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Combined effects of biostimulants, N level and drought stress on yield, quality and physiology of greenhouse-grown basil

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A B S T R A C T

Precise nitrogen (N) supply is an agronomic practice of crucial importance to achieve optimal crop performance without compromising product quality. However, excessive use of synthetic N fertilizers may have deleterious effects on both agroecosystem and human health. Thus, the development and use of strategies aiming to ameliorate the losses caused by water constraints and N deficiency are essential for fostering resilient and sustainable agroecosystems. In this regard, the impact of three drought stress levels (DS) [100%, 80% and 60% of the field capacity (FC)] in combination with four N supply rates (0, 50, 100 and 150 kg ha^{-1}) on sweet basil cultivated in a protected environment was investigated. The interactive biostimulatory action of Kelpstar® seaweed extract (SWE) and Tyson® protein hydrolysate (PH) was also explored. The study focused on the effects of these treatments on yield, physiological attributes, functional traits, and volatile compounds profile. Drought stress led to a reduction in yield by 12.5% and 21.1% under irrigation at 80% and 60% FC, respectively, compared to well-watered plots (100% FC). Furthermore, drought stress levels linearly decreased total leaf area (-15.4% and -26.2% for DS80 and DS60, respectively), stomatal conductance (-14.2% and 34.1% for DS80 and DS60, respectively), nitrogen use efficiency (NUE) (4.0% and 10.0% for DS80 and DS60, respectively), and volatile compounds, such as trans-2-hexanal, 1-octen-3-ol and α-bergamotene. Conversely, an increase in N application rate positively influenced yield (8.6% and 12.2% for N100 and N150, respectively), total leaf area (22.2% and 16.5% for N100 and N150, respectively), specific leaf area (SLA), total chlorophyll (7.7% for N150), nitrate content, and the presence of specific volatile compounds, such as 1-octen-3-ol and α-bergamotene, when compared to no N application. Seaweed extract application caused an upsurge in yield (+17.5%), stomatal conductance (+25.8%), WP (+13.5%), total chlorophyll (+2.3%), nitrate (+3.4%), phenolics (+14.2), ascorbic acid (+28.2), as well as, 1-octen-3-ol, β-cis-ocimene, linalool and eugenol, compared to the control. Similarly, plant protein hydrolysate increased yield (+16.1), stomatal conductance (+10.4), WP (+13.7), total chlorophyll (+4.3), phenolics (+10.7%), ascorbic acid (+9.7%), β-cis-ocimene and eugenol, compared to the control. Notably, the increased yield, improved quality, and enchanced physiological traits observed after biostimulant application, especially under drought stress or N deficiency conditions, underscore the potential role of biostimulants in increasing resilience of basil plants. Thus, the foliar application of SWE and PH offer a valuable strategy for enhancing plant yield and quality under sub-optimal conditions, while simultaneously enhancing water and N use efficiency.

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1. Introduction

Sweet basil (*Ocimum basilicum* L.) belongs to the *Lamiaceae* family. Due to its properties, is a unique element for old-fashioned Italian plates such as "pesto". Beyond its culinary significance, sweet basil is also used in the pharmacosmetic sector (Barátová et al., 2015; Ciriello et al., 2021a,b) due to its known ability to synthesize low molecular weight organic compounds that contribute to its characteristic aroma (Dias et al., 2016). The high functional value of basil is mostly linked to an assorted phenolic profile that - like flavoring - is intensely influenced by the genotype × environment interactive effect (Jakovljević et al., 2019; Ciriello et al., 2021b). Phenols, not only affect the qualitative aspects of vegetables, but also possess antioxidant and antiseptic properties, making them potential substitutes to synthetic additives in the agri-food sector (Filip, 2017).

Climate changes and extreme ecological circumstances combined with the consumer demand for high-quality vegetables have driven farmers and agronomists towards alternative cropping schemes (Ciriello et al., 2021a; Teklić et al., 2021). In this context, water insufficiency - generated by climate change - is a crucial abiotic stress for the vegetable production sector, as it harmfully affects global food accessibility (Snyder, 2017; IPCC, 2019). Thus, as reported by Vila-Traver et al. (2021), a proper management of irrigation water is essential to maintain a steady food supply (Vila-Traver et al., 2021). The effect of water deficiency on plants depends on its intensity and duration and, as observed by Okçu et al. (2005), it is highly related to the plant's developmental stage. Drought stress damagingly affects plant water relations (França et al., 2000), water use efficiency (WUE) (Wu et al., 2008), photosynthesis, water and nutrient uptake (Ansari et al., 2018; Praba et al., 2009), as well as plant growth, productivity and quality.

Successful vegetable crop cultivation demands the implementation of precise agronomic practices (D'Anna and Sabatino, 2013; Miceli et al., 2019; Consentino et al., 2020; Sabatino et al., 2021, 2022) coupled with optimal nutrient supply - mainly nitrogen (N), phosphorous (P) and potassium (K) - to ensure growth, productivity and high quality(Solaiman and Rahbbani, 2006; Zaidi et al., 2015; Sabatino et al., 2020). Nitrogen participates in several physiological and metabolic processes and is a building block of proteins, enzymes and nucleic acids (Maathuis, 2009; Albornoz, 2016). Nitrogen availability affects morpho-physiological plant traits, which in turn affect marketability and visual quality (Broadley et al., 2000). Biesiada and Kuś (2010) found an increase of herb yield in sweet basil as N rate increased. Additionally, Golcz et al. (2006) stated that N supply significantly increase sweet basil yield, chloroplast pigment content and essential oil production. Nonetheless, excessive N fertilization, frequently used by farmers, can result in nitrate accumulation in plant tissues, triggering harmful effects on the agro-ecosystem through N leaching also posing potential risks to human health (Ward, 2009; Galloway et al., 2002).

The incessant use of N-based synthetic fertilizers combined with water shortage may threaten the ecosystem sustainability. Therefore, aligned with the European Commission guidelines, plant biostimulants are considered promising and eco-friendly approaches to enhance vegetable plant performance (Sabatino et al., 2022; Consentino et al., 2022). Among plant biostimulants, seaweed and plant extracts can regulate plant primary and secondary metabolism in order to increase overall plant performance within diverse growing environments, whether favourable or sub-optimal (Lucini et al., 2018; Consentino et al., 2021). Seaweed extracts, particularly those derived from the brown macro-algae, are employed for their content in signaling molecules (polysaccharides, betaines, nutrients and phytohormones) which improve overall plant fitness (Khan et al., 2009; Craigie, 2011). The improvement of crop productivity, under unfavorable conditions through the seaweed extracts supply, is associated with several mechanisms, including the stimulation of enzymes involved in the carbon (C) and N metabolic pathways, the Krebs cycle and glycolysis, the elicitation of phytohormones and the enhancement of mineral uptake and accumulation through root morphology modifications (Battacharyya et al., 2015).

Colla et al. (2017), reported an encouraging influence of plant proteins hydrolysate on plants exposed to different distresses, including drought. This positive effect was predominantly related to the build-up of defending and/or bioactive compounds characterized by an antioxidant, hormone-like or osmotic activity, that alleviate the harshness of crop yield reduction under stress. The action of plant protein hydrolysate is related to the biosynthesis and accumulation of some compounds, such as proline and glycine betaine (Colla et al., 2017). Moreover, there is evidence that plant proteins hydrolysates act as stimulators of the C and N metabolism (via key enzymes involved in the N absorption and regulation) and of the enzymes enclosed in the tricarboxylic acid cycle (Colla et al., 2015a; du Jardin, 2015; Nardi et al., 2016). Researches (Colla et al., 2014 and 2015a) showed that plant proteins hydrolysates may, also, interact - through bioactive peptides - with hormonal activities, eliciting plant growth and yield (Ertani et al., 2009; Matsumiya and Kubo, 2011; Colla et al., 2014; Lucini et al., 2015). In this regard, biostimulants, such as seaweed and plant extracts can enhance crop yield due to their aptitudes to boost nitrogen use efficiency (NUE), prompting mineral and water absorption and utilization efficiency. Plant responses to climate variables and agronomic practices are highly dependent on plant species and cultivation environments. Despite numerous studies investigating the effects of individual stressors on crop yield and quality, little is known about the interactive effects of drought stress, N doses and biostimulants on yield and qualitative features of sweet basil.

For the aforesaid premises, the aim of the present study was to evaluate the effect of three irrigation levels (100%, 80% and 60% of field capacity), four N fertilization regimes (0, 50, 100 and 150 kg ha^{-1}) and two biostimulants (Kelpstar ® seaweed extract and Tyson ® protein hydrolysate) on 'Gervaso' F_1 sweet basil. The results of this research might be useful to balance the resources supply, to expand the crop yield performance of sweet basil as well as to reduce the ecological impact of its cultivation. Furthermore, identifying the optimal combination of drought stress levels, N rate, and biostimulant application that may result in reduced chemical inputs and maximized resource use efficiency, without jeopardizing crop yield and quality, is of paramount importance in the present study. Overall, this research represents an important step towards sustainable cultivation of sweet basil.

2. Materials and methods

2.1. Research location, plant material and cultivation practices

The study was carried out near Termini Imerese (Palermo province), at an experimental field (GPS coordinates: 37° 59′ 11′' N, 13° 40′ 59′' E, altitude 12 m) of the Department of Agricultural, Food and Forest Science (University of Palermo, Italy). The experiment was accomplished inside a greenhouse (25 × 50 m) with galvanized iron structure, covered with polyethylene film (0.05 mm). The experimental soil was tilled during the winter period, then it was mulched with green polyethylene (0.05 mm) and provided with a drip irrigation system. The soil was basically sandy clay loam at pH 6.4 and EC 0.7 dS m^{-1}, composed by 0.8% of total nitrogen and 2.2% of organic matter. On 8 April 2022, sweet basil "Gervaso F1" (Fenix seed, Belpasso, Catania, Italy) plug plants at the stage of 4–6 true leaves were transplanted at a density of 8 plant m^{-2}. During the experiment, all agronomic practices recommended for sweet basil cultivation were followed (Tesi, 2010). Briefly, during the growing cycle plants received 34 kg P_2O_5 ha^{-1}, 144 kg K_2O ha^{-1}, 70 kg CaO ha^{-1} and 24 kg MgO ha^{-1}. The fertilization was calculated on the basis of the mineral elements in soil. Plants were harvested by cutting above the third stem node. Copper and sulfur were applied to combat cryptogamic diseases, whereas, *Bacillus thuringiensis* was used to combat lepidopteran larvae.

2.2. Determination of soil hydrological properties

The soil water content corresponding to field capacity (FC) was determined by the pressure plate extractors (Dane and Hopmans, 2002) for pressure head value corresponding to −1 m. For each treatment, two replicated samples were prepared by compacting the 2 mm fraction into 5 × 1 cm samplers at the bulk density values measured on the undisturbed soil. Equilibrium with the applied pressure head was assumed when the samples stopped draining for at least 24 hrs. The volumetric water content related to the equilibrium condition was determined by the thermogravimetric method after oven-drying the samples at 105 °C for 24 hrs. All the measurements were performed under controlled conditions, setting the temperature at 22 ± 1 °C.

2.3. Treatments and experimental design

Plants were cultivated under different N rates (NR) and distinct drought stress levels (DS). For the N treatments, four different doses (kg ha^{-1}) were disposed: 0 (not nitrogen fertilized), 50, 100 and 150, taking into consideration the soil N content. The N was administered via fertigation using ammonium nitrate (Nitrosol 34, Mugavero fertilizers®) containing 34% of total nitrogen (17% of nitric and 17% of ammoniacal). The fertigation treatments were supplied starting 20 days after transplanting, and every 15 days after each harvest. Regarding the DS, three different levels were disposed: 100%, 80% or 60% of the FC. The soil water content was recorded hourly via a data logger (ZL6 data logger, Meter®, Germany) equipped with Teros 10 soil moisture sensor (placed 6 cm deep), and data were read daily. When necessary, water was added to maintain the soil moisture level previously set (100%, 80% or 60% FC). The drought stress treatment started 20 days after transplant (plant establishment phase). The two biostimulant treatments (B), seaweed extract (Kelpstar®, Mugavero fertilizers) and plant protein hydrolysed (Tyson®, Mugavero fertilizers) were administered via foliar spray at a dosage of 3 mL L^{-1} (recommended dose). The composition of both biostimulants is presented in supplementary Table S1 and Table S2. Treatments started 10 days after transplant and were accomplished every ten days (plants received three biostimulant applications for each harvest). Each biostimulant application was accomplished applying 0.5 L m^{-2} of solution. Control plants received only water.

The experimental factors were arranged in split-split plot experimental design. The main plots contained the three DS (100%, 80% and 60% FC), the plots comprised the four different NR (0, 50, 100 and 150 kg ha^{-1}), whereas the biostimulant treatments [control (ctrl), seaweed extract (SWE) and plant protein hydrolysates (PH)] were disposed in the sub-plots. Treatments generated 36 combinations (3 DS × 4 NR × 3 B) and were replicated three times, each combination enclosed 8 plants, for a total of 864 plants (8 plants × 36 treatments × 3 replicates).

2.4. Yield, dry matter, total leaf area, stomatal conductance, and total chlorophyll determinations

Plants were weighed after harvest to obtain yield values. A total of three harvests were always made before plant flowering stage (on 04 May 2022, 06 June 2022 and 06 July 2022, respectively). Values were presented as kg m^{-2}. Dry matter was determined by drying basil samples in a thermo-ventilated oven at 105 °C. Dry matter data were expressed as percentage. Total leaf area was determined on three plants per replicate, leaf pictures were obtained using a digital scanner set at 300 dpi. The scan image was then analysed with ImageJ software. Data were showed as cm^2. Stomatal conductance was measured using a porometer (AP4, Delta-T devices, Cambridge United Kingdom) on 5 leaves belonging to three plants, chosen from each replicate, and the values expressed as mmol m^2 s^{-1}. Stomatal conductance was measured on undamaged mature leaves (from 5th-6th node) on the fifth day after the biostimulant application, at 12:00 AM. Total chlorophyll content was determined on five undamaged and completely expanded leaf sample (from 5th-6th node), chosen from each replicate, using the method of Costache et al. (2012). Briefly, 1 g of fresh sample was mixed with methanol (90%), then the values were obtained via a spectrophotometer using a wavelength of 653 and 666 nm. Values were presented as mg g^{-1} fresh weight (FW).

2.5. Water productivity (WP), specific leaf area (SLA) and nitrogen use efficiency (NUE) calculations

Water productivity was calculated as follows: WP = yield (t) / water supplied (m^3). Specific leaf area was assessed using the formula: SLA = leaf area (cm^2) / leaf dry weight (g). Nitrogen use efficiency was determined as follows: NUE = yield (t) / N application rate (kg).

2.6. Nitrates, phenolics and ascorbic acid measurements

For nitrates determination, the method of Formisano et al. (2021) was used. Briefly, 0.25 g of grounded dried samples were extracted in ultrapure water and were analysed by ion chromatography. For each treatment, three samples per replicate were examined and the results were presented as mg kg^{-1} FW.

Phenolic content (mg GAE 100 g^{-1} DW) was assessed by the Folin-Ciocâlteu methodology (Meda et al., 2005). In brief, leaves were mixed with water, Na_2CO_3 and Folin-Ciocâlteau reagent, after that the mix was left at 24 °C for 30 min and the absorbance was evaluated at 750 nm.

Ascorbic acid (mg 100 g^{-1} FW) was determined in leaf samples using a Reflectometer Merck RQflex10 Reflectoquant® and Reflectoquant Ascorbic Acid Test Strips.

2.7. Volatiles profile

The volatiles profile was determined using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS), as reported by De Pasquale et al. (2007). Briefly, two grams of basil parts were placed into the vials, set at temperatures of 70 °C for 20 min. After that, an SPME needle was inserted through the septum and headspace volatiles were absorbed on the exposed fiber for 10 min. The loaded fiber was then desorbed in the gas chromatograph inlet port for 2 min. Measurements were replicated three times for each combination of treated basil samples. Moreover, three runs as control were performed to see what comes off the cleaned fibers and also what comes off to the fibers when they are exposed to clean and hot vials.

GC–MS analyses were performed using a Hewlett-Packard 5890 GC system interfaced with an HP 5973 quadrupole mass spectrometer. Each compound from basil samples were identified with the NIST 11 mass spectral database and were confirmed by comparing mass spectra and retention times with those of standards (all 99% purity —Fluka, Sigma-Aldrich Chemie, Switzerland). A standard mixture of identified molecules in hexane was used as an external standard to verify retention times for each compound.

2.8. Statistical analysis and heat map

Prior to statistical analysis, the data were tested for the basic assumption of ANOVA. The normal distribution was verified via Shapiro-Wilk test, whereas the homoscedasticity using Levene test. Collected data were analysed by ANOVA following the split-split- plot experimental design, using the software SPSS 28. Consequently, a three-way ANOVA was executed setting DS, NR and B as fix factors. Mean separation was performed via the Tukey HSD test at $p \leq 0.05$. Moreover, to summarize the outcome on volatile profile, a heatmap was realized using ClustVis online software (https://biit.cs.ut.ee/clustvis/).

3. Results

3.1. Yield and plant traits

Basil yield was affected by the main factors and by their interaction (Table S3) as it progressively varied passing from DS100 to DS60 (from 6.02 to 4.75 kg m^{-2}, −21%) and from NR0 (5.09 kg m^{-2}) up to the NR100-NR150 range (5.62 kg m^{-2} on average, +10%). Both biostimulants increased sweet basil yield (Fig. 1). When the DS100 was considered, the highest yield was achieved through the application of SWE at NR50, NR100 and NR150 and PH at NR0 and NR150 (6.43 kg m^{-2}, on average). This level did not differ when PH was applied to the DS60-NR150 plants (6.27 kg m^{-2}) (Fig. 1).

The leaf dry matter was promoted within the DS80-DS60 range (Table 1), peaking when these levels received no N supply, i.e. in the case of DS80-NR0 and DS60-NR0 plants (14.1%, on average) (Table 2).

Regarding total leaf area, the increasing DS and NR levels had opposite effects (Table 1). Indeed, total leaf area was reduced within the DS100-DS60 range (from 2521 to 1859 cm^2, −26%), but increased passing from NR0-NR50 (1962 cm^2, on average) to NR100-NR150 (2380 cm^2, on average). As biostimulant application, the highest total leaf area was achieved when DS100-NR100 and DS100-NR150 were treated with SWE and PH, respectively (3804 cm^2, on average) (Table 3).

3.2. Physiological variables

The stomatal conductance was progressively reduced by the increasing DS (up to −34%, comparing DS100 and DS60), and N supply (mainly passing from NR50 to NR100, −21%) (Table 1), with a significant interaction among these main factors (Table S3). This was evident at DS60 where, by increasing the N fertilization, the stomatal conductance passed from 611.1 (N0) to 521.7 mmol m^{-2} s^{-1} (averaged over N50-N150, −14.6%) (Fig. 2).

On the other hand, both biostimulants promoted this variable, mostly in the case of the SWE application (Table 1). Indeed, taking unsprayed controls as a reference, the SWE application was the main booster of stomatal conductance at DS100 (where it passed from 754.8 to 918.8 mmol m^{-2} s^{-1}, +21.7%), DS80 (from 601.1 to 806.9 mmol m^{-2} s^{-1}, +34.2%) and DS60 (from 496.9 to 604.8 mmol m^{-2} s^{-1}, +21.7%) (Fig. 2). A different response to DS was recorded for WP, as it was promoted by 86% passing from DS100 to DS60 (from 0.475 to 0.885 t m^{-3}) (Table 1). Moreover, when the N rate and biostimulant were concerned, under DS60 WP was maximized mainly in the combination N150-PH (1095 t m^{-3}) followed by NR150-SWE (1024 t m^{-3}), N0-PH (0.985 t m^{-3}) and NR100-PH (0.969 t m^{-3}) (Fig. 3).

Within the DS100-DS60 range, opposite effects were recorded for SLA (which decreased by 28.71 cm^2 g^{-1}) and total chlorophylls (which increased by 25 μg g^{-1} FW), whereas both variables were promoted passing from NR0 to NR150 (by 2.85 cm^2 g^{-1} and 144 μg g^{-1} FW, respectively) (Table 1). On the other hand, both biostimulants interactively promoted these variables, especially for the combinations N100-SWE (141.4 cm^2 g^{-1}) and NR150-PH (119.7 cm^2 g^{-1}) (Table 3). The NR150-PH combination gave the highest total chlorophylls in all the imposed DS levels, namely DS100, DS80 and DS60 (2054, 2063 and 2083 mg kg^{-1} FW, respectively) (Table 3).

Concerning NUE, reduced plants performances were recorded between the extreme levels of DS (−10%) and N supply (−71%) (Table 1). The PH and SWE applications fostered NUE mostly when matched to the NR50 level, and at DS100 (1.27 and 1.19 kg t^{-1}, respectively), DS80 (1.29 kg t^{-1}, on average) and DS60 (1.13 kg t^{-1}, on average) (Fig. 4a).

3.3. Main compositional variables

Among the main compositional variables, the leaf nitrate content was promoted by the increasing DS and N supply, as it varied from 2129.9 (DS100) to 2265.3 mg kg^{-1} FW (DS60, +6%) and from 1898.7 (NR0) to 2641.3 mg kg^{-1} FW (NR150, +39%) (Table 4). Regarding the biostimulants, SWE and PH had contrasting effects on nitrate content (Table 4), with the ANOVA revealing a significant 'DS × NR × B' interaction (Table S3).

This was evident when considering mostly the NR150 level, for which, compared to control, the nitrate content at DS100, DS80 and DS60 was progressively promoted by SWE (+75.63, +110.60 and +162.90 mg kg^{-1} FW, respectively) and reduced by PH (−302.47, −515.17 and −480.53 mg kg^{-1} FW, respectively) (Fig. 4b). On the other hand, leaf phenolics and ascorbic acid contents peaked at DS60 (65.9 mg

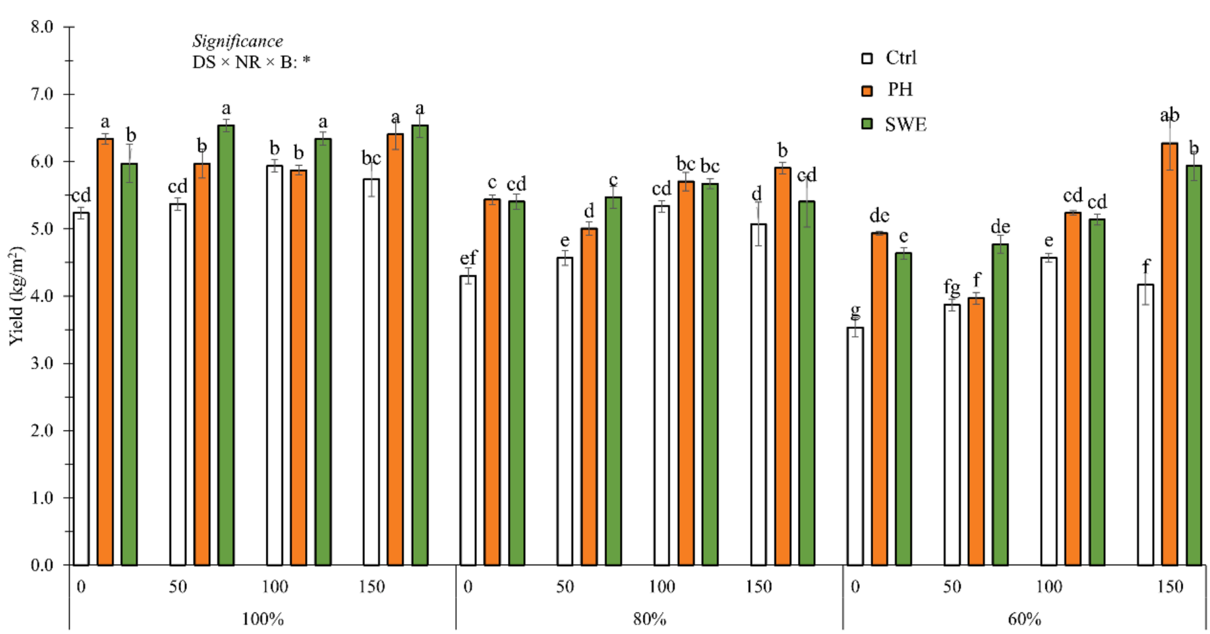

Fig. 1. Simultaneous effect of drought stress levels (DS), nitrogen rates (NR) and biostimulants (B) on sweet basil plants yield. 100%, 80% and 60% indicate the percentage of soil water content in relation to the field capacity. 0, 50, 100 and 150 are the nitrogen rates, expressed as kg ha^{-1}. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey HSD test. Bars indicate mean values ± standard error. *: significant at $p \leq 0.05$.

Table 1
Main effects of drought stress levels (DS), nitrogen rate (NR) and biostimulant (B) on sweet basil plants yield, dry matter percentage, total leaf area, stomatal conductance, water productivity (WP) specific leaf area (SLA), total chlorophyll and nitrogen use efficiency (NUE).

Treatments	Yield (kg m^{-2})	Dry matter (%)	Total leaf area (cm^2)	Stomatal conductance (mmol m^2 s^{-1})	WP (t m^{-3})	SLA (cm^2 g^{-1})	Total Chlorophylls (μg g^{-1} FW)	NUE (t Kg^{-1})
DS (% FC)								
100	6.02a	11.29b	2521.4a	824.8a	0.475c	88.02a	1915b	0.723a
80	5.27b	13.08a	2133.0b	707.4b	0.648b	69.41b	1889c	0.694b
60	4.75c	13.23a	1859.1c	544.1c	0.885a	59.31c	1940a	0.650c
NR (kg ha^{-1})								
0	5.09b	12.61a	1994.2b	716.4a	0.672a	69.47b	1875c	
50	5.06b	12.75a	1930.3b	678.6b	0.632b	67.92b	1833d	1.174a
100	5.53a	12.15a	2437.5a	687.3b	0.683a	79.27a	1932b	0.549b
150	5.71a	12.63a	2322.7a	686.1b	0.689a	72.32ab	2019a	0.343c
B								
Ctrl	4.81b	12.47a	2140.6a	617.6c	0.607b	71.09a	1873c	0.630b
SWE	5.65a	12.35a	2094.2a	776.9a	0.699a	72.43a	1916b	0.717a
PH	5.58a	12.79a	2278.7a	681.9b	0.702a	73.22a	1955a	0.719a

FC: field capacity. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test.

Table 2
Simultaneous effect of drought stress levels (DS) and nitrogen rates (NR) on sweet basil plants dry matter percentage and stomatal conductance.

DS (% FC)	NR (kg ha^{-1})	Dry matter (%)	Stomatal conductance (mmol m^2 s^{-1})
100	0	9.7d	830.9a
	50	11.7c	820.2a
	100	12.1c	830.8a
	150	11.7c	817.4a
80	0	14.0a	707.3b
	50	13.1b	701.9b
	100	12.6b	716.8b
	150	12.6bc	703.8b
60	0	14.2a	611.1c
	50	13.5ab	513.8d
	100	11.7c	514.3d
	150	13.6ab	537.1d

Means with different letters are significantly dissimilar according to Tukey's HSD test.

GA 100 g^{-1} DW) and DS80 (25.81 mg 100 g^{-1} FW), respectively, whereas these variables were promoted by both biostimulants, in an N-dependent way (Fig. 5).

In this sense, the highest phenolics content was achieved when both NR0 and NR50 were treated with SWE (67.23 mg GA 100 g^{-1} FW) and then with PH (65.74 mg GA 100 g^{-1} FW) (Fig. 5a), whereas the former biostimulant maximized the ascorbic acid content mainly within the NR0-NR50 interval (29.62 mg 100 g^{-1} FW, on average), then at NR100 and NR150 (28.21 and 26.43 mg 100 g^{-1} FW, respectively) (Fig. 5b).

3.3. Volatile composition

Overall, the volatile composition of the leaf extracts was significantly affected by the tested factors, with the 7 organic constituents proving different responses to the imposed growth conditions (Table 5).

Indeed, when DS was concerned, the content of 4 VOCs, namely trans-2-hexenal, 1-octen-3-ol, β-cis-ocimene and α-bergamotene was the highest at DS100 (1.34, 2.58, 3.61 and 15.94%, respectively), whereas eugenol was maximized at DS60 (37.30%) (Table 5). Differently, eucalyptol and linalool were promoted at DS80 (16.15 and 36.88%, respectively) (Table 5). The N supply boosted the accumulations of 1-octen-3-ol and α-bergamotene up to N150 (2.83 and 14.97%, respectively) and those of β-cis-ocimene and eugenol up to N100 (3.61 and 31.98%, respectively), whereas trans-2-hexanal, eucalyptol and linalool peaked under the N-unfertilized conditions (1.30, 15.55 and 37.52%, respectively) (Table 5). Regarding the biostimulants, the content of 3

Table 3
Simultaneous effect of drought stress levels (DS), nitrogen rates (NR), and biostimulant (B) on sweet basil plants total leaf area, specific leaf area (SLA) and total chlorophyll.

DS (% FC)	NR (kg ha^{-1})	B	Total leaf area (cm^2)	SLA (cm^2 g^{-1})	Total Chlorophylls (mg g^{-1} FW)
100	0	Ctrl	1049e	41.1g	2.00cd
		PH	2779c	94.3cd	2.00cd
		SWE	1990de	82.8de	2.00cd
	50	Ctrl	2205d	83.7de	1.69m
		PH	2408cd	79.1de	1.87hi
		SWE	2005de	64.5f	1.82i
	100	Ctrl	3505b	116.3bc	1.78jk
		PH	2106de	73.1ef	1.97e
		SWE	4006a	141.4a	1.92f
	150	Ctrl	3174bc	105.4c	1.86h
		PH	3603ab	119.7b	2.05ab
		SWE	1428e	54.8fg	2.01cd
80	0	Ctrl	1809de	59.5fg	1.74l
		PH	2680c	99.9cd	1.84i
		SWE	2477cd	90.7d	1.79jk
	50	Ctrl	1839de	67.4ef	1.78jk
		PH	1922de	67.6ef	1.87gh
		SWE	1542e	65.5ef	1.83i
	100	Ctrl	2490cd	59.9fg	1.87gh
		PH	2026de	54.0fg	1.97de
		SWE	2067de	69.9ef	1.93f
	150	Ctrl	2710c	77.6e	1.96e
		PH	2183de	57.6fg	2.06b
		SWE	1852de	63.3fg	2.02c
60	0	Ctrl	1514e	55.6fg	1.84hi
		PH	1838de	51.9g	1.85hi
		SWE	1812de	49.4g	1.81j
	50	Ctrl	1732e	65.3f	1.89gh
		PH	1930de	57.4fg	1.89g
		SWE	1790de	60.8fg	1.85hi
	100	Ctrl	1681e	64.4f	1.99d
		PH	2036de	66.0ef	1.99d
		SWE	2022de	68.5ef	1.95ef
	150	Ctrl	1981de	56.9fg	2.08a
		PH	1834de	57.9fg	2.08a
		SWE	2140de	57.5fg	2.04bc

FC: field capacity. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test.

VOCs, namely trans-2-hexanal, eucalyptol and α-bergamotene, were higher in the untreated controls (1.35, 15.51 and 15.36%, respectively), whereas the SWE application fostered the accumulation 1-octen-3-ol and linalool (up to 2.61 and 35.92%, respectively); differently, the highest content of β-cis-ocimene and eugenol were noticed in PH-treated

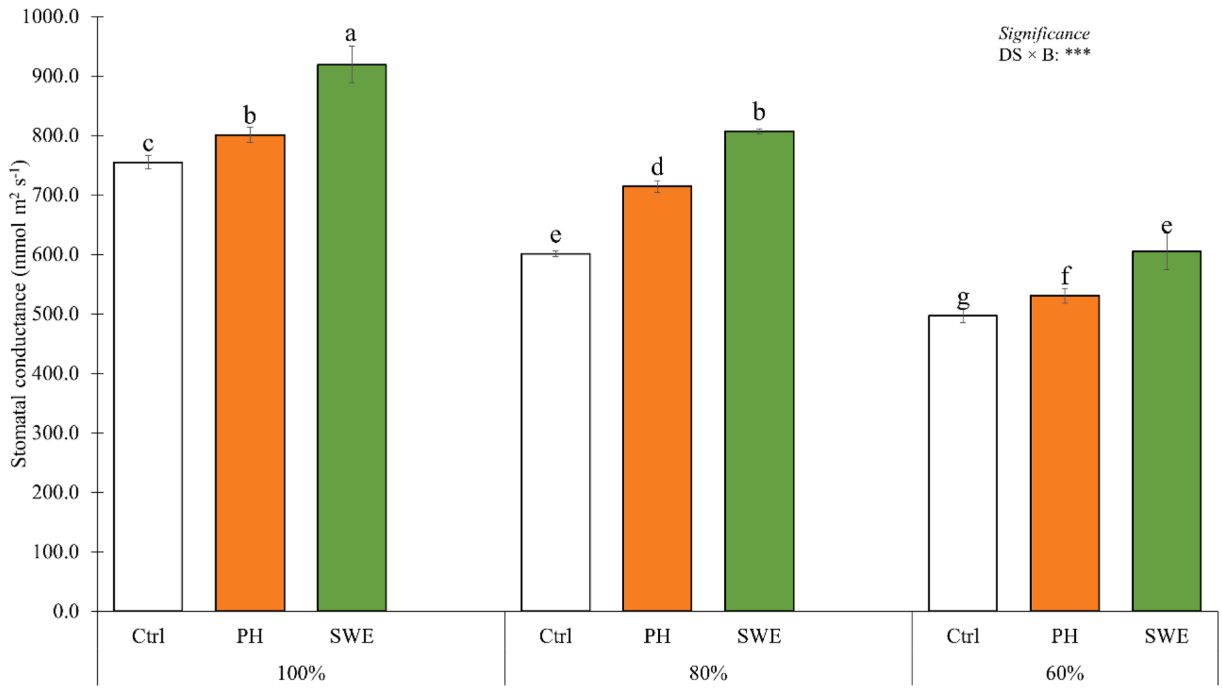

Fig. 2. Simultaneous effect of drought stress levels (DS) and biostimulants (B) on sweet basil plants stomatal conductance. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test. Bars indicate mean values ± standard error. ***: significant at $p \leq 0.001$.

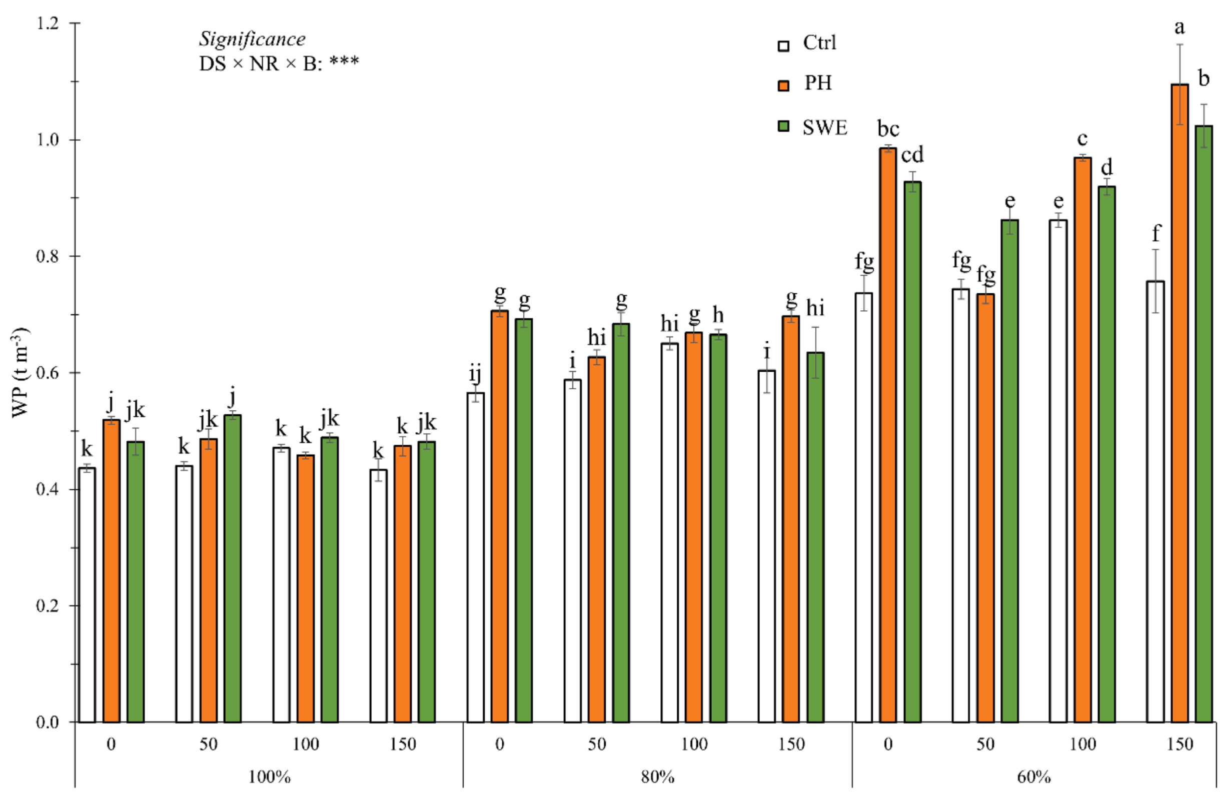

Fig. 3. Simultaneous effect of drought stress levels (DS), nitrogen rates (NR) and biostimulants (B) on sweet basil plants water productivity (WP). 100%, 80% and 60% indicate the percentage of soil water content in relation to the field capacity. 0, 50, 100 and 150 are the nitrogen rates, expressed as kg ha^{-1}. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test. Bars indicate mean values ± standard error. ***: significant at $p \leq 0.001$.

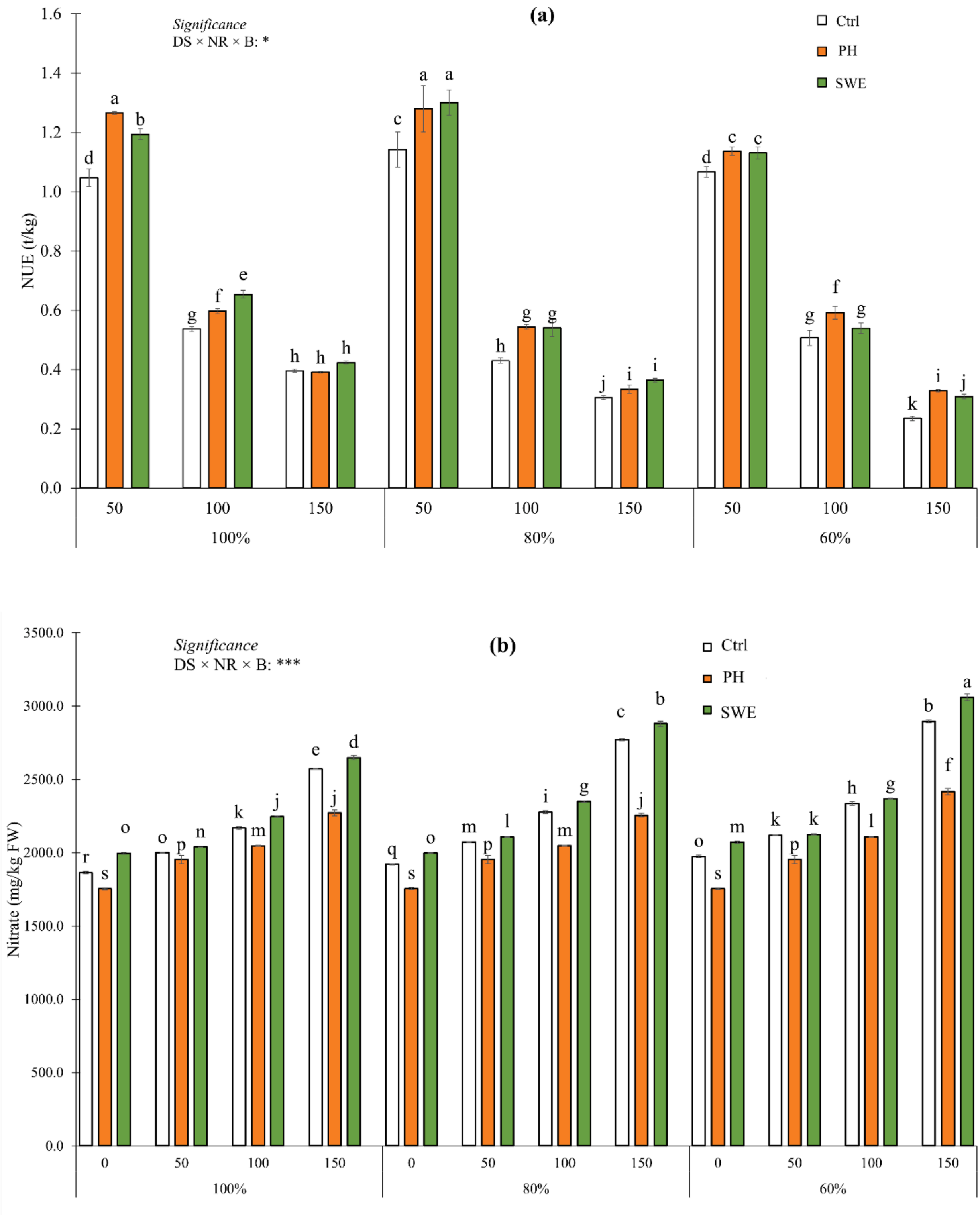

Fig. 4. Simultaneous effect of drought stress levels (DS), nitrogen rates (NR) and biostimulants (B) on sweet basil plants nitrogen use efficiency (a) and nitrate content (b). 100%, 80% and 60% indicate the percentage of soil water content in relation to the field capacity. 0, 50, 100 and 150 are the nitrogen rates, expressed as kg ha^{-1}. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test. Bars indicate mean values ± standard error. *: significant at $p \leq 0.05$.

plants (3.55 and 33.52%, respectively) (Table 5).

A data heat-map analysis of all assessed volatiles was performed to realize a graphical appraisal of the influences determined by the experimental factors on basil extracts (Fig. 6).

The heat-map output consisted of two dendrograms, Dendrogram 1 sited on the top containing all the 36 experimental combinations (3 DS × 4 NR × 3 B), and Dendrogram 2, located on the left side, comprising all volatile contents influencing this distribution. Dendrogram 1 presented two main clusters: the first one, on the right, grouped 20 combinations, mostly including DS60 (10 out of 12 combinations), N50 (8

Table 4
Main effects of drought stress levels (DS), nitrogen rate (NR) and biostimulant (B) on sweet basil plants nitrate, phenolics, and ascorbic acid content.

Treatments	Nitrate (mg kg^{-1} FW)	Phenolics (mg gallic acid 100 g^{-1} DW)	Ascorbic acid (mg 100 g^{-1} FW)
DS (%FC)			
100	2129.9c	58.1c	24.89b
80	2199.1b	61.4b	25.81a
60	2265.3a	65.9a	24.34c
NR (kg ha^{-1})			
0	1898.7d	64.1a	25.83a
50	2035.9c	63.9a	25.89a
100	2216.5b	61.2b	24.69b
150	2641.3a	58.0c	23.65c
B			
Ctr	2248.0b	57.1c	22.20c
SWE	2323.9a	65.2a	28.47a
PH	2022.4c	63.2b	24.37b

FC: field capacity. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test.

out of 9) and PH (8 out of 12). This cluster showed two sub-groups, the first on the right, grouping most of the DS60 (8), N50 (5) and PH (5) combinations of cluster 1, which were aggregated mainly on the basis of their higher eugenol and lower β-cis-ocimene contents. The second sub-group, on the left, included mostly the DS100 (6) and control (5) combinations of cluster 1, characterized by their higher content of α-bergamotene (Fig. 6). The second main cluster, which included the remaining 16 combinations, was divided into two sub-groups too. The first one, on the right, grouped most of the DS100 (4), N100 (4) and PH (4) combinations of cluster 2. These were aggregated on the basis of their tendentially higher contents of β-cis-ocimene, trans-2-hexanal and 1-octen-3-ol, with these last two volatiles being highly accumulated in the combinations DS100-N0-Control and DS100-N150-SWE, respectively (Fig. 6). The second sub-group, on the left, was characterized by a higher presence of N0 combinations (4), along with for the absence of N100 and PH. The samples of this sub-group showed the least content of eugenol (mostly in the combinations DS100-N0-SWE and DS80-N0-Control) along with the highest contents of eucalyptol (primarily in DS80-N0-Control and DS60-N0-Control) and linalool (in DS80-N0-Control and DS100-N0-SWE) (Fig. 6).

4. Discussion

Nowadays, the horticultural sector is facing the dual challenge of addressing food availability for an increasing global population while increasing resource use efficiency and reducing the ecological impact of vegetable production (Colla et al., 2017). A pioneering agronomic technique to address these challenges is the use of biostimulants, by reducing the use of synthetic fertilisers (Ottaiano et al., 2021) and improving water productivity (Sabatino et al., 2023). Results from this study revealed a significant effect of drought stress intensity (DS) × nitrogen rate (NR) × biostimulant (B) application on yield. The improved yield of the biostimulated plants seems to be related to the higher total chlorophylls (Miri, 2009). Tyson® protein hydrolysate may boost plant physiological activities as it contains tryptophan, a precursor of indole-3-acetic acid which is responsible for the growth and development of shoots and roots. Similar results were reported by Di Mola et al. (2019) who, by investigating the influence of vegetal- and SWE-based biostimulants on yield and quality of baby lettuce grown under various N rates, found that - regarding yield - biostimulants had a buffer effect on N-deficiency. These findings could be related to the SWE composition characterized by polysaccharides, phenolic compounds, osmolytes and phyohormones (Battacharyya et al., 2015). Furthermore, biostimulated plants showed a higher stomatal conductance than control plants, and while the relationship between phytohormones and stomatal control is still unclear (Figueiredo-Lima et al., 2018), the yield increase could be attributed to the interaction of different phyohormones, which appear to play a role on stomatal conductance (Skelton et al., 2017). Both biostimulants are thus useful tools to mitigate N deficiency in sweet basil plants under well-watered or drought stress conditions.

In this study, basil plants exposed to 80% or 60% FC and fertigated with the lowest N dosage (not-fertilized) had the highest dry matter percentage. This increase in dry matter percentage of drought stressed plants could be attributed to a phenomenon known as "condense effect". Indeed, plants grown under drought stress had lower root hydraulic conductivity for water uptake than those cultivated under optimal water conditions (Aroca and Ruiz-Lozano, 2012). Moreover, as showed by the analysis of variance (ANOVA), nitrogen rate (NR) interacted with drought stress (DS) to modulate plant responses. When plants were irrigated with 100% FC, NR of 50, 100 or 150 significantly boosted the dry matter percentage compared with the control. However, when plants were subjected to drought stress, an opposite trend was recorded. It is well known that N supply significantly boosts dry matter percentage (Consentino et al., 2022), thus we may hypothesize that in our experiment the low water availability caused a low plant N uptake and, consequently, a lower effect of it on dry matter percentage.

The ANOVA results for total leaf area indicated a significant interaction among the experimental factors (DS, NR and B). Post-hoc analysis highlighted that plants from well-watered plots (100% FC), treated with 100 kg N ha^{-1} and sprayed with SWE had the highest total leaf area. Our data also pointed out that total leaf area decreased as DS increased. As stated by Giordano et al. (2021), the reduction in total leaf area is a plant defense mechanism that results in decreased absorption of solar radiation and, concomitantly, reduced transpiration. Furthermore, plants fertigated with 100 or 150 kg N ha^{-1} exhibited the highest total leaf area. These outcomes are consistent with those of Di Mola et al. (2019), who found that the leaf area index (LAI) of baby lettuce increased with increasing N rate.

Our data on the DS × NR interaction, revealed that the highest stomatal conductance values were recorded in plants irrigated with 100% FC, followed by those irrigated with 80% FC. These findings could be ascribed to a reduction in transpiration via stomata closure when plants are exposed to low water potential in the root zone, which represents a plant defense mechanism (Barbieri et al., 2012). However, the inconsistent response of plants grown under 60% FC to the various nitrogen rates was presumably due to an increased soil electrical conductivity, above basil's tolerance threshold values. Our stomatal conductance dataset also pointed out a significant DS × B interaction. Overall, plants supplied with SWE and PH showed higher stomatal conductance than control plants, demonstrating the biostimulant efficiency in drought stress mitigation. These results are in line with those reported by Van Oosten et al. (2017) who, by investigating the effects of biostimulants on the alleviation of plant abiotic stresses, showed that both SWE and PH can enhance stomatal conductance in drought stressed plants.

ANOVA analysis for WP showed a significant DS × NR × B interaction. WP values increased linearly with the increase of DS; concomitantly, biostimulant application enhanced WP. However, the highest WP values were recorded in plants exposed to severe drought stress (60% FC), supplied with 150 kg N ha^{-1} and sprayed with PH, followed by those from the same DS × NR combination but sprayed with SWE. As already showed for leaf area and stomatal conductance, this response was probably due to an adaptive plant mechanism to reduce the water loss during drought stress conditions (Craufurd et al., 1999). Regarding the SWE, our findings tie well with those of Santaniello et al. (2017), who reported that SWE application significantly ameliorate WUE values in drought stressed *Arabdopsis* plants. Moreover, the results of this study are in line with Choi et al. (2022), who found that PH application significantly increases WUE values in lettuce and tomato compared to the untreated controls.

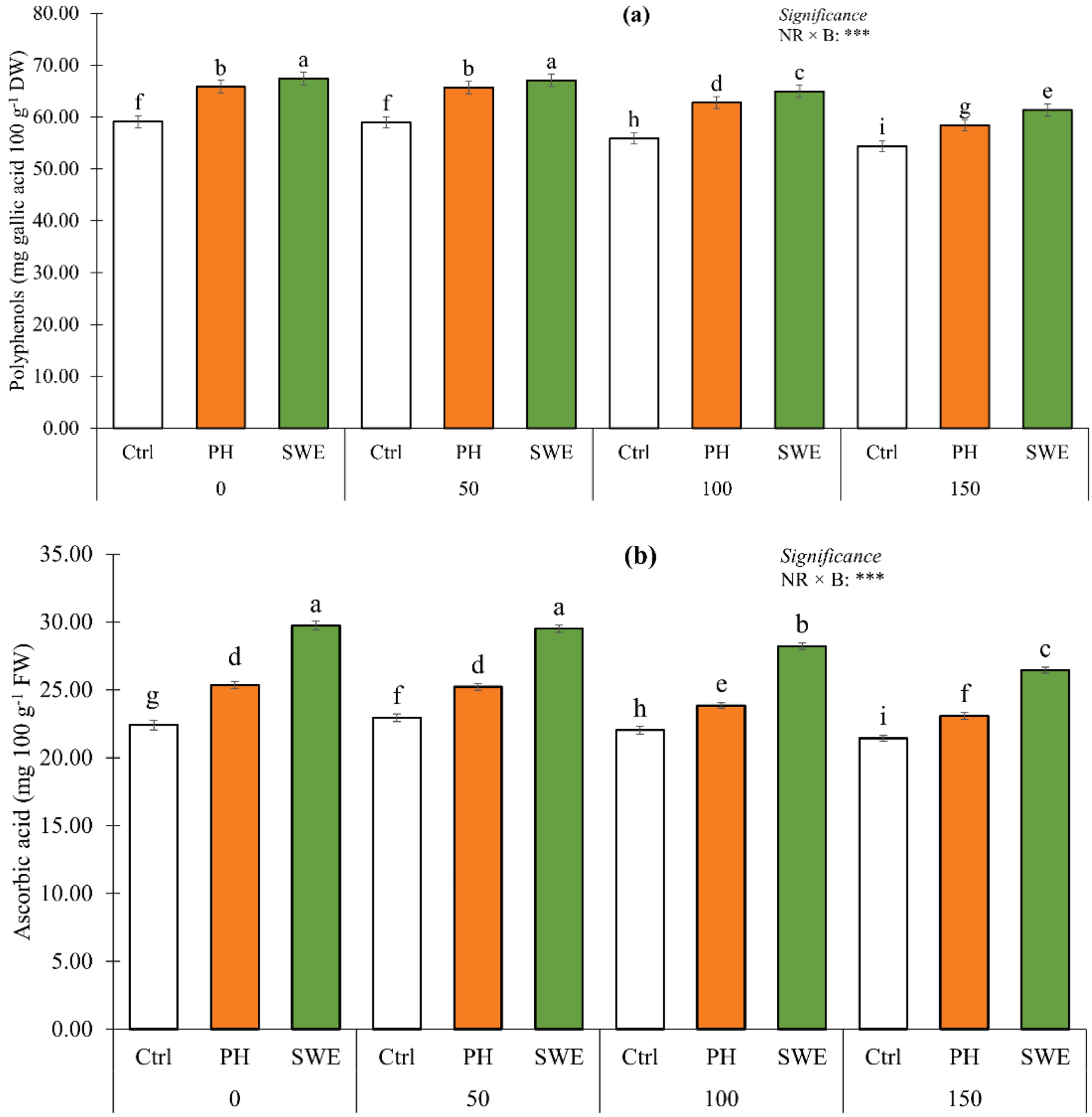

Fig. 5. Simultaneous effect of nitrogen rates (NR) and biostimulants (B) on sweet basil plants polyphenols (a) and ascorbic acid (b) content. 0, 50, 100 and 150 are the nitrogen rates, expressed as kg ha^{-1}. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test. Bars indicate mean values ± standard error. ***: significant at $p \leq 0.001$.

As for SLA, ANOVA displayed a significant effect of the interaction among the three experimental factors (DS, NR and B). Particularly, data on SLA supported the trend established for total leaf area, remarking an inverse relation between leaf area and weight per cm^2. Our data revealed that SLA values decreased as DS increased. This result was linked to an augmented dry matter percentage of plants exposed to moderate (80% FC) or severe (60% FC) drought stress. Concomitantly, plants supplied to the highest N rates (100 or 150 kg ha^{-1}) had the highest SLA values, while, those exposed to 0 or 50 kg N ha^{-1} underlined the lowest SLA. There findings are totally in line with those of Di Mola et al. (2019), who found that specific leaf weight (SLW) - expressed as leaf dry weight (mg) per unit area (cm^2) - decreases as N rate increases. When averaged over DS and NR, ANOVA did not show a significant effect of the biostimulants. These results partially overlap with those of Di Mola et al. (2019), who found that two out of three biostimulants do not significantly influence SLW. The various biostimulants effects reported in literature, testified a genotype-specific response to the different plant-based biostimulants. Consequently, further crop-specific studies are required to investigate the biostimulant efficiency on vegetable crops.

Concerning total chlorophyll content, statistical analysis revealed a significant effect of the three factors (DS, NR and B). Plants subjected to a moderate drought stress (80% FC) had low total chlorophyll compared to well-watered plants (100% FC) likely due to a plant defense mechanism involving a degradation of absorbing pigments, such as chlorophyll, which in turn decreases the energy absorption in plant photosynthetic system (Herbringer et al. 2002). However, plants exposed to severe drought stress (60% FC) had increased total chlorophyll content compared to control plots. In this respect, since total chlorophyll was expressed on fresh weigh basis and considering that plants grown under severe drought stress conditions showed a lower water content, we may assume that a "condense effect" occurred,

Table 5
Main effects of drought stress levels (DS), nitrogen rate (NR) and biostimulant (B) on sweet basil plants volatile profile.

Treatments	Trans-2-hexanal (%)	1-Octen-3-ol (%)	Eucalyptol (%)	b-cis-Ocimene (%)	Linalool (%)	Eugenol (%)	a-Bergamotene (%)
DS (%FC)							
100	1.34a	2.58a	14.01c	3.61a	34.82b	27.70b	15.94a
80	1.04c	2.26b	16.15a	3.58a	36.88a	24.94c	15.15b
60	1.27b	2.24b	14.79b	2.74b	31.08c	37.30a	10.58c
NR (Kg ha^{-1})							
0	1.30a	2.04c	15.55a	3.17c	37.52a	27.51d	12.90d
50	1.09c	2.08c	14.26c	3.09d	34.09b	30.73b	14.66b
100	1.25b	2.47b	15.14b	3.61a	32.51c	31.98a	13.03c
150	1.22b	2.83a	14.99b	3.35b	32.92d	29.70c	14.97a
B							
Ctr	1.35a	2.34b	15.51a	3.14c	34.83b	27.48c	15.36a
SWE	1.20b	2.61a	14.99b	3.23b	35.92a	28.93b	13.12b
PH	1.11c	2.12c	14.46c	3.55a	32.04c	33.52a	13.20b

FC: field capacity. Ctrl: control; PH; plant protein hydrolysates; SWE: seaweed extract. Means with different letters are significantly dissimilar according to Tukey's HSD test.

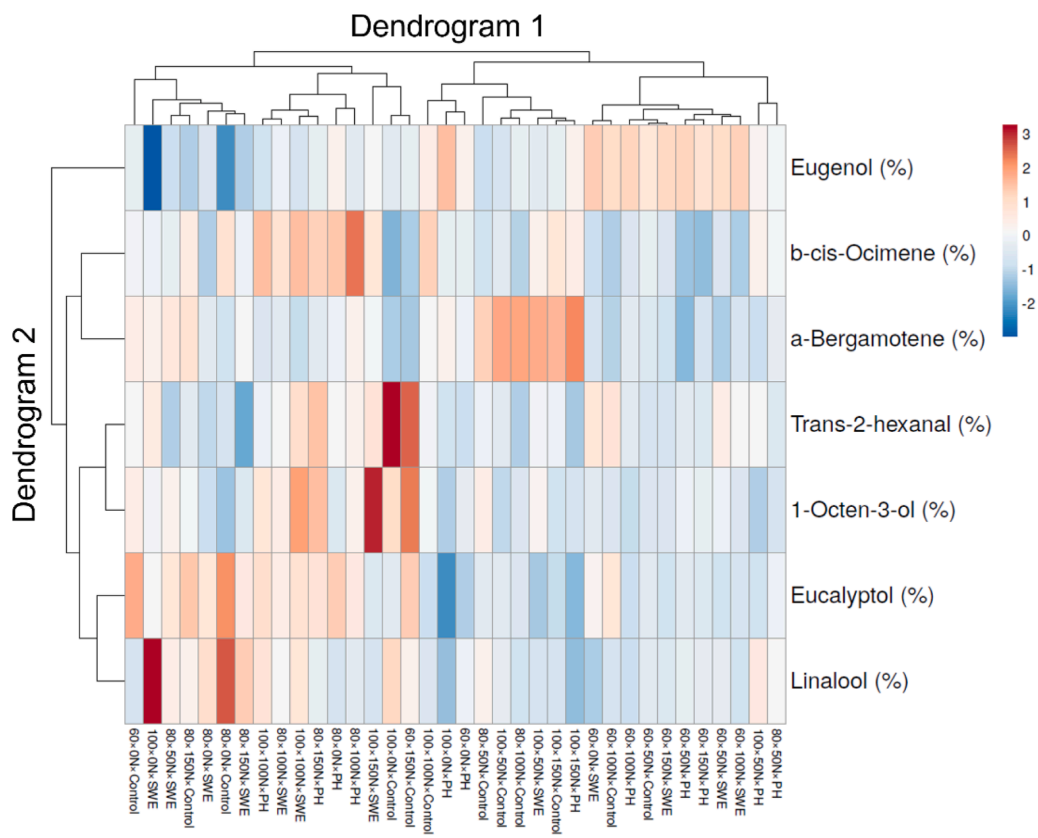

Fig. 6. Heatmap analysis showing the volatile profile of sweet basil plants subjected to drought stress levels (100%, 80% or 60% of the FC), various nitrogen rates (0, 50, 100 or 150 kg/ha) and biostimulant (seaweed extract and plant protein hydrolysates). Heatmap was realized via the online program ClustVis, https://biit.cs.ut.ee/clustvis/ (accessed on 01/12/2022). SWE: seaweed extracts; PH: plant protein hydrolisates.

resulting in an incremented total chlorophyll content. When averaged over drought stress and biostimulants, high N rates positively affected total chlorophyll content. These results are in line with those obtained by Consentino et al. (2022) on lettuce. Regardless of both DS and NR, biostimulants significantly improved total chlorophyll content in sweet basil leaf tissue compared to the control with PH-treated plants having the highest total chlorophyll content. The boosted photosynthesis observed in PH-treated plants may be related to the action of amino acids on the photosynthetic system (Sharma et al., 1989), contributing in biochemical and physiological functions like carbon fixation and formation of chlorophylls' porphyrin ring (Hermans et al., 2011).

The analysis of variance for NUE revealed a significant effect of the DS × NR × B interaction. Plants fertigated with 50 kg N ha^{-1} had a higher NUE than plants supplied with 100 or 150 kg N ha^{-1}. In addition, sweet basil plants sprayed with the biostimulants showed a higher NUE than control plants, with the exception of well-watered plants fertigated with the highest N rate. However, plants maintained to 100% or 80% FC, supplied with 50 kg N ha^{-1} and sprayed with PH and those exposed to moderate drought (80% FC) and supplied with PH or SWE had the highest NUE. Sestili et al. (2018) reported improved tomato growth with PH supply at both optimal and sub-optimal N rates. Interestingly, the same authors reported that PH at low N levels upregulated gene expression for amino acid transporter and glutamine synthetase, leading to a higher N uptake, resulting in a positive plant growth. In leafy vegetables - such as sweet basil – this is can be translated to an increase in yield and, consequently, an improvement in NUE. Data also showed that

NUE decreased as drought stress increased. These findings coincide with those of Hoang et al. (2019) who found that water constraints decrease NUE. In terms of biostimulants application, our results could be associated to the fact that PH and SWE supply modified root morphology of treated plants, resulting in an improved efficiency of plant mineral uptake and, thus, plant growth (Battacharyya et al., 2015).

Leafy green vegetables, such as sweet basil, are - generally - prone to accumulate nitrate in the edible part, which can be dangerous for human health (Du et al., 2007; Gorenjak and Cencič, 2013). The present study found that, regardless of the N rates and biostimulant application, leaf nitrate content increased as DS increased. These results can be attributed to the reduced activity of the enzyme nitrate reductase, which is involved in the conversion of nitrate to ammonium and is decreased by water stress (da Silva et al., 2011). Consequently, the decreased activity of this enzyme causes an accumulation of nitrate in leaves of sweet basil. When averaged over DS and B, nitrate content increased with increasing N supply. On the other hand, when averaged over DS and NR, SWE increased nitrate content, while the reverse was true for PH. The ability of PH to store less nitrate in sweet basil leaf tissue, might be associated with a molecular mechanism such as the up-regulation of genes involved in N metabolism (nitrate reductase) leading to an increased assimilation of nitrates into amino acids (Tsouvaltzis et al., 2014; Colla et al., 2018).

Data on phenolics showed a significant effect of the NR × B interaction. The highest values were observed in plants treated with SWE and fertigated with a NR0 or NR50. Results displayed that phenolics increases with the intensity of the drought stress. These findings could be explained as a defense mechanism from the oxidative damage caused by the drought stress as stressed plants have an increased secondary metabolism activity (Luna et al., 2015; Šamec et al., 2021). Findings also revealed that, regardless of DS and B, plants fertigated with a N0 and N50 had the highest phenolics concentration, while those supplied with the highest N dose had the lowest ones. This behavior may be attributed to the fact that plants fertigated with a NR100 and NR150 are less stressed than plants fertigated with NR0 or NR50. Our study also pointed out that both biostimulants significantly increased phenolic concentration in sweet basil plants compared with the control. In particular, SWE performed better than PH in enhancing phenolic content. Findings are corroborated by La Bella et al. (2021), who reported that SWE significantly enhances the phenols content of spinach plants compared to the control. This increase could be linked to key enzyme activity (chalcone isomerase) involved in phytochemical homeostasis and changes in plant mineral uptake (Rouphael et al., 2017; Rouphael et al., 2018).

The ascorbic acid content was significantly influenced by the interaction NR × B. The highest values were found in plants treated with SWE and fertilized with a NR0 or NR50, while the lowest ones were determined in non-biostimulated plants supplied with the highest NR. Regardless of NR and B applications, the highest ascorbic acid concentrations were measured in plants grown under 80% FC. As stated by Cruz de Carvalho (2008), ascorbic acid plays an essential role in increasing plant stress tolerance. Indeed, it repairs the damages caused by reactive oxygen species and boosts the plant defense against oxidative stress produced by drought (Taha et al., 2020). Moreover, the results showed that NR above 50 kg ha^{-1} significantly decreased ascorbic acid values. These findings are in line with those of Di Mola et al. (2020) who revealed a significant decrease of ascorbic acid concentration in leafy vegetables when fertigated with nitrogen. In our study, biostimulant application significantly boosted ascorbic acid concentration compared to the control. As already explained for phenolics, the increased production of ascorbic acid can be ascribed to the effects of enzymes involved in phytochemical homeostasis and in the modulation of plant nutritional status.

Hassanpouraghdam et al. (2010) classified the volatile components of sweet basil in two clusters, terpenes and phenylpropenes, which are biosynthesized via two diverse metabolic pathways. Sweet basil 'Gervaso' F_1 hybrid is featured by a higher incidence of linalool, which is an oxygenated monoterpene, considered a responsible molecule for a perceived fruity-flowery flavor (Ortiz et al., 2011). Linalool biosynthesis is mediated by enzymes, such as linalool synthase (LIS) and 1,8-cineol synthase, and considering that their activities are extremely sensitive to environmental conditions, linalool in sweet basil could be affected by the genotype × growing conditions interaction (Pinto et al., 2019). Our data highlighted that linalool increased in plants exposed to moderate drought stress. These results tie well with the findings of Simon et al. (1992) and with those reported by Khalid (2006), who found that drought stress augmented the concentration of linalool in *O. basilicum*. However, our results also showed that linalool decreased in sweet basil plants when exposed to a severe drought stress compared with plants from control plots (100% FC). Thus, for the aforesaid consideration it seems that, when plants are exposed to moderate drought stress the enzymes involved in linalool biosynthesis are elicited. Contrariwise, when plants are exposed to severe drought stress, the enzymes involved in linalool production are inhibited. The second volatile compound in 'Gervaso' F_1 basil is represented by eugenol which is considered an antioxidant component conferring the spicy taste (Jordán et al., 2017; Nurzyńska-Wierdak, 2012). Regardless of the NR and B, severe drought stressed plants showed the highest eugenol values, whereas, a decrease in terms of α-bergamotene was assessed when DS increased. A higher level of biosynthesis of this sesquiterpene might be connected to the higher potassium uptake (Nurzyńska-Wierdak and Borowski, 2011), which might occur in less stressed plants. As asserted by Onofrei et al. (2018), plant nutrition affects the quanti-qualitatively aspects of the plant aromatic profile. In our study, the different N dose significantly affected the content of trans-2-hexenal, 1-octen-3-ol, eucalyptol, β-cis-ocimene, linalool, eugenol and α-bergamotene. The content of this latter component was positively influenced by the increased N application. Furthermore, a similar trend was recorded for 1-octen-3-ol and β-cis-ocimene. On the other hand, biostimulants significantly affected the whole aromatic profile of 'Gervaso' F_1 hybrid. Data showed that plants supplied with SWE had an increase in linalool content compared to the control plants, while, biostimulants reduced the content of trans-2-hexanal, eucalyptol and α-bergamotene. Since, as reported by Aktsoglou et al. (2021), mineral availability affects the content of volatile oils and considering that seaweed and plant extracts significantly modify plant mineral uptake, the use of biostimulants resulted in significant variations of sweet basil volatile profile.

5. Conclusion

The stand-alone or combinatorial effect of different drought stress intensity, nitrogen rate and biostimulants supply (Kelpstar® seaweed extract and Tyson® protein hydrolysate) on sweet basil was appraised in order to attain valuable insights for optimizing agronomic practices, such as irrigation and N fertilization. These practices play a significant role in enhancing resource use efficiency (water and N), which in turn have significant repercussion on plant yield, quality and physiological traits. Our study showed that both biostimulants increased yield, NUE, WP, polyphenols, ascorbic acid, stomatal conductance and total leaf area compared to the control, even when plants were exposed to sub-optimal, supra-optimal or unfavorable conditions. Concomitantly, the use of PH led to reduced nitrate leaf content. Furthermore, SWE application increased the linalool content of sweet basil, while both biostimulants enhanced the eugenol, resulting in a positive variation of the two must abundant volatile components. Based on the findings, the application of SWE and PH is a promising, useful and eco-friendly agronomic strategy for increasing yield and quality of sweet basil grown under water constraints and/or N-deficit in greenhouse. This study suggests that the use of biostimulants as means to alleviate drought stress in plants subjected under N deficiency, can be considered as a valid approach to reduce chemical inputs and maximize resource use efficiency.

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.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2023.100268.

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