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Effect of water regime, nitrogen level and biostimulants application on yield and quality traits of wild rocket [*Diplotaxis tenuifolia* (L.) DC.]

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ABSTRACT

Wild rocket is an expanding vegetable crop, especially as a fresh-cut product that requires high quality standards. These can be achieved through appropriate management of agronomic practices such as water supply and nitrogen nutrition, in combination with emerging techniques such as the application of biostimulating substances. Hence, this study is focused on evaluating the response of the wild rocket to the application of two biostimulants in relation to different water regimes and nitrogen levels. The combined effect of two watering regimes (*WR*) (restoration of 50% and 100% of crop evapotranspiration-ETc, named WR_{50} and WR_{100}, respectively); three N levels (NL) (0, 75 and 150 kg ha^{-1} N, named N_0, N_{75} and N_{150}, respectively); two biostimulants (BS) based on seaweed extract (SW), Azoxystrobin (AZ) and an untreated control (C) are investigated. The split-split plot experimental design with three replicates was used by arranging *WR* in the main plots, NL in the subplots and BS in the sub-subplots. Experiment was carried out in an unheated greenhouse during two growth cycles. Water shortage caused root biomass/total biomass ratio (RR) increase by 11.3% and 31.1% drop in yield, mainly because of the reduction in leaf size and number. Total phenols (TP), total antioxidant activity (TAA), total carotenoids (TCa) and nitrate (Ni) content were higher in WR_{50} in respect to WR_{100} by 10.4%, 18.7%, 12.0%, and 35.5%, respectively, while total chlorophyll (TCh) was lower by 5.3%. The increase of NL between N_0 and N_{150} raised yield (222%), TCh (32.5%) and Ni (288.3), but reduced RR (43%), TP (16.2%), TAA (21.4%) and TCa (31.0%). SW and AZ increased yield respectively by 10.3% and 16.9%, mainly because raised leaf number for SW and leaf size for AZ. Moreover, SW increased RR by 7.2%, while AZ reduced it by 17.2%. SW and AZ improved TP, TAA, TCh and TCa respectively by 8.8%, 13.1%, 10.2% and 23.8% and by 10.8%, 19.7%, 16.0% and 35.6%. BS reduced Ni content by 21.5 (SW) and 36.8% (AZ). SW and AZ have shown good biostimulating efficacy by increasing yield and improving quality, reducing the harmful effects of water deficit. Tested BSs can be a useful tool for wild rocket farmers, especially under scarce water resources.

1. Introduction

In recent years, the cultivation of some leafy vegetables harvested as baby leaf has become very widespread because they are highly appreciated for marketing as fresh-cut products, whose market trend is constantly growing. Among these species, the wild rocket [Diplotaxis tenuifolia (L.) DC.] is one of the most used because of its organoleptic and nutritional characteristics (Caruso et al., 2019). In fact, it has a bitter and sometimes pungent taste deriving from the presence of glucosinolates (Halkier and Gershenzon, 2006), and is also rich in fiber, iron, vitamin C and phenols (Barillari et al., 2005; Cavaiuolo and Ferrante, 2014). The wild rocket grows spontaneously in all Italian regions up to 1000 m of altitude and its cultivation was started about two decades ago. The wild rocket is a very rustic species that grows in harsh conditions (e.g. water deficit, poor soils). However, to obtain high, uniform, and good quality production suited to the market demand, it is necessary to apply

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appropriately agronomic practices. In particular, the crop requires an adequate water regime as it suffers from both an excess of water and even a moderate water deficit. In the latter case, a decrease in production quality may occur due to the increase in fibrousness and nitrate content, despite some beneficial compounds for health as phenols and antioxidant activity can increase (Schiattone, 2018). As with many brassicas, whose family also includes the wild rocket, good levels of nitrogen in the soil are required to ensure good quality production. However, the excessive presence of this nutrient can give rise to some drawbacks such as increased sensitivity to some fungal diseases, enticement due to the presence of low-fiber tissues and an increase in nitrate content (Schiattone, 2018). In addition, high nitrogen level increase the risk of aquifer pollution (Hammad et al., 2017). Among the agronomic tools that could improve the quality standard we can include biostimulating compounds whose availability on the market is growing. These compounds can also help limiting the negative effects of any situations of water deficit and inadequate nitrogen availability (Colla et al., 2015; Li et al., 2022).

The application of biostimulants is a part of modern strategies that have been spreading in recent years to improve the sustainability of production systems in agriculture. Biostimulants represent a promising tool capable of improving the quality parameters of various plant products, nutritional efficiency, and tolerance to abiotic stress (Colla et al., 2015; Khan et al., 2018). Biostimulants are compounds of different origin (humic and fulvic acids, protein hydrolysates and other compounds containing N, chitosan and other biopolymers, inorganic compounds, seaweed extracts, ketones, etc.) in which there are one or more bioactive molecules that can influence the physiological and metabolic processes of the plant by increasing their production performances, also helping to limit the damage produced by abiotic stress (du Jardin, 2015).

There are several types of seaweed based biostimulants containing inorganic and organic substances in variable quantities in relation to the species of algae and the extraction method. Inorganic compounds including N, P, K, Fe, Mg, Zn, Ca, Na and S, some organic compounds including osmolytes (e.g., mannitol, betaines and betaine analogs), amino acids, bioactive secondary metabolites, polysaccharides, vitamins, phytohormones (e.g. polyamines, brassinosteroids, gibberellins, cytokinins, auxins, acid acetic indole, abscisic acid) and phenols (El Boukhari et al., 2020). The biological activity of the extract can vary considerably due to a large number of potentially active compounds, the variability of the source material and the extraction methods (Khairy and El-Shafay, 2013; Puglisi et al., 2020). Hence, it is extremely difficult to discriminate the biostimulating effects between the different active compounds (El Boukhari et al., 2020).

Among the substances with a biostimulating action we can also include strobilurins, the compounds of natural origin from which various structural variants have been synthesized (e.g.: Azoxystrobin, Kresoxim-methyl, Pyraclostrobin), and whose fungicidal action is widely exploited in the phytosanitary defense of many crops. Numerous studies have been published that also demonstrate a complementary biostimulating action on different species (Amaro et al., 2020; Boari et al., 2019; Candido et al., 2020; Cantore et al., 2016; Schiattone et al., 2021).

In the scientific literature, there are few documents in which the effect of water regime, nitrogen level, and biostimulants based on seaweed extracts or Azoxystrobin on wild rocket has been studied. Consequently, this research is focused on evaluating the interactive effect of these factors on yield and some qualitative characteristics of the wild rocket grown for two crop cycles in an unheated greenhouse. It is expected to support farmers with useful information for the agronomic management of the crop that ensures high quality standards and low environmental impact.

2. Material and methods

2.1. Characteristics of experimental site

The trial was carried out at Mediterranean Agronomic Institute of Bari (IAMB), near Valenzano (BA, Italy) (41° 03' N, 16 ° 52' E; 72 m a.s.l.), from 15 November 2016–28 February 2017, in an unhetaed greenhouse, covered with ethylene vinyl acetate film (200 μm thick). The study area is characterized by a Mediterranean climate.

The wild rocket was grown in cylindrical pots (0.34 m in diameter and 0.3 m in height) suitably equipped with saucers, each containing 20 L of soil (type Lithic-Ruptic-Inceptic-Haploxeralfs) (USDA, 2006) of good fertility taken in the same locality, with the following physical and chemical characteristics: sand (2 >ϕ>0.02 mm) 20.6%, silt (0.02 > ϕ >0.002 mm) 48.8%, clay (ϕ <2 μ) 30.6%; bulk density 0.00123 kg cm^{-3}; soil moisture at wilting point (−1.5 MPa, Richard Pressure Plate Extractor) 23.2 cm^3 cm^{-3}, and at field capacity 37.1 cm^3 cm^{-3}; pH 7.5, organic matter (Walkley–Black method) 19.2 g kg^{-1}, total N (Kjeldahl method) 1.16 g kg^{-1}, available P_2O_5 (Olsen method) 37.2 mg kg^{-1}, exchangeable K_2O (ammonium acetate method) 345.4 mg kg^{-1}, total limestone 22.4 g kg^{-1}, active limestone 10.3 g kg^{-1}, ESP 1.3%, electrical conductivity of saturated paste extract (ECe, 2:1) 0.35 dS m^{-1}.

To avoid soil compaction because of watering, perlite at 5:1 (v/v) ratio was mixed to the soil of the superficial layer (0–5 cm).

2.2. Climatic trend

During the wild rocket growth cycles, minimum (T_{min}) and maximum (T_{max}) air temperature ranged between − 2.1 and 16.9 °C and between 1.4 and 23.5 °C, respectively. Very low temperatures were recorded in early January, when T_{min} was negative for several days and T_{max} did not exceed 5.5 °C. During the second growth cycle T_{min} fluctuated around 7 °C with a stable trend throughout the period, while until harvest T_{max} showed rising trend with averaged value of about 16 °C (Fig. 1a).

Solar radiation had a stable trend (about 7.5 MJ m^{-2} d^{-1}) from planting time until the end of January. Later, the trend showed an increase of solar radiation up to 14 MJ m^{-2} d^{-1} around the period of second harvest (Fig. 1b).

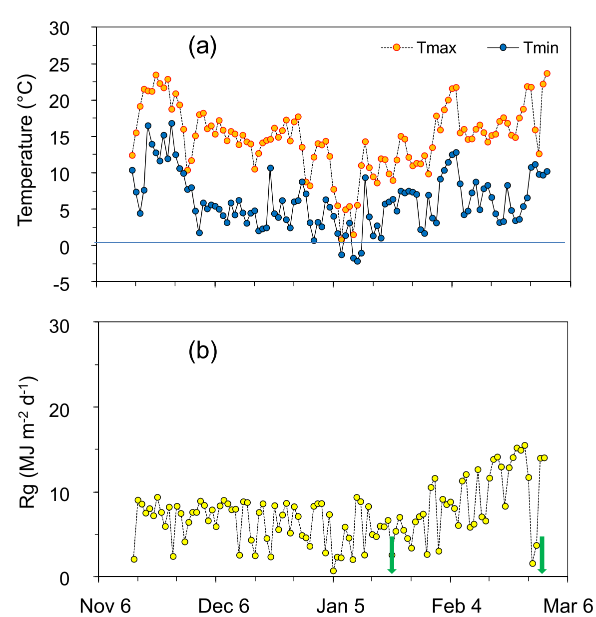

Fig. 1. Daily values of the maximum (T_{max}) and minimum (T_{min}) air temperature (a) and global solar radiation (Rg) (b) during the growth cycles of wild rocket. Arrows indicate harvest dates.

2.3. Treatments, experimental design and crop management

The trial involved the comparison of three treatments in combination between them: 2 watering regimes (W*R*) (restoration of 50% and 100% of crop evapotranspiration-ETc, named WR_{50} and WR_{100}, respectively); 3 N levels (NL) (0, 75 and 150 kg ha^{-1} N, named N_0, N_{75} and N_{150}, respectively); two biostimulants (BS) based on seaweed extract (Bioproject SM23 Foliar-BioKimia® International Srl) (SW), Azoxystrobin (Ortiva®, Syngenta) (AZ) and the untreated control (C). The experimental layout was a split-split-plot with three replications, arranging W*R* in the main plots, NL in the subplots and BS in the sub-subplots. This last consisted in one pot.

Fertilization was carried out as follows: 3.34 and 1.25 g pot^{-1} of P_2O_5 (19% superphosphate) and K_2O (51% potassium sulfate), corresponding respectively to 70 kg ha^{-1} of both nutrients, distributed before transplantation in all treatments and then buried at a depth of 5–10 cm. Furthermore, treatments N_{75} and N_{150} were fertilized, respectively, with 1.73 and 3.46 g pot^{-1} of ammonium sulfate 21% (40 and 80 kg ha^{-1} of N) before transplantation and with 1.51 and 3.02 g pot^{-1} of the same fertilizer (35 and 70 kg ha^{-1} of N) just after the first harvest. Weed control was performed by hand few days after their emergence to minimize competition with wild rocket plants.

Wild rocket was planted on November 15th, 2016, and, exploiting the regrowth capacity of this species after harvest, two growth cycles were carried out: the first one ended on January 17th, while the second on February 28th. Seedlings at the 5th true leaf stage, prepared in the nursery in 228-hole polystyrene alveolate trays, were used for the transplant. In each pot 5 tufts of 3 seedlings each have been transplanted, suitably spaced. Ortiva® (1.5 mL L^{-1}) and Bioproject SM23 Foliar (1.5 mL L^{-1}) were sprayed on the leaves 20, 50, 84, and 94 days after transplant (DAT).

During the two growth cycles there were no phytosanitary problems. In fact, only one insecticide application was required (Confidor® 200 SL - Bayer CropScience S.r.l. - Italy) to fight an initial attack of aphids (Myzus persicae) during the first growth cycle.

Evapotranspiration (ET) was measured by the water balance method weighing the pots (every 4–5 days) which were considered to be weighing lysimeters, and using the following equation (Schiattone et al., 2017):

$$ET = \frac{(W_n - W_{n+m}) + (W_I - W_{Dp})}{\rho_w} \times \frac{1}{N_d}$$

where, ET is the daily evapotranspiration (L), W_n and W_{n+m} are two consecutive weights (kg) of the pot, W_I is the supplied water (kg), W_{Dp} is the drained water (kg), ρ_w is the specific weight of the water (1 kg L^{-1}), N_d represents the number of days elapsed between the two weighings. The biomass increases between the two weighings were considered negligible (Ghaemi and Rafiee, 2016), so they were not considered in the ET calculation procedure.

To meet the water needs of the wild rocket, fresh water was supplied over soil surface by hand, avoiding wetting of leaves. Based on the threshold value (p = 45) of other Brassicaceae (Allen et al., 1998), irrigation was carried out when 45% of the available water (AW) ran out in the WR_{100} treatment. Any percolation water was collected in the saucers, which was subsequently weighed and used for the subsequent watering of the same pot, to recover any loss of nutrients due to leaching.

2.4. Yield and biometric features of plants

Harvests were performed on 63 and 105 DAT, respectively for the first and second growth cycle. At the first harvest, two of the five tufts of plants were uprooted with the roots. Instead, the other three tufts of plants were cut about 2 cm above the collar with a knife. After removing the soil under running water and drying with absorbent paper, the plants were placed in numbered plastic bags and protected from light with a black plastic film. Once the samples were transferred to the laboratory, the roots and the corresponding epigeal part were weighed separately. For the calculation of the yield and the average weight of the plants, all five tufts of plants present in the pots were considered. Of course, only three of the five initial plant tufts were present in the second growth cycle. When harvested, the latter were uprooted with the entire root system. After washing and drying, the epigeal part was separated from the roots for the subsequent weighing.

At each harvest, total, marketable and waste (yellowed, necrotic leaves or damaged by insects or pathogens) yield were assessed. The number of leaves per plant was assessed on five plants per pot. The leaf area was determined by measuring, by means of the LAI meter (Li-COR, 3100, Lincoln Nebraska, USA) the area of the entire sample collected from each pot, and then to relating it to the number of plants and the average number of leaves per plant. Above ground dry biomass (AGDB) and root dry biomass (RDB) were assessed by multiplying the total yield by the percentage of leaf dry matter, and root fresh weight by their percentage of dry matter. Average leaf length and width, the ratio of leaf lamina length to total leaf length (lamina + petiole) were determined on a sample of ten leaves per each pot.

2.5. Qualitative parameters

On the marketable product, the following qualitative parameters were determined: chromatic features, leaf (DM_l) and root (DM_r) dry matter, total chlorophyll (TCh), total carotenoids (TCa), total phenols (TP), total antioxidant activity (TAA), nitrates (Ni).

Chromatic features - For each experimental unit, just after harvesting, ten representative leaves were selected and the chromatic parameters L* , a* and b* measured. L* represents the brightness, a* and b* the chromatic coordinates indicating, respectively, the red-green and yellow-blue components. Values of +a* indicate the direction of red (redness), -a* the direction of green (greenness), +b* the direction of yellow (yellowness), –b* the direction of blue (blueness) (CIE, 1986). The measurements were made with a colorimeter (CR-400, Konica Minolta, Osaka, Japan; using Spectra Magic NX software) in the apical part of the upper surface of the leaf blade, avoiding the central rib.

Leaf and root dry matter - For the determination of DM_l, a sample of marketable product of approximately 150 g was used, while for the DM_r a smaller amount (20–40 g) was used. The vegetable material, after weighing, was placed in a thermo-ventilated oven at a temperature of 60 °C until reaching steady weight (about 48 h). The DM_l and DM_r obtained were expressed as a percentage to fresh.

Total phenols, antioxidant activity - For each treatment, about 20 g of fresh marketable product were homogenized and subjected to reflux extraction on a water bath (2 times per hour), with methanol (1:5 w/v). After filtration through a Whatmann filter paper, the methanolic extract was concentrated under vacuum and then, used for the determination of total phenols and antioxidant activity. The total phenols were determined spectrophotometrically by the Folin-Ciocalteu method (Singleton et al., 1999) as described by Sergio et al. (2020) and expressed as mg of caffeic acid equivalent (CAE) per gram of dry matter.

TAA was determined by means of the analysis of the radical cations ABTS (2,2'-azino-bis-3-ethylbenzothiazolin-6-sulfonic) as described by Sergio et al. (2021) and expressed as g Trolox 100 g^{-1} DM.

Nitrates - A sample of about 10 g of dry leaves has been finely ground by means of a mill (IKA, Labortechnik, Staufen, Germany) fitted with a 1 mm sieve. Analyses were performed on a 0.5 g sub-sample, with ion chromatography (Dionex DX120; Dionex Corporation, Sunnyvale, CA), with a conductivity detector, using an IonPack AG14 pre-column and an IonPack separation column AS14 (Bonasia et al., 2008). The nitrate concentration was determined based on standards prepared with sodium nitrate ($NaNO_3$).

Total chlorophyll, total carotenoids - For each treatment, a sample of about 30 g of leaf blades was finely chopped in a mortar after adding liquid nitrogen and then stored at − 20 °C. To carry out the extraction, this material was homogenized with 80% acetone (0.2 g mL^{-1}), kept in a dark cold chamber at 4 °C for 24 h and then centrifuged for 10 min at 14,000 rpm. Three spectrophotometric readings (at 662, 646 and 470 nm) were performed on the recovered supernatant. The method is based on the property of chlorophyll to absorb light in the red zone of the visible spectrum. The absorbance values obtained were then used to calculate TCh and TCa concentrations according to Wellburn (1994). All extraction procedures were performed in low light conditions.

2.6. Statistical analysis

The data collected in each growth cycle were subjected to the analysis of variance (ANOVA) according to the split-split plot experimental design, using the SPSS 17 software; mean values were separated with the Student-Newman-Keuls (SNK) test (P = 0.05).

3. Results

Crop evapotranspiration (ET) did not differ significantly among nitrogen levels and biostimulating treatments, while variations were observed between irrigation regimes.

During the two growth cycles, the daily ET presented a sinusoidal trend mainly due to a drastic change in the leaf area that occurred after the first harvest (Fig. 2a). In WR_{100} the ET went from about 1.0 mm d^{-1} in the first days after transplantation to about 2.5 mm d^{-1} at the end of December. In the first ten days of January (the coldest period), this parameter fluctuated around 1 mm d^{-1} and then rose again up to about 2 mm d^{-1} of the harvest period. This was followed by a reduction and, therefore, a rapid increase as the leaf area and the air temperature raised, up to 5 mm d^{-1} a few days before the second harvest. In the WR_{50}, daily ET presented the same trend found in WR_{100} with values on average 37% lower, because of the water limitation regime (Fig. 2a).

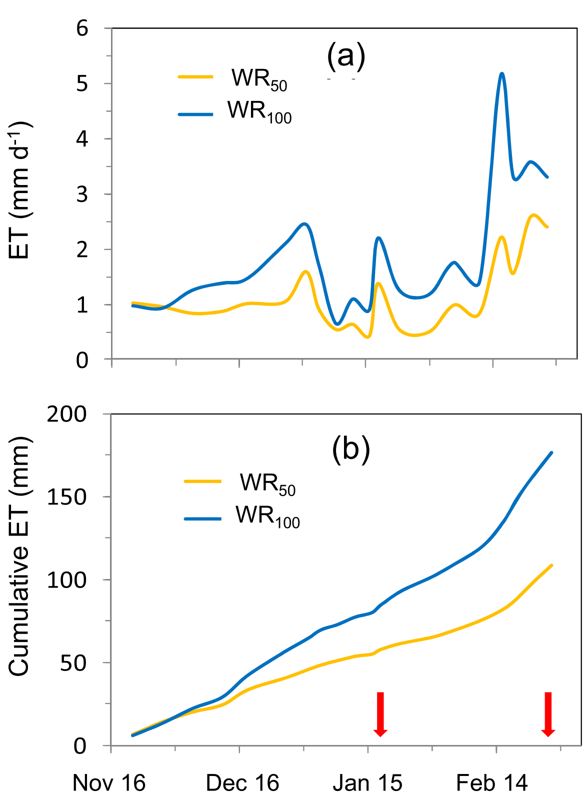

Fig. 2. Trend of daily (a) and cumulative (b) values of evapotranspiration (ET) for the two irrigation regimes during the growth cycles of wild rocket. Arrows indicate harvest dates. WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc.

The total value of the ET was 57.5 and 50.9 mm (WR_{50}) and 84.4 and 92.0 mm (WR_{100}), respectively for the first and second growth cycle (Fig. 2b).

The soil water reserve was also not significantly affected by nitrogen levels and biostimulant treatments, while it varied in relation to water regimes. In the WR_{100} treatment, during the growth cycles, this parameter was maintained above the readily available water (RAW) with periodic fluctuations, between 0% and 44% of the available water (AW), resulting from waterings. In WR_{50}, the water reserve was always lower than that found in WR_{100}, falling below RAW from the first decade of December and remaining in this condition for almost the entire duration of the wild rocket growth cycles, with fluctuations between 12% and 80% of the AW (Fig. 3).

Obviously, the irrigation volumes, supplied during 19 waterings, were also different between the irrigation regimes, with seasonal irrigation volume (total of the two growth cycles) equal to 85 and 168.5 mm for WR_{50} and WR_{100}, respectively.

The application of different management practices affected the wild rocket production and related parameters in a diverse way and magnitude. The marketable yield, on average, was equal to about 834 and 627 g m^{-2}, respectively in the first and second harvest (Table 1). The main biometric parameters showed a variable behavior in both growth cycles. In particular, between the first and second harvest, as an average of all treatments: i) the leaf area decreased from 11.0 to 5.4 cm^2 $leaf^{-1}$, ii) the plant weight and the leaf number varied, respectively, from 3.7 g $plant^{-1}$ and 8.5 n. $plant^{-1}$ to 3.5 g $plant^{-1}$ and 15.7 n. $plant^{-1}$, iii) the length of the leaf blade and the total leaf length have been reduced, respectively, from 10.5 and 17.6 cm to 9.4 and 13.8 cm, with a percentage ratio between the two parameters, which ranged from 59.6% to 69.2%; iv) the leaf width decreased from 2.5 to 1.5 cm; v) the ratio between root biomass and total biomass (root + shoot) increased from 17.3% to 24.3%.

The water supply 50% lower than the optimal water requirement resulted in a 26.3% and 35.8% yield decline in the first and second growth cycle, respectively. The differences in yield between the water regimes were mainly determined by the variations in leaf size and, to a lesser extent, by the leaf number per plant (Table 2). In WR_{50}, the root weight, as average of two harvests, was reduced by 17.5%, but the root biomass/total biomass ratio increased by 11.3%.

The yield of wild rocket raised with the increase of nitrogen intake. In N_{150}, it was 112% and 333% higher than in N_0, respectively in the first and second growth cycle. However, in both growth cycles, there was a

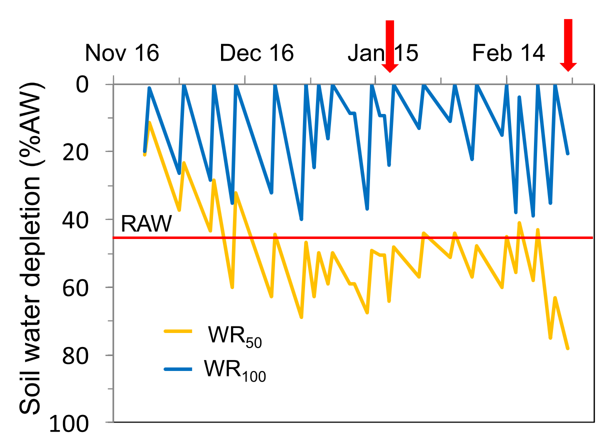

Fig. 3. Trend of the soil water depletion, as a percentage of available water (AW), during the growth cycle of wild rocket for the irrigation regimes. Arrows indicate harvest dates; the horizontal red line indicates the threshold value (p) of ready available water (RAW) attributed to the wild rocket in analogy to the other Brassicaceae (Allen et al., 1998). WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc.

Table 1
Effect of water regime, nitrogen level and the application of biostimulants on yield and some morphological characteristics of wild rocket in two harvests. WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc; N_0 = 0 kg ha^{-1} of N, N_{75} = 75 kg ha^{-1} of N, N_{150} = 150 kg ha^{-1} of N; C = control, AZ = application of Azoxystrobin, SW = application of seaweed extract based biostimulant.

Treatments	Marketable yield	Waste	Roots	Dry root biomass/dry total biomass	Aboveground dry biomass	Root dry biomass	Aboveground plant fresh weight	Root fresh weight
	(g m^{-2})	(%)	(g m^{-2})	(%)	(g m^{-2})	(g m^{-2})	(g plant^{-1})	(g plant^{-1})
1st harvest								
Water regime (WR)	**	*	*	*	**	*	**	*
WR_{50}	707.6	7.4	163.3	18.2	87.2	54.7	3.1	0.6
WR_{100}	959.9	6.5	199.5	16.4	111.9	61.8	4.3	0.8
Nitrogen level (NL)	**	**	**	**	**	**	**	*
N_0	521.4c	13.7 a	153.7c	22.2 a	69.0c	49.4c	2.4c	0.6c
N_{75}	873.3 b	4.5 b	179.1 b	15.0 b	104.8 b	57.7 b	3.8 b	0.7 b
N_{150}	1106.6 a	2.6 b	211.3 a	14.6 b	124.7 a	67.7 a	5.0 a	0.8 a
Biostimulants (BS)	*	ns	*	**	*	*	*	*
C	758.1 b	7.6	181.1 b	18.1 a	94.4 b	58.2 b	3.1 b	0.7 b
AZ	888.4 a	6.1	166.3c	14.3 b	98.8 ab	53.4c	4.2 a	0.6c
SW	854.8 a	7.2	196.7 a	19.5 a	105.4 a	63.2 a	3.9 a	0.8 a
WR x NL	*	ns	ns	ns	*	ns	*	ns
WR x BS	ns	ns	ns	ns	ns	ns	ns	ns
NL x BS	ns	ns	ns	ns	ns	ns	ns	ns
WR x NL x BS	*	ns	ns	ns	*	ns	*	ns
2nd harvest								
Water regime (WR)	**	*	**	*	**	*	*	*
WR_{50}	490.5	7.5	149.8	25.6	62.8	46.4	2.9	0.8
WR_{100}	764.0	5.2	180.4	22.9	87.3	49.4	4.1	1.0
Nitrogen level (NL)	**	**	**	**	**	**	**	*
N_0	231.9c	13.3 a	124.3 b	34.2 a	32.9c	40.0 b	1.5c	0.8 b
N_{75}	645.2 b	3.6 b	183.0 a	22.1 b	80.4 b	54.1 a	3.6 b	1.0 a
N_{150}	1004.7 a	2.2c	188.1 a	16.5c	111.9 a	49.7 a	5.4 a	1.0 a
Biostimulants (BS)	*	ns	*	*	*	*	*	ns
C	580.1c	7.2	163.3 b	25.3 a	69.7c	47.2 b	3.1c	0.9
AZ	676.6 a	6.2	155.4 b	22.0 b	81.1 a	45.0c	3.9 a	0.9
SW	625.1 b	5.6	176.7 a	25.5 a	74.4 b	51.5 a	3.5 b	0.9
WR x NL	**	ns	ns	ns	*	ns	*	ns
WR x BS	ns	ns	ns	ns	ns	ns	ns	ns
NL x BS	ns	ns	ns	ns	ns	ns	ns	ns
WR x NL x BS	*	ns	ns	ns	*	ns	*	ns

ns, *, ** indicate non-significant or significant F-test at $P < 0.05$ and $P < 0.01$, respectively. Different letters indicate significantly different values according to the SNK test ($P = 0.05$).

harmful effect of the higher nitrogen dose on yield of the less watered plants (WR_{50}) (*WR* x NL interaction) (Fig. 4).

As happened for the water regime, the NL effect was also determined to a greater extent by the variation in leaf morphology, especially the leaf area and, secondly, by the leaf number per plant. The root biomass also raised in relation to the increase in the N level, with an average rise of 35% observed in the two harvests, passing from N_0 to N_{150}. The percentage ratio of root biomass/total biomass decreased significantly by increasing N dose (on average by 43% between N_0 and N_{150}).

The application of the seaweed extract based biostimulant resulted in 10.3% average yield increase compared to the control, while with Azoxystrobin yield raised by 16.9%. For SW, yield increase was mainly determined by the greater leaf number per plant and, secondly, by the greater leaf area. Azoxystrobin, on the other hand, caused the increase in the leaf area to a greater extent and, to a lesser extent, the increase in the leaf number. The negative effect on yield, previously described, of the greater nitrogen intake in water deficit conditions, did not occur in the Azoxystrobin treated plants (*WR* x NL x BS interaction) (Table 1, Fig. 5). A significant interaction was also observed for the leaf size (Table 1).

In the first harvest, SW increased root biomass and root biomass/total biomass ratio of 29.2% and 7.2%, respectively, while in the second harvest, the effect was not significant. With the application of Azoxystrobin, on the other hand, there was a tendency towards a reduction in root biomass, in particular for the root biomass/total biomass ratio, which was reduced by 21.3% and 13.1%, respectively, in the first and second harvest.

The examined qualitative parameters were influenced by treatments compared (Table 3). The leaf dry matter (DM_l) on average was 9.5% higher in water deficit conditions. Furthermore, this parameter has undergone a progressive reduction with the increase of the N level. In particular, going from N_0 to N_{150}, the DM_l values, on average, dropped by 15.5%. The SW did not have a significant effect on the DM_l, while AZ caused a reduction of 10.2% in the first harvest, only.

Total phenol content and total antioxidant activity showed the same trend as DM: these parameters increased under water deficit and lower nitrogen intake. In fact, TP and TAA were 10.4% and 18.7% higher in WR_{50} than in WR_{100} and decreased, respectively, by 16.2% and 21.4% between N_0 and N_{150}.

Table 2
Effect of water regime, nitrogen level and the application of biostimulants on some leaf characteristics of wild rocket in two harvests. WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc; N_0 = 0 kg ha^{-1} of N, N_{75} = 75 kg ha^{-1} of N, N_{150} = 150 kg ha^{-1} of N; C = control, AZ = application of Azoxystrobin, SW = application of seaweed extract based biostimulant.

Treatments	Leaf number	Leaf size	Total leaf length (TL)	Leaf blade length (BL)	BL/TL	Leaf width
	(n. $plant^{-1}$)	(cm^2 $leaf^{-1}$)	(cm)	(cm)	(%)	(cm)
1st harvest						
Water regime (WR)	ns	**	*	*	ns	ns
WR_{50}	8.1	9.8	16.5	9.8	59.5	2.2
WR_{100}	8.9	12.2	18.6	11.1	59.6	2.7
Nitrogen level (NL)	*	**	**	*	ns	*
N_0	6.9c	8.5c	14.0c	8.3c	59.5	1.8 b
N_{75}	8.8 b	11.6 b	18.1 b	10.8 b	59.5	2.6 a
N_{150}	9.8 a	12.9 a	20.5 a	12.2 a	59.7	3.0 a
Biostimulants (BS)	*	*	*	*	ns	*
C	7.7 b	10.1 b	16.3 b	9.9 b	60.8	2.3 b
AZ	8.8 a	12.3 a	19.1 a	11.0 a	57.8	2.7 a
SW	9.1 a	10.8 b	17.3 b	10.4 b	60.2	2.4 b
WR x NL	ns	*	ns	*	ns	*
WR x BS	ns	ns	ns	ns	ns	ns
NL x BS	ns	ns	ns	ns	ns	ns
WR x NL x BS	ns	*	ns	*	ns	ns
2nd harvest						
Water regime (WR)	*	**	*	ns	ns	ns
WR_{50}	14.9	4.7	13.1	9.2	71.2	1.4
WR_{100}	16.4	6.1	14.4	9.6	67.2	1.5
Nitrogen level (NL)	**	**	*	*	ns	*
N_0	11.3c	3.3c	10.4 b	7.6 b	73.0 a	1.1 b
N_{75}	15.6 b	5.9 b	15.0 a	9.6 ab	64.7 b	1.6 a
N_{150}	20.0 a	7.0 a	15.9 a	11.0 a	69.8 a	1.6 a
Biostimulants (BS)	*	*	ns	*	ns	ns
C	14.4c	5.0 b	13.7	9.1 b	67.6	1.4
AZ	16.7 a	6.0 a	13.9	9.9 a	71.2	1.5
SW	15.9 b	5.3 b	13.6	9.2 b	68.7	1.4
WR x NL	ns	*	ns	*	ns	ns
WR x BS	ns	ns	ns	ns	ns	ns
NL x BS	ns	ns	ns	ns	ns	ns
WR x NL x BS	ns	*	ns	*	ns	ns

ns, *, ** indicate non-significant or significant F-test at $P < 0.05$ and $P < 0.01$, respectively. Different letters indicate significantly different values according to the SNK test (P = 0.05).

Furthermore, a positive effect of the application of SW and AZ was found on these last two parameters. In fact, TP and TAA, compared to the control, increased on average by 8.8% and 13.1% with the application of SW, and by 10.8% and 19.7% with the application of AZ, respectively.

The chlorophyll content decreased by 5.3% under water deficit and increased by 32.5% from N_0 to N_{150}. Furthermore, the application of SW and Azo resulted in an average increase of this parameter by 10.2% and 16.0%, respectively.

The water and nitrogen deficit favored the accumulation of carotenoids. In fact, the total carotenoids in WR_{50} were 12.0% higher than in WR_{100} and decreased progressively with the increase in the N level, reaching − 31% in N_{150} compared to N_0. Furthermore, the application of SW and AZ favored the accumulation of the latter compounds with an average increase, compared to the control, of 23.8% and 35.6%, respectively.

The examination of leaf color parameters values highlights the effect of WR and NL on L* , and of all the treatments compared on a* and b* .

The greater availability of water and nitrogen made the leaves brighter as evidenced by the L* values which were 10.7% higher in WR_{100} compared to WR_{50}, and 9.2% in N_{150} compared to N_0.

The greater availability of water and N, and the application of SW and AZ have made the leaves greener because of the higher chlorophyll content, as was also highlighted by the SPAD values detected during the growth cycles (Candido et al., 2022) and the TCh measured at harvest. In fact, the value of a* , on average, was lower (more negative) by 13.9% in WR_{100} compared to WR_{50}, by 41.6% in N_{150} compared to N_0, by 26.3% and 19.8% in SW and AZ, compared to control.

The b* values, on the other hand, were 11.8% higher in WR_{50} than in WR_{100}, while they decreased by 17.4% between N_0 and N_{150}. Furthermore, in the first harvest, the application of SW reduced this last parameter by 15.3%, compared to the control.

In both harvests, the leaf nitrate content varied between about 800 and 4700 mg kg^{-1} FM. The water deficit increased Ni, on average, by 35.5%. In addition, the Ni raised considerably with the increase in NL. In fact, compared to N_0, this parameter increased by 99.1% and 288.3%, respectively in N_{75} and N_{150}. SW and AZ resulted in a reduction in Ni of 21.5% and 36.8%, respectively.

4. Discussion

The soil water regime has proved to be one of the main factors that affects yield and quality of wild rocket. The decrease in yield due to water shortage occurred because of concomitant reduction in leaf size and leaf number per plant, as observed by other authors on wild rocket, lettuce and spinach (Bozkurt et al., 2009; Mahmood et al., 2004; Mirdad, 2009). The reduction of transpiring area that occurs under water deficit is one of the strategies implemented by plants to cope with these situations and represents an adaptation to xeric environments (Parolin, 2001; Taiz and Zeiger, 2002). These morphological variations occur

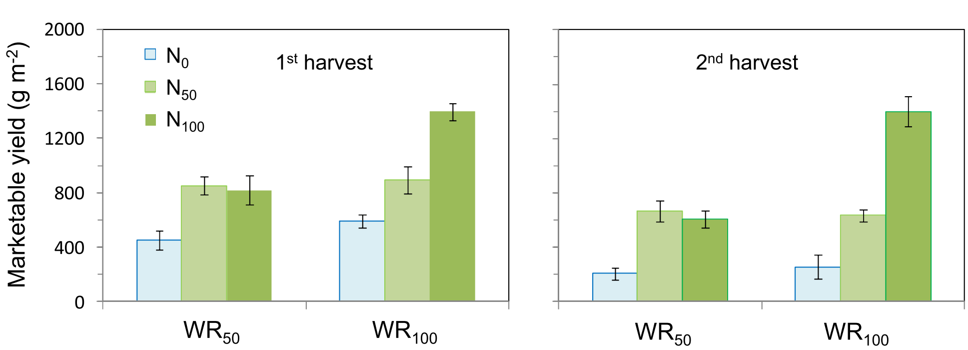

Fig. 4. Water regime x nitrogen level interaction on the marketable yield of wild rocket in two harvests. Vertical bars indicate standard deviation. WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc; N_0 = 0 kg ha^{-1} of N, N_{75} = 75 kg ha^{-1} of N, N_{150} = 150 kg ha^{-1} of N.

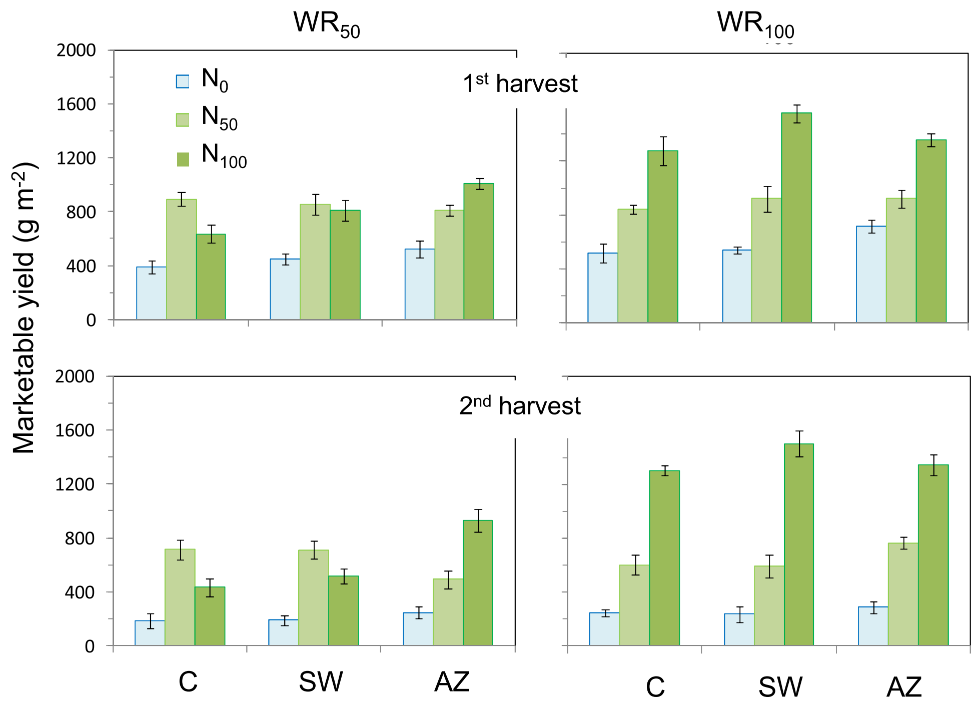

Fig. 5. Water regime x nitrogen level x biostimulants interaction on the marketable yield of wild rocket in two harvests. Vertical bars indicate standard deviation. WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc; N_0 = 0 kg ha^{-1} of N, N_{75} = 75 kg ha^{-1} of N, N_{150} = 150 kg ha^{-1} of N. C = control, AZ = application of Azoxystrobin, SW = application of seaweed extract based biostimulant.

Table 3
Effect of water regime, nitrogen level and biostimulant application on leaf dry matter (DM_l) content, nitrates (Ni), total phenols (TP), total antioxidant activity (TAA), total chlorophyll (TCh), total carotenoids (TCa), and on the color parameters of the wild rocket for in two harvests. WR_{100} = restoration of 100% of the ETc, WR_{50} = restoration of 50% of the ETc; N_0 = 0 kg ha^{-1} of N, N_{75} = 75 kg ha^{-1} of N, N_{150} = 150 kg ha^{-1} of N; C = control, AZ = application of Azoxystrobin, SW = application of seaweed extract based biostimulant. FM = fresh matter, DM = dry matter.

Treatments	DM_l	Ni	TP	TAA	TCh	TCa	Color parameters		
	(g 100 g^{-1} FM)	(mg kg^{-1} FM)	(mg CAE g^{-1} DM)	(g Trolox 100 g^{-1} DM)	(µg g^{-1} DM)	(µg g^{-1} DM)	L*	a*	b*
1st harvest									
Water regime (*WR*)	*	*	*	*	*	**	*	*	*
WR_{50}	12.7	3311.1	14.2	0.43	794.4	183.3	36.2	-11.9	19.8
WR_{100}	12.0	2622.2	13.4	0.37	845.5	160.0	39.6	-14.0	18.1
Nitrogen level (NL)	**	**	*	*	**	**	*	*	*
N_0	13.5 a	1383.3c	14.9 a	0.44 a	725.0c	200.0 a	36.5 b	-10.6 b	21.0 a
N_{75}	12.1 b	2796.7 b	14.0 ab	0.41 a	826.7 b	171.7 b	37.4 b	-13.8 a	18.3 b
N_{150}	11.4c	4720.0 a	12.5 b	0.35 b	908.3 a	143.3c	39.8 a	-14.7 a	17.5 b
Biostimulants (BS)	*	*	*	*	*	*	ns	*	*
C	12.8 a	3350.0 a	13.1 b	0.38 b	776.7 b	151.7c	37.9	-11.5 b	20.3 a
AZ	11.5 b	2358.3 b	14.3 a	0.42 a	856.7 a	188.3 a	38.0	-13.5 a	19.4 ab
SW	12.8 a	3191.7 a	14.0 a	0.40 ab	826.7 a	175.0 b	37.7	-13.9 a	17.2 b
All interactions	ns	ns	ns	ns	ns	ns	ns	ns	ns
2nd harvest									
Water regime (*WR*)	*	**	*	**	*	**	*	*	*
WR_{50}	13.5	2340.0	15.2	0.48	761.0	186.4	35.0	-10.8	18.1
WR_{100}	12.0	1617.8	13.3	0.39	797.8	168.9	39.2	-11.9	16.0
Nitrogen level (NL)	*	**	*	*	**	**	*	*	*
N_0	14.3 a	811.7c	15.5 a	0.48 a	645.0c	213.8 a	35.5 b	-9.0 b	18.8 a
N_{75}	12.6 b	1591.7 b	14.3 b	0.44 ab	791.7 b	177.5 b	37.0 ab	-11.9 a	17.0 b
N_{150}	11.3c	3533.3 a	13.0c	0.38 b	901.7 a	141.7c	38.8 a	-13.0 a	15.4c
Biostimulants (BS)	ns	*	*	*	*	**	ns	*	ns
C	12.8	2726.7 a	13.3 b	0.38 b	696.7 b	140.5c	37.2	-9.5 b	17.6
AZ	12.7	1535.0 b	14.8 a	0.48 a	848.3 a	206.7 a	36.9	-11.9 a	16.9
SW	12.7	1685.0 b	14.7 a	0.45 a	793.3 a	185.8 b	37.4	-12.5 a	16.6
All interactions	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns, *, ** indicate non-significant or significant F-test at $P < 0.05$ and $P < 0.01$, respectively. Different letters indicate significantly different values according to the SNK test ($P = 0.05$).

especially when water shortage is prolonged. In fact, the first consequence of the water deficit is the reduction of stomatal conductance and assimilation rate, as has been observed in this trial (data not shown), which leads to a lower biomass production (Flexas et al., 2006; Hammad et al., 2018; Romero-Aranda et al., 2001). Furthermore, the water deficit has changed the allocation of assimilates between the aerial part and roots, resulting in a greater root biomass than aboveground biomass. This behavior confirms the capability of plant to adapt to the lower availability of resources, which the root system absorbs and transfers to the aerial part (Liu and Stützel, 2004). These results agree with the literature in which it is reported that plants subjected to prolonged water deficit tend to allocate a greater quantity of assimilates in the roots than in the aerial part, and that the alteration of the relationship between aerial part and roots derives from either the increase in root growth or the reduction of growth of the aerial part compared to the root (Franco, 2011; Kerbiriou et al., 2013).

The higher nitrogen doses resulted in an increase in yield of over 100% and 300% compared to the non-fertilized control, in the first and second harvest, respectively. A notable difference between two harvests is attributable to the fact that in the first growth cycle, wild rocket of the unfertilized control treatment benefited from the nitrogen reserve in the soil. Nevertheless, in the second growth cycle, the plants experienced greater N deficiency since most of the nitrogen reserve had been already depleted. The positive effect of nitrogen on yield was observed in a greater extent by the increase in the leaf number and, secondarily, by the leaf size. This result agrees with the findings reported by Schiattone et al. (2018) on wild rocket and with observations made on other leafy vegetables (Wang and Li, 2004; Zhang et al., 2015) or other species (Awais et al., 2017; Rahman et al., 2021).

However, the increase in nitrogen intake caused a detrimental effect on yield under water deficit, as already observed on wild rocket by Schiattone et al. (2018). Similarly, Liu et al. (2012) reported that the high nitrogen intake causes an increase in leaf sensitivity to water stress that led to a decrease of growth and yield. In fact, water scarcity in addition to directly limiting the growth process and plant survival, indirectly influences the plant's response to nutrients (Song et al., 2010). Hence, it can be affirmed that a supply of N commensurates with the water regime to which the crop is expected to be subjected, would help to improve its response to water shortage (Arora et al., 2001).

The increase in the nitrogen level, in addition to the increase of aboveground biomass, also led to a greater development of root biomass. However, the ratio between root biomass and total biomass presented very high values in the non-fertilized control and decreased with the increase in nitrogen supply. As well as for the water deficit, in the case of N deficiency the plants change the allocation of assimilates in favor of the roots whose greater growth supports the plant's capacity for the absorption of nutrients. Similar conclusions are reported in literature for different species, demonstrating the important role of root growth in balancing any lack of nutrients and/or water (Bonifas et al., 2005; Hammad et al., 2017).

The application of the seaweed extract based biostimulant resulted in an increase of yield in accordance with the rise of net assimilation (data not shown). The increase of photosynthesis is attributable to the higher content of chlorophyll whose biosynthesis was probably stimulated by the biostimulant (Jannin et al., 2013). The root biomass has also increased with the application of seaweed extract based biostimulant such as the ratio between roots and aerial part. Therefore, a better water and nutrient absorption efficiency may have contributed to improve yield of plants treated with the biostimulants. The biostimulating effect of seaweed extracts are well documented on a large number of species (Baltazar et al., 2021). However, the causes are not yet well known. Some authors have attributed some of the biostimulating effects to the presence of phytohormones (Ertani et al., 2018). However, this hypothesis has been rejected by other studies that have highlighted the irrelevant content of phytohormones in seaweed extracts, also taking into account the even lower quantity of extract that is distributed on the vegetation (Stirk et al., 2014). Instead, other authors have shifted the attention to various polysaccharides found in considerable concentrations in the extracts (Shekhar Sharma et al., 2014). Some biostimulating and abiotic stress mitigation effects could be attributed to them (Baltazar et al., 2021).

Azoxystrobin also resulted in a significant increase in yield which, as for the seaweed extract based biostimulant, is the direct consequence of the positive effect of this substance on net assimilation (data not shown). The higher leaf chlorophyll content could be the main cause of the increased photosynthesis as well as the improvement of the water status of the plant due to the control exerted on the stomatal conductance by the phytohormones, whose production seems to be stimulated by Azoxystrobin (Grossmann and Retzlaff, 1997; Venancio et al., 2003).

Another factor that could contribute to the increased yield following the application of Azoxystrobin is the stimulation of nitrate reductase activity (Amaro et al., 2018; Barbosa et al., 2011; Ishikawa et al., 2012). In fact, this enzyme plays a fundamental role in the nitrogen organication process which leads to the formation of new organic molecules such as nucleotides and amino acids (Köehle et al., 2002).

The greater tolerance to water deficit of plants treated with Azoxystrobin can be attributed to the effect on the biosynthesis of the phytohormones involved in stomatal regulation and, therefore, on the plant's water status (Boari et al., 2017; Cantore et al., 2016). Contrary to the seaweed extract based biostimulant, in the case of Azoxystrobin, there was a reduction in the roots/aerial part ratio. The higher production levels were obtained because of the greater net assimilation, as well as a different economy in the allocation of assimilates.

The leaf dry matter increased in conditions of water deficit and decreased with the increase in the nitrogen level. Similarly, Bonasia et al. (2017) and Schiattone et al. (2017) reported a rise in the leaf dry matter of Diplotaxis genotypes subjected to salt stress. In the case of low-to-moderate salinity, the effects are attributable to the consequences of water shortage induced by salinity. An increase in dry matter following the water deficit was also found on lettuce and cabbage (Karam et al., 2002; Xu and Leskovar, 2014), while for spinach the same parameter was inversely correlated to the nitrogen dose provided (Liphadzi et al., 2006).

Water deficit led to a reduction in the leaf chlorophyll content and an increase in carotenoids as shown by the SPAD data and color characteristics. These results agree with the findings reported by Schiattone et al. (2017) on wild rocket. On various leafy vegetables, Luoh et al. (2014) reported contrasting effects of water deficit on carotenoid content while Misra and Srivastava (2000) observed a reduction in two parameters.

Chlorophyll raised with the increase of nitrogen level while an opposite effect on carotenoids was observed. In literature, conflicting results are reported on the effects of N availability on these parameters. Kopsell et al. (2007) reported that the increase in the N level does not affect chlorophyll and carotenoids in the fresh tissues of cabbage leaves, but for the latter parameter the trend is reversed if the value is expressed on the dry matter basis.

In our case, phenols and antioxidant activity increased under water and nitrogen deficit, as also reported by other authors (Koyama et al., 2012; Luoh et al., 2014).

The application of the seaweed extract based biostimulant and Azoxystrobin increased chlorophyll and carotenoids in accordance with results reported on wild rocket by Candido et al. (2022) and Schiattone et al. (2021). Similarly, Kulkarni et al. (2019) observed chlorophyll and carotenoids increase in spinach after seaweed extract application. Jannin et al. (2013) reported that biostimulating compounds based on seaweed extracts favor the biosynthesis of chloroplasts and the reduction of chlorophyll degradation.

Strobilurins also favor the increase in chlorophyll, as observed by other authors (Bonasia et al., 2013; Liang et al., 2018), which can be attributed to the reduction of the synthesis of ethylene, a hormone known to be involved in the processes of senescence and degradation of chlorophyll (Abeles et al., 1992), and the increase of cytokinins, involved in the biosynthesis of chlorophyll (Sundqvist et al., 1980).

The Azoxystrobin and the biostimulant based on seaweed extract resulted in an increase in total phenols and antioxidant activity. Notoriously, this last parameter is closely related to the phenolic content since phenols are among the main antioxidant compounds present in the plant.

The experimental findings confirmed the increase in phenols as a response to the application of seaweed extracts (Battacharyya et al., 2015), as for example on spinach (Rouphael et al., 2018), cherry (Correia et al., 2020; Gonçalves et al., 2020), soybean (do Rosário Rosa et al., 2021), common bean (Kocira et al., 2020) and cucumber (Trejo Valencia et al., 2018).

Following the application of Azoxystrobin, Conversa et al. (2014) observed an increase in phenols on baby leaf spinach, while Bonasia et al. (2013) did not observe any effects.

Leaf nitrate content was below the limits imposed by the EU (Reg. UE n. 1258/2011) in all treatments. This confirms the results obtained on wild rocket by Schiattone et al. (2017) and demonstrates that, in the climatic conditions of southern Italy, even in greenhouse where the light intensity is reduced, the problem of high nitrate content is more limited, compared to the northern latitudes. In these environments, the scarce availability of light and the low air temperature slow down the nitrogen metabolism favouring the increase of nitrates in plant (Bonasia et al., 2017; Weightman et al., 2012).

Among the treatments studied, the water deficit and the increase in nitrogen level favored a greater accumulation of nitrates in the leaves of the wild rocket. This is consistent with the literature data, according to which the water deficit reduces the activity of nitrate reductase (Santamaria et al., 1999; Weightman et al., 2012), as well as the higher N content in the rhizosphere favors its absorption by the roots and, therefore, its accumulation in the aerial part (Chen et al., 2004; Devienne-Barret et al., 2000; Weightman et al., 2012).

The application of the seaweed extract based biostimulant and Azoxystrobin reduced the leaf nitrates. This can be attributed to the better physiological conditions of the treated plants which had a greater photosynthetic activity. In the case of Azoxystrobin, the results of this study agree with several other studies that attribute to Azoxiytrobin a stimulating action of nitrato reductase activity (Bonasia et al., 2013; Conversa et al., 2014; Joshi et al., 2014).

5. Conclusions

The research focused on the biostimulating effect of seaweed extract (SW) and Azoxystrobin (AZ) in combination with two irrigation regimes and three nitrogen levels, to improve the production of wild rocket. As it was expected, the water deficit negatively affected yield of wild rocket, however improving some qualitative features such as phenols, carotenoids and antioxidant activity, worsening others such as dry matter and nitrates. Yield increased with the raise in the N level, but the latter worsened the effects of the water deficit and some qualitative parameters such as phenols, antioxidant activity, carotenoids and nitrate content.

The tested biostimulants showed good efficacy on yield for increasing the number of leaves (SW) or their size (AZ). Furthermore, they reduced the negative effects of water and nitrogen deficit. The application of two biostimulants has improved the quality of wild rocket for the increase of phenols, antioxidant activity, carotenoids and chlorophyll, and the reduction of nitrates.

Biostimulating products based on Azoxystrobin or seaweed extracts can be a beneficial strategy for wild rocket growers to increase yield, improve quality and limit the negative effects of water deficit. It should be emphasized that Azoxystrobin is a pesticide already marketed for the fight against various fungal diseases. Therefore, its use can be promoted as an alternative to other fungicides, given the complementary biostimulating action.

Further research is desirable to evaluate the optimal doses and methods of application of the two biostimulants tested. In addition, further studies should be carried out to identify the active compound(s) of the seaweed extract.

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

The data that has been used is confidential.

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