treatments, SwE enhances plant aerial weight. Findings are also in line with those of La Bella et al. [23] who, examining the influence of the SwE of *E. maxima* and molybdenum enrichment on yield, quality and NUE in spinach plants, highlighted that SwE application boosts growth plant features. Without regard to the SwE treatments, the $G4$ and $G5$ landraces performed better than the other tested genotypes, while the $G3$ landrace had the lowest plant growth features. As reported by Weiner [39], this was probably because plants may grow efficiently until they achieve the threshold size for reproduction. Once they accomplish this size, a certain fraction of sources is assigned to reproduction. Indeed, the $G3$ landrace revealed the earliest female flower emission. On the other hand, when averaged over genotype, SwE application delayed first female flower emission, in accordance with the aforesaid vegetative vs. reproductive competitive activity.

There are researches on the favourable effect of diverse plant biostimulants on the marketable yield of various vegetables [22,28]. In this respect, the results of the present study are in agreement with those reported by Ali et al. [40], who underlined that *Ascophyllum nodosum*-based SwE improved tomato yield in a soilless system by 54% compared with the control. These results were related to the *A. nodosum* SwE polysaccharides content which in turn enhances yield promoting endogenous hormone homeostasis [41]. Outcomes agree with those of Colla et al. [28] who, studying the effect of different classes of biostimulants on yield and fruit quality of tomato cultivated under greenhouse, found that SwE 'Kelpak' increase marketable yield compared with the control. Furthermore, the results are sustained by Hussain et al. [42] who found that SwE of *Durvillaea potatorum* and *A. nodosum* augment tomato marketable yield. Data are also consistent with those of Hassan et al. [43] and La Bella et al. [23]. In this study, the marketable fruit increase prompted by the SwE

application was due to a higher fruit mean mass rather than to the higher number of marketable fruits. These results are in contrast with those of Colla et al. [28], who reported that SwE treatment improves the number of fruits per plant but did not affect fruit mean mass. Thus, we may hypothesise that the plant yield response to SwE application is genotype-dependent. G4 and G5 landraces revealed the lowest fruit yields. Averaged over genotype, SwE supply elicited young shoot production, both in terms of yield and number. In this respect, there are reports that brown seaweed extracts comprise phytohormones (IAA, cytokinin, GA, polyamines and ABA) [25,44,45]. Consequently, we may assume that the growth eliciting effects of SwE are linked to their effect. Wally et al. [46] stated that the SwE phytohormone-like action might also be triggered by chemical compounds included in the extract rather than by the phytohormones themselves. On the other hand, irrespective of the SwE application, G4 and G5 landraces had the highest young shoot yield traits. Thus, it seems that young shoot production is a genotype-associated trait and is negatively related to fruit production. Results showed that SwE treatment increased NUE_{ys} . These findings are coherent with those of Di Mola et al. [46] who investigated the effect of plant-based biostimulants on nitrogen use and uptake efficiency, yield and quality of leafy vegetables cultivated under different nitrogen regimes, found that treated plants had a higher NUE than untreated ones. Moreover, the results are in agreement with previous studies concerning the influence of *E. maxima* SwE application and molybdenum supply on yield, quality and NUE of spinach [23]. Outcomes showed that the SwE application enhanced fruit firmness. Overall, this is consistent with previous research on the influence of plant-based biostimulants on different tomato cultivars [47]. As reported by Basile et al. [47] and Cozzolino et al. [48] the SwE effect on fruit firmness is related to the higher Ca uptake and accumulation of SwE treated plants compared with the control. Indeed, as pointed out by Hocking et al. [49], calcium-pectin cross-links play an imperative function in defining the resistance of cell walls and, thus, the characteristics of the physical and structural fruit. Furthermore, since it is assumed that auxins partake in Ca transport and fruit uptake [49], the present study suggested that the SwE may have an auxin-like action in *L. siceraria*, improving calcium nutrition. Regardless of the SwE application, statistics showed a significant influence of the genotype on fruit firmness. Findings showed that SwE did not significantly affect young shoot SSC. In this respect, the results concur with those stated by Colla et al. [28] and La Bella et al. [23] who found no significant effect of the SwE on SSC on tomato and spinach, respectively. ANOVA displayed a significant effect of the genotype on young shoot SSC values. A similar response was previously reported for zucchini squash by Rouphael et al. [50] and Rouphael et al. [51], and it was related to a reduction in water accumulation in the fruit without influence on the biosynthesis and accumulation of organic solutes. Thus, since G2, G4 and G5 landraces gave the highest SSC young shoot yield and considering that G2, G4 and G5 were more productive than the other genotypes in terms of young shoots, but less performing in terms of fruit yields, it seems that the aforesaid landraces had a resource translocation mainly toward to the shoots rather than to the fruits compared to the G1 and G3 landraces.

The results displayed that the SwE application reduced N concentration in young shoots. In this respect, there are contrasting reports. Krouk et al. [52] and Castaings et al. [53] state that different *A. nodosum*-based SwE upregulated the expression of a nitrate transporter gene NRT1.1, which enhance nitrogen sensing and auxin transport. On the contrary, Rouphael et al. [38], found that SwE application does not significantly influence N concentration in tomato leaves. Thus, we may hypothesise that the plant N uptake and accumulation response to SwE supply, is significantly related to genotype and plant site. Results on minerals revealed that SwE treatment augmented P, K, Ca and Mg concentrations. These findings are partially coherent with those of Rouphael et al. [12], who found that *E. maxima* SwE application improve K and Mg concentrations in spinach plants. Furthermore, the outcomes concur with those of La Bella et al. [23], who evidenced that SwE treatment increase P, K and Mg concentration in spinach. As highlighted by Battacharyya et al. [29], this improved minerals uptake and build-up could be linked to a modification of the root

architecture, resulting in an enhanced plant mineral uptake. Moreover, Soppelsa et al. [54] pointed out that commercial SwE includes a compound named kahydrin, which modifies plasma membrane proton pumps and elicit the H⁺ ions excretion into the apoplast determining rhizosphere acidification, resulting in a higher metal ions plant availability [52,53].

Among plant secondary metabolites, ascorbic acid and polyphenols provide benefits to human health and, furthermore, play a crucial role in numerous plant life aspects [55]. The current study showed that SwE application upgraded ascorbic acid and polyphenols concentrations in young shoots of bottle gourd. These results sustained the outcomes of Roupheal et al. [12] and La Bella et al. [23] on spinach and those of Abbas et al. [56] on onion. As reported by Ertani et al. [57] and Roupheal et al. [58], the enhancement of bioactive components, such as ascorbic acid and phenols, could be related to the chalcone isomerase activity, which is a key enzyme in phytochemical homeostasis [59].

5. Conclusions

In the current study, SwE application boosted plant growth traits, fruit and young shoot yield, NUE_{ys}, mineral profile and functional components of young shoots. Concurrently, Sicilian bottle gourd landraces showed a relevant range of genetic variability. Overall, the results suggested that combining G4 and G5 landraces with 3 mL L⁻¹ SwE profoundly upgraded young shoot yield, plant height, number of leaves young shoot yield and NUE_{ys} index. Quality traits such as SSC, minerals concentration, ascorbic acid and polyphenols content were also improved.

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