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ORIGINAL PAPER

Root-promoting Biostimulant Enhances Salinity Tolerance in Wild and Cultivated Rocket Salads

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Abstract

Rocket salads (*Diplotaxis* spp. and *Eruca* spp.) are leafy vegetables appreciated for their typical taste and nutritional value. When exposed to salt stress, these plants undergo morpho-physiological and metabolic changes. The aim of the study was to investigate the efficacy of a "root-promoting biostimulant" (Radifarm®) applied during germination (Experiment 1) and during the growth cycle (Experiment 2) on two rocket species under salt stress. Experiment 1 explored if Radifarm® can protect seed from salt stress in early-stage development. Different salt levels (0, 150 and 200 mM NaCl) were combined with different Radifarm® concentrations (0, 0.5, 1, 2.5, 5 mL L^{-1}). Experiment 2 investigated how Radifarm® can promote plant growth after transplantation when irrigated with saline water (0, 150, and 200 mM NaCl) until harvest. Experiment 1 showed that salt stress significantly affected the germination of rocket salads. The addition of Radifarm® did not improve the germination of *D. tenuifolia* grown under any salt conditions, but it was beneficial for *E. sativa* when the highest level of Radifarm® was applied. In Experiment 2, the application of Radifarm® significantly reduced the symptoms of salt stress in both species. In *E. sativa*, salt stress affected all growth parameters (plant height, leaf number and area). However, under 200 mM NaCl, plants fully recovered when Radifarm® was applied. The same recovery was observed for chlorophyll content in both species. Radifarm® also contributed to increase protein and lipid content compared to plants under salt stress. This study showed that Radifarm® was able to protect both species from salt stress.

Highlights

- Salinity resistance of rockets was evaluated at germination and maturity stages.
- Radifarm® biostimulant induced salt tolerance in wild and cultivated rockets.
- Biostimulant-treated rockets showed increased protein and lipid content.

Keywords *Diplotaxis tenuifolia, Eruca sativa* · NaCl · Radifarm®, abiotic stress

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1 Introduction

Abiotic stress is a serious constraint on crop productivity, causing up to 50% of production losses in most major crops (Bulgari et al. 2019a; Bray et al. 2000). Salinity is considered one of the most important stress factors affecting both crop yield and quality worldwide (Arzani 2008). Nowadays, 1.1 × 109 ha of land is affected by salinity and this value is supposed to increase up to 1.5 million ha annually (Hossain 2019). Salinity could be generated through natural processes (primary) or could be induced by human related activity such as fertilization and wastewater treatment (secondary salinity) (Daliakopoulos et al. 2016). In recent years, under extreme weather conditions, salt intrusion is consistently affecting coastal agriculture (Tarolli et

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al. 2023). Overall, plants under salt stress exhibit several disorders that have a significant impact on crop and on plant food productivity. Many physiological processes, such as seedling germination, plant growth and development, photosynthesis and water/nutrient uptake could be negatively affected by salinity (Mishra et al. 2023; Shaheen et al. 2013; Pompeiano et al. 2014; Shayyad-Amin et al. 2016). Obviously, the type and the level of stress symptoms occurring in plants depend on crop type, growth stage and the specific sensitivity to salinity conditions. In addition, salt stress generates oxidative stress and the production of reactive oxygen species (ROS) (Colla et al. 2010), which activate the complex abiotic stress response in the plant. Salt-tolerant plants overcome salt stress involving multiple and complex physiological, molecular and genetic networks, contributing to: (a) reduce the concentration of salt ions, (b) increase and/or production of compatible solutes, (c) protect plant cellular alteration (such as membrane structure), (d) induce abiotic response of secondary metabolites (i.e. hormones, antioxidants) (Gupta and Huang 2014; Cavaiuolo et al. 2015; Hao et al. 2021). Beside the primary salinization also the irrational use of fertilizer can induce soil salinization causing a reduction of crop productivity (Rütting et al. 2018). Hence, multiple strategies are necessary to improve crop production, crop residue utilization and soil fertility management.

Rocket salads, *Eruca sativa* (L.) DC and *Diplotaxis tenuifolia* (L.) DC, are annual species belonging to the Brassicaceae family and are mainly cultivated in the Mediterranean regions. Their nutritional value, high content of vitamin C, mineral salts and peculiar bitter taste caused by health-promoting molecules (glucosinolates) are just some of the characteristics responsible for the growing interest in these vegetables (Hnilickova et al. 2017). Rocket salads as well as several Brassica species are considered moderately salt tolerant (Shariatinia et al. 2021). However, different levels of tolerance are reported based on species, cultivar and even stage of development (Hnilickova et al. 2017). In literature, NaCl has been reported to cause severe damage to *D. tenuifolia* at the germination stage (reduction in the percentage of germinated seeds), while fewer stress symptoms occur during full plant development (Bianco and Boari 1996). In contrast, in *E. sativa* (cv "Folha Larga" and "Cultivada"), the increase in salinity induces a reduction in fresh and dry biomass in susceptible cultivars (Jesus et al. 2015), while no loss of biomass occurred in the more tolerant cultivar (Ashraf 1994).

Salinity has also been shown to affect numerous biochemical functions of *D. tenuifolia* and *E. sativa*, such as metabolism and nutrient uptake, which in turn affects nutrient quality and the content of phytochemicals in the edible part of the plant. For example, the content of glucosinolates clearly depends on the growing conditions: it increases after a moderate salt treatment and decreases at higher NaCl levels (Petretto et al. 2019). In view of the increasing challenges posed by salt stress in agriculture, exploring innovative strategies to improve the resilience of plants has become essential. Among all, the use of biostimulants is drawing attention. Biostimulants are substances known for their ability to stimulate natural processes in plants inducing positive effects on plant growth, nutrition, and biotic and abiotic stress tolerance. It has been reported that the application of biostimulants is a strategy that can improve plant tolerance to salt stress (Emilia et al. 2020; Campobenedetto et al. 2021; Rouphael et al. 2022) and could contribute to protect crops under salt stress from yield losses (Yakhin et al. 2017; Gedeon et al. 2022). Based on the European Biostimulants Industry Council (EBIC), Radifarm® (Valagro, Atessa (Chieti) Italy) is classified as a biostimulant for crop and ornamental species (Abdelkader et al. 2021; Parađiković et al. 2017). Its composition includes:

- total Nitrogen (N): 3.0%.
- organic Nitrogen (N): 1.0%.
- ureic Nitrogen (N): 2.0%.
- potassium oxide (K_2O): 8.0%.
- water soluble organic Carbon (C): 10.0%.
- chelated Zinc EDTA (Zn): 0.1%.
- vitamins.
- amminoacids and proteins.
- polisaccarides.
- betaines.
- saponins.

Overall plant responds to salinity activating morphological, anatomical and ultrastructural modifications. As reported by Arif et al. (2020), roots play an important role in salinity tolerance; root diameter, length, and number during salinity stress increase in order to improve water and nutrient uptake during the abiotic stress. Radifarm® is reported as biostimulant for the overcome of the transplantation shock in crop species, because it induces a faster recover of plant growth and development in transplanted plants (Dong et al. 2020). The information available on Radifarm® about the stimulant effect on root development seems to be promising in cultivation areas affected by salinization. The aim of the present research was: (i) quantifying the effect of the application of Radifarm® on the germination of two rocket salads (*Eruca sativa* and *Diplotaxis tenuifolia*) under increasing salt concentration of the irrigation water and (ii) quantifying the effect of the application of different doses of Radifarm® at rocket salads transplantation on harvestable biomass and the quality of biomass. The periodic application of Radifarm® is reported to improve the full attachment of transplanted or replanted plants, but limited information is available on the

impact of Radifarm® in salt stress tolerance acquisition. To address this gap of information, *E. sativa* and *D. tenuifolia* species were tested under salinity in presence or absence of Radifarm®. The main hypothesis is that treating rocket species with Radifarm ® a raising of plant salt tolerance can occur. This research present with two main objectives: (1) test wild and cultivated rocket response to increasing salt concentration in irrigation water during germination (with and without Radifarm®); (2) test the effect of Radifarm® in salt tolerance acquisition and in nutritional value of the two species at the harvest of plant. Two different experiments were conducted to answer the different research questions. The effect of Radifarm® was evaluated in Experiment 1 by measuring germination and seedling parameters, while in Experiment 2 the biomass yield, secondary metabolites, nutrients content in the leaves at the time of harvest were measured.

2 Materials and Methods

2.1 Plant Material and Growing Conditions

Diplotaxis tenuifolia L. cv Dragon Tongue was purchased from Mexfi Graines while *Eruca sativa* (Mill.) cv. Nemat seeds derived from the CREA-CI collection (Lazzeri et al. 2013). Both seeds were used in Experiment 1 and 2.

2.2 Experiment 1: Germination Experiment

E. sativa (ES) e *D. tenuifolia* (DT) seeds were incubated at RT (22 ± 1 °C) for 1 h in a water suspension at 5 concentrations of Radifarm®, using the biostimulant as priming treatment:

-water: rad0 (Control).
-0.5 mL L^{-1} of biostimulant: rad1.
-1.0 mL L^{-1} of biostimulant: rad2.
-2.5 mL L^{-1} of biostimulant: rad3.
-5.0 mL L^{-1} of biostimulant: rad4.

Seeds were then washed with sterile water using a double layer of sterile gauze (Medicomp 10 × 10 cm) as support to prevent their leakage. After filtration seeds were placed on double-layered Whatman No. 1 filter paper moistened with 2 mL of distilled sterile water within 90-mm diameter plastic Petri dishes; plates were then incubated in dark hoven at two different temperatures, based on the species needs: 20 ± 1 °C and 25 ± 1 °C for ES and DT, respectively. Seeds were irrigated with the following solutions:

-Tap water: salt0.
-150 mM NaCl: salt150.
-200 mM NaCl: salt200.

Overall, Experiment 1 was laid out as a completely randomized design with two factors: (1) biostimulant and (2) salt concentration in irrigation water. Factor 1 had five levels already mentioned above, while factor 2 had three salt levels above described. For each dish 20 seeds were placed, and each dish was replicated 5 times. Dishes were irrigated every 3 days (ES) and every 2 days (DT) according to the saline treatment assigned.

2.2.1 Germination Parameters and Seedlings Biometric Traits

Germination of both species was monitored daily, and the following indexes were calculated: germination percentage (G), and synchrony of the germination process (Z).

Germination percentage (G) was calculated as:

$$G = \frac{\sum_{i=1}^{k} n_i}{N} \times 100$$

n_i = number of seeds germinated at interval i and N = total number of seeds germinated.

Synchrony of the germination process (Z) was calculated as:

$$Z = \frac{\sum_{i=1}^{k} C_{ni,2}}{c_{\sum_{i=1}^{k} ni,2}}$$

where $C_{ni,2}$ is the combination of the seeds germinated in the main time, two by two, thus ranging between 0 (when at least two seeds could germinate, one at each time) and 1 (when germination of all seeds occurs at the same time).

Beside germination parameters also plant development (number of germinated seeds developed in seedlings, expressed as %) (PD), seedling length (mm) (SL), average fresh weight (g) of seedling for each plate (FW) and the number of secondary roots (SR) for seedling were recorded.

2.3 Experiment 2: Transplantation Experiment

Greenhouse experiment was conducted in winter/spring 2020 in Sassari, Sardinia, Italy (IT) at the Ottava experimental station of the University of Sassari (40◦46′ 47” N, 8◦29′ 45” E). Greenhouse was unheated and the average minimum air temperature during the trial was 5 °C while the average maximum air temperature was 16 °C, and the average heliophany was ~421.45 min/day. Seeds were sown in trays (84 holes) filled with peat soil with the addition of

1.72 g of diammonium-phosphate 18-46-0. The amount of fertilizer used in the plug-trays was set based on the nutrient requirements of rocket salads in field trials (100 kg/ha) and was provided with a proportioned amount of mineral fertilizer 18-46-0, adjusted for the plug-trays area. Once plants had reached stage 13–14 of BBCH (Lancashire et al. 1991), they were transplanted with the entire soil adhering to the roots. Each experimental unit consisted of a plastic fruit box (30×50×15 cm) lined with non-woven fabrics and filled with 5 kg of Brill® Semina (Agrochimica), reaching the height of 10 cm of substrate for each experimental unit. Twelve plants were transplanted per each experimental unit keeping distance of 7 cm on the same row and 10 cm between rows. The experiment was laid out as a completely randomized design with two factors: biostimulant and irrigation water. Two levels of biostimulant were set: presence of absence. When Radifarm® was applied, it was added only once at the transplant stage, distributing 250 mL of Radifarm® solution (5 mL L^{-1} - rad4) directly as foliar spray. Considering the quite fast-growing cycle and the cost of Radifarm® application we decided to apply the biostimulant only one time in order to contain the cost of wild and cultivated rocket product. The biostimulant concentration was selected based on the manifold instruction of the product. Three levels of irrigation water were set after the application/not application of Radifarm®: 0, 150 mM, 200 mM NaCl. Following is reported the irrigation solutions used in Experiment 2, in absence (T1-T3) or presence (T4-T6) of Radifarm®:

Treatment 1 (T1): 0 NaCl + 0 Radifarm®.
Treatment 2 (T2): 150 NaCl + 0 Radifarm®.
Treatment 3 (T3): 200 NaCl + 0 Radifarm®.
Treatment 4 (T4): 0 NaCl + Radifarm®.
Treatment 5 (T5): 150 NaCl + Radifarm®.
Treatment 6 (T6): 200 NaCl + Radifarm®.

The same design was applied to the two species: *E. sativa* (ES) and *D. tenuifolia* (DT). Transplanted plants were irrigated twice a week with 500 mL of irrigation solution based on the treatment used (Supplementary Table 1). The Experiment 2 ended when plants reached the commercial maturity (BBCH 19), 40 days after transplantation.

2.4 Biometric Traits and Biomass Production

To monitor both rocket salads growth and development, the following biometric parameters were recorded twice a week until the plants were ready for the cut: plant height, PH (cm); leaf number, LN. At the end of the experiment the following parameters were added: Leaf area LA (cm^2) which included the whole leaves per plant and LA/LN (cm^2) considered as the ratio between the LA and the NL per plant. LA (cm^2) was measured using a planimeter (LI-COR, model 3100 area meter). For LA and LA/LN, four replicates per experimental unit were used. At the end of the experiment, both leaves fresh weights (LFW), and dry weights (LDW) were recorded and expressed in grams (g) in order to evaluate plant biomass. Dry weight was obtained after drying the plant tissue for 72 h at 60 °C.

2.5 Optical Sensor for Measurement of Relative Chlorophyll and Flavonoid Content

Relative chlorophyll content (CHL), flavonoid (FL) content and Nitrogen balance index (NBI) were recorded once a week using Dualex™ (FORCE-A, Orsay, France). The relative CHL and FL content in leaves were estimated by the instrument based on the CHL fluorescence excitation spectra (Cerovic et al. 2005). For each three fully expanded and healthy leaves per plants were performed four measurements. Four plants per each experimental unit were involved in the weekly measurement.

2.6 Leaf Nutritional Parameters

2.6.1 Reagents and Standards

Concentrated sulfuric acid 96%, orthophosphoric acid 85%, potassium chloride, phenol, copper sulfate, sodium hydroxide, sodium potassium tartrate (all of RPE ACS Reagent Ph Eur grade), and methanol (LC-MS grade) were purchased from Carlo Erba (Val de Reuil Cedex, France). Chloroform (RPE ACS Reagent Ph Eur grade), sodium carbonate, Folin-Ciocalteau reagent, standards of gallic acid, albumin, and vanillin, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (conductivity lower than 18.2 MΩ) was distilled and filtered through a Milli-Q system (Millipore, Bedford, MA, USA).

2.6.2 Sample Preparation

For chemical analyses, both rocket salad samples were freeze-dried using a freeze dryer LIO 5P DGT (Cinquepascal, Trezzano s/Naviglio (MI)). Lyophilized samples were thinly pulverized in a home-style coffee grinder and mixed thoroughly. The samples were stored in the dark at room temperature and under low RH conditions until the preparation of the extracts.

2.6.3 Determination of Carbohydrate

Total carbohydrates were determined by colourimetric analysis according to the slight modification protocol of

Dubois et al. (1956). Freeze-dried and pulverized samples (10 mg) were extracted with 10 mL of distilled water. The suspension was kept in an ultrasonic bath for 30 min. The extract or standard (glucose) (200 µl) were added to 200 µl of phenol 5% (w/v) and 1 mL of sulfuric acid concentrated. After 30 min of incubation (room temperature), the optical density (OD) was measured against a blank at 490 nm using a spectrophotometer Varian Cary 50 and 1 cm wide disposable cuvettes. Quantitative analysis was carried out using an external standard calibration method with glucose as the standard. All the analyses were conducted in triplicate, and the results were expressed in mg/kg FW ± SD of glucose.

2.6.4 Determination of Total Protein

Protein determination was performed according to the protocol of Lowry et al. (1951) with slight modification. Sample (10 mg) was extracted with 10 mL of distilled water. The suspension was shaken with a vertical mixer. The extract (500 µl) or standard (albumin) were added to 500 µl of sodium hydroxide 1 N for 5 min at 100 °C. To the sample were added 2.5 mL of sodium carbonate 5% (w/v), copper sulphate 0.5% (w/v) and sodium potassium tartrate 1% (w/v). After 10 min were added 500 µl of Folin-Ciocalteau reagent 1 N. After 30 min of incubation (room temperature), the OD was measured against a blank at 750 nm using a 1 cm wide disposable cuvettes. Quantitative analysis was carried out using an external standard calibration method (albumin). The results were expressed in mg/kg FW ± SD of albumin.

2.6.5 Determination of Lipids

Lipids extraction was carried out by combining and adapting to the samples the protocols of Chen and Vaidyanathan (2013) and Bligh and Dyer (1959). Sample (15 mg) was added with 100 µL di PBS and 1.5 mL of sodium hydroxide 1 N containing 25% of methanol. The suspension was shaken with a vertical mixer and heated at 100 °C. The sample was centrifuged, 1 mL of supernatant was withdrawn and added to methanol/chloroform 1:2 (v/v) and 0.5 mL of potassium chloride 0,88%. The suspension was shaken with a vertical mixer and centrifuged. Lipids determination was performed according to the slight modification protocol (Mishra et al. 2014). One mL of chloroform phase was dried and then added 100 µL of concentrated sulfuric acid for 10 min at 90 °C. The sample was added to 2.4 mL of phospho-vanillin reagent 68% (w/v). After 10 min of incubation (room temperature), the OD was measured against a blank at 530 nm using a 1 cm wide disposable cuvettes. Quantitative analysis was carried out using an external standard calibration method (oil containing 100% fat). The results were expressed in mg/kg FW ± SD of oil.

2.6.6 Determination of Total Polyphenols

Total polyphenols determination was performed according to the slight modification protocol of Singleton and Rossi (1965). 250 µl of the extract or 100 µl standard (gallic acid) were added to 500 µl of Folin-Ciocalteau reagent for 5 min (room temperature); after were added 3 mL of sodium carbonate 10% (w/v) and ultrapure water to a volume of 10 mL. After 90 min of incubation (room temperature), the OD was measured against a blank at 725 nm using a 1 cm wide disposable cuvettes. Quantitative analysis was carried out using an external standard calibration method (gallic acid). The results were expressed in mg/kg FW ± SD of gallic acid.

2.7 Statistical Analysis

Statistical analyses were performed using RStudio Team (2020) (packages lme4, emmeans, multicomp). Given the heteroscedasticity of studied variables after Bartlett's test, these were processed using a generalized linear model with a quasi-Poisson distribution using a logit link function in the case of FW, DW, PH, SL, SR CHL, FL, NBI, LA and LA/LN. Conversely, a quasi-binomial distribution using a logit link function was used for Z, G and PD. The significance of the differences between the mean values of the treatments was assessed using Tukey's test at $P < 0.05$.

3 Results

3.1 Experiment 1. Germination and Seedling Development as Influenced by Salt and Biostimulant Treatments

Different germination results were observed for the two rocket species. Germination of ES ended after 8 days, while it ended after 12 days in DT. The percentage of germination (G) decreased significantly for both species with increasing NaCl concentration in the irrigation water (Fig. 1). In ES, the lowest G was found under 200 mM with almost all levels of Radifarm® (from rad0 to rad3). The lowest G (24%) was measured when rad2 was added to 200 mM water. The only increase in G was registered under salt200 with rad4 (76% of G). Under 150 mM, G showed no significant differences according to biostimulant level (average G of 86.6%). The highest G was recorded under salt0 and rad4 (98%). For salt0, no significant difference was observed with the addition of different levels of biostimulants, resulting in an average G of 96%. DT was very sensitive to salinity. Under both salt150 and salt200, G was not statistically different regardless the amount of biostimulant tested and was generally low (between 3% and 11% of G). Under salt0, G increased,

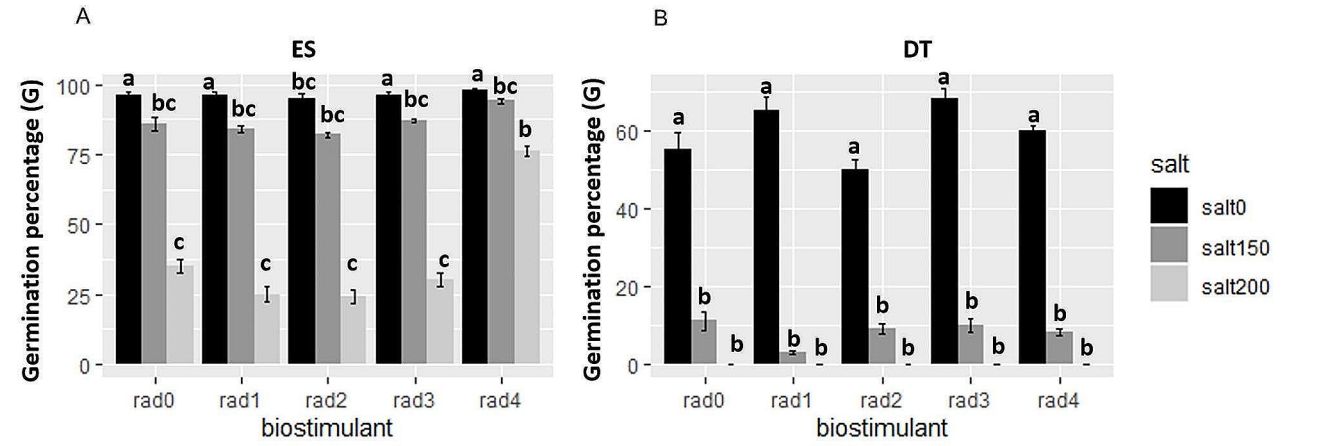

Fig. 1 Effect of different saline irrigations and biostimulant concentration on germination percentage (G) of *Eruca sativa* (ES) (**A**) and *Diplotaxis tenuifolia* (DT) (**B**) seeds. Germination percentage (G) is reported on the y-axis, while the biostimulant levels are reported in x-axis. Three salt levels, 0; 150 mM NaCl; 200 mM NaCl (salt0, salt150 and salt200 respectively) were used during the whole experiment (see figure legend). Five levels of Radifarm® 0; 0.5; 1.0; 2.5; 5.0 mL L^{-1} (rad0, rad1, rad2, rad3 and rad4 respectively) were tested for each treatment; see material and methods for further details. Vertical bars represent standard error, and different combinations of lower-case letters indicate significantly differing means ($P < 0.05$, Tukey's Test)

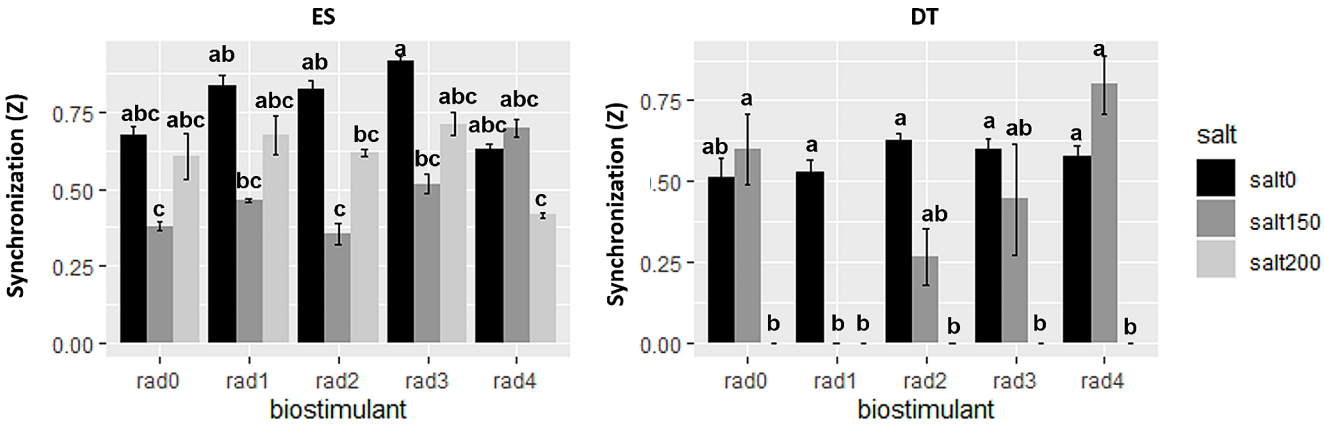

Fig. 2 **Fig.2.**Effect of different saline irrigations and biostimulant concentrations on synchronization (Z) of germination process of *Eruca sativa* (ES) (A) and *Diplotaxis tenuifolia* (DT) (B) seeds. Synchronization (Z) of germination process is reported on the y-axis, while the biostimulant levels are reported in x-axis. Three salt levels, 0; 150 mM NaCl; 200 mM NaCl (salt0, salt150 and salt200 respectively) were used during the whole experiment (see figure legend).Five levels of Radifarm®; 0.5; 1.0; 2.5; 5.0 mL L^{-1} (rad0, rad1, rad2, rad3 and rad4, respectively); see material and methods for further details. Vertical bars represent standard error and different combinations of lower-case letters indicate significantly differing means ($P < 0.05$, Tukey's Test)

but no statistical differences were observed with respect to biostimulant concentration. G ranged from 47 to 68%.

About synchrony of the germination process (Z) (Fig. 2), the values in ES changed as a function of both salt concentration and biostimulant level. Overall, the highest value was found under salt0 and rad3 while the lowest values were recorded under salt150 when rad0 and rad 2 were applied and under salt200 in combination with rad4. In DT, the effect of Radifarm® on Z was evident under salt150. Indeed, its application contributed to increase synchrony. Unfortunately, Radifarm® application did not contribute to protect seeds from salt stress during germination.

Table 1 reported the seedling parameters recorded. In ES, in presence or in absence of Radifarm® (at all levels) a significant reduction of PD, SL, SR and FW was observed as a consequence of the salt treatments (salt150; salt200). The number of developed seedlings over the total number of seeds (PD) ranged from a minimum of 45% (rad1, salt200) to a maximum value of 100% (rad1, salt0; rad3, salt0 and rad4, salt 0). Overall, no significant difference was found between water and salt150 treatments' with or without Radifarm®. A wide variation of seedling length (SL) was observed among the different treatments. SL ranged from 8.5 mm (rad2, salt200) to 139.96 mm (rad0, salt0). A significant reduction of SL was observed from water to salt

Table 1 Biometric seedling parameters evaluated in *Eruca Sativa* (ES) and *Diplotaxis tenuifolia* (DT). In the table are reported the effect of different treatment with Radifarm® (0, 0.5, 1, 2.5, 5 mL L^{-1}, respectively from rad0 to rad4) and salinity level of irrigation water (water, 150 mM and 200 mM NaCl) on plant development (PD) (%), seedling length (SL, in mm), number of secondary roots (SR), seedling fresh weight (FW in g). Each species has been analyzed separately. Parameters are expressed as average ± standard deviation. Different letters show significant differences ($p < 0.05$) after the Tukey HSD test for post hoc comparisons for each species

Species	Treatment	Salinity	PD (%)	SL (mm)	SR	FW (g)
ES	rad0	Water	90.00 ± 5.48e	139.96 ± 29.37f	2.00 ± 0.20ab	0.322 ± 0.170 cd
		150 mM NaCl	70.00 ± 12.45de	62.14 ± 16.03bc	3.20 ± 0.17ab	0.150 ± 0.080bc
		200 mM NaCl	55.00 ± 12.75bc	12.94 ± 6.55a	0.00a	0.035 ± 0.020a
	rad1	Water	100.00 ± 6.52e	114.44 ± 52.87f	4.40 ± 0.51ab	0.300 ± 0.134 cd
		150 mM NaCl	75.04 ± 6.12de	71.34 ± 9.89c	6.60 ± 0.91ab	0.182 ± 0.107bc
		200 mM NaCl	45.00 ± 13.69a	10.10 ± 6.82a	0.00a	0.036 ± 0.024a
	rad2	Water	95.02 ± 8.66e	135.4 ± 10.73af	7.60 ± 0.51b	0.360 ± 0.031 cd
		150 mM NaCl	85.01 ± 4.47de	69.18 ± 6.11c	4.60 ± 0.16ab	0.209 ± 0.010c
		20 0mM NaCl	20.03 ± 12.45ab	8.50 ± 5.42a	0.00a	0.072 ± 0.078ab
	rad3	Water	100.00 ± 5.48de	126.64 ± 19.38de	5.00 ± 0.10ab	0.344 ± 0.032 cd
		150 mM NaCl	85.01 ± 6.52de	58.56 ± 12.15bc	1.09 ± 0.08ab	0.201 ± 0.038bc
		200 mM NaCl	50.01 ± 12.45ec	10.08 ± 3.68a	0.00a	0.042 ± 0.012a
	rad4	Water	100.00 ± 2.74e	138.68 ± 17.77ef	5.60 ± 0.28ab	0.410 ± 0.047d
		150 mM NaCl	95.00 ± 4.18de	93.48 ± 27.19d	7.50 ± 0.68ab	0.318 ± 0.083 cd
		200 mM NaCl	70.00 ± 9.62d	53.9 ± 12.41b	4.50 ± 0.80ab	0.211 ± 0.042bc
DT	rad0	Water	55.00 ± 17.32 cd	27.03 ± 3.99c	0.00	0.040 ± 0.002abc
		150 mM NaCl	11.00 ± 10.25b	0.00a	0.00	0.002 ± 0.000ab
		200 mM NaCl	0.00a	0.00a	0.00	0.000a
	rad1	Water	65.00 ± 16.96d	30.76 ± 5.97c	0.00	0.060 ± 0.002bc
		150 mM NaCl	2.00 ± 0.73ab	0.46 ± 0.06a	0.00	0.001 ± 0.000a
		200 mM NaCl	0.00a	0.00a	0.00	0.000a
	rad2	Water	43.00 ± 5.88c	17.86 ± 1.67b	0.00	0.028 ± 0.001ab
		150 mM NaCl	7.00 ± 0.58ab	1.08 ± 0.41a	0.00	0.003 ± 0.000 a
		200 mM NaCl	0.00a	0.00a	0.00	0.000a
	rad3	Water	68.00 ± 13.50 cd	37.86 ± 2.13c	0.00	0.140 ± 0.017c
		150 mM NaCl	6.00 ± 0.51ab	1.62 ± 0.70a	0.00	0.003 ± 0.000ab
		200 mM NaCl	0.00a	0.00a	0.00	0.000a
	rad4	Water	60.00 ± 6.12 cd	27.98 ± 8.60c	0.00	0.043 ± 0.001abc
		150 mM NaCl	7.00 ± 0.58ab	0.76 ± 0.07a	0.00	0.030 ± 0.000ab
		200 mM NaCl	0.00a	0.00a	0.00	0.000a

200 at all levels of rad treatments. Evaluation of the number of secondary roots (SR) and fresh weight (FW) produced quite similar results. Indeed, SR ranged from 0 (rad 0,1,2,3 at salt200) to 7.60 (rad2, salt0); while the FW varied from 0.035 g (rad0, salt0) to 0.410 g (rad4, salt0). The addition of Radifarm® led to an increase of SR in rad4, compared to the control condition (rad0, salt0). In presence or in absence of salt, when rad4 was applied SR showed no significant difference among the treatments. However, in presence of rad3 and rad4, no significant differences were found between water and salt treated plants, while salt200 still induced a significant reduction of FW in both Radifarm® levels.

In DT, the addition of Radifarm® showed no significant effect on almost all seedling parameters considered. The clearest effect was observed in the FW. In fact, FW ranged from 0 g (rad0-rad5, salt200) to 0.140 g (rad3, salt0). In the presence of rad4, an increase in FW was observed at salt150 compared to salt0.

3.2 Experiment 2

3.2.1 Effect on Plants' Growth in Greenhouse Experiment

Overall, the addition of Radifarm® mitigated salt stress symptoms in both species (Table 2; Fig. 3). In ES, the irrigation with NaCl had a significant effect on PH, LN and LA/LN, significantly decreased their values. The addition of Radifarm® (T4-T6) in presence of salt reduce salt stress even under salt 200.

In ES, both FW and DW showed a strong decrease in biomass under T2 and T3. FW under 150 mM and 200 mM NaCl dropped from 8.35 g (T1) to 1.05 g and 1.12 g in T2 and T3, respectively. Similarly, DW shifted from 0.97 g (T1)

Table 2 Biometric data result of *E. sativa* (ES) and *D. Tenuifolia* (DT) irrigated with three salt levels (NaCl 0, 150 mM and 200 mM), in presence and absence of biostimulant (Radifarm®). Each combination of salt and biostimulant was indicated by a code (T1 to T6). In the table are reported the average value and the standard deviation of 3 plant height (PH, in cm), leaf number (LN), fresh and dry weight (FW and DW respectively, in g) and leaf area for leaf (LA/LN)(cm^2); further details are indicated in material and methods section. Each species has been analyzed separately. Different letters show significant differences ($p < 0.05$) after the Tukey HSD test for post hoc comparisons for each species

Code	Specie	Salt	Biostimulant	PH (cm)	LN	FW (g)	DW(g)	LA/LN
T1	ES	Water	-	17.12 ± 1.93d	8.25 ± 1.93c	8.35 ± 0.99d	0.97 ± 0.04c	15.29 ± 4.71d
T2		150mM_NaCl	-	7.25 ± 0.35a	4.00 ± 0.35a	1.05 ± 0.36a	0.08 ± 0.02a	2.52 ± 0.24a
T3		200mM NaCl	-	6.90 ± 0.53a	3.33 ± 0.53a	1.12 ± 0.36a	0.16 ± 0.01a	3.75 ± 0.32a
T4		Water	Radifarm®	14.05 ± 0.82c	8.50 ± 0.82c	3.91 ± 0.67c	0.50 ± 0.02b	11.11 ± 1.24 cd
T5		150mM NaCl	Radifarm®	10.82 ± 0.99b	6.00 ± 0.99b	1.59 ± 0.43ab	0.25 ± 0.04ab	5.96 ± 0.90ab
T6		200 Mm NaCl	Radifarm®	12.12 ± 0.2bc	8.25 ± 2.02c	3.23 ± 0.61bc	0.46 ± 0.07b	8.29 ± 1.92bc
T1	DT	Water	-	11.95 ± 0.49b	10.50 ± 0.49b	4.73 ± 0.76c	0.45 ± 0.01c	6.26 ± 0.74c
T2		150mM NaCl	-	8.56 ± 0.95a	4.00 ± 0.95a	1.05 ± 0.01a	0.08 ± 0.02a	1.55 ± 0.02a
T3		200mM NaCl	-	7.40 ± 0.84a	4.00 ± 0.84a	1.74 ± 0.23ab	0.16 ± 0.01ab	2.19 ± 0.22a
T4		Water	Radifarm®	10.77 ± 0.79b	9.67 ± 0.79b	2.25 ± 0.09b	0.44 ± 0.02c	4.41 ± 0.46b
T5		150mM NaCl	Radifarm®	10.50 ± 1.11b	11.67 ± 1.11b	1.51 ± 0.27ab	0.22 ± 0.08b	5.07 ± 0.43bc
T6		200mM NaCl	Radifarm®	8.45 ± 1.26a	9.25 ± 1.26b	1.14 ± 0.43ab	0.17 ± 0.08ab	4.12 ± 0.15b

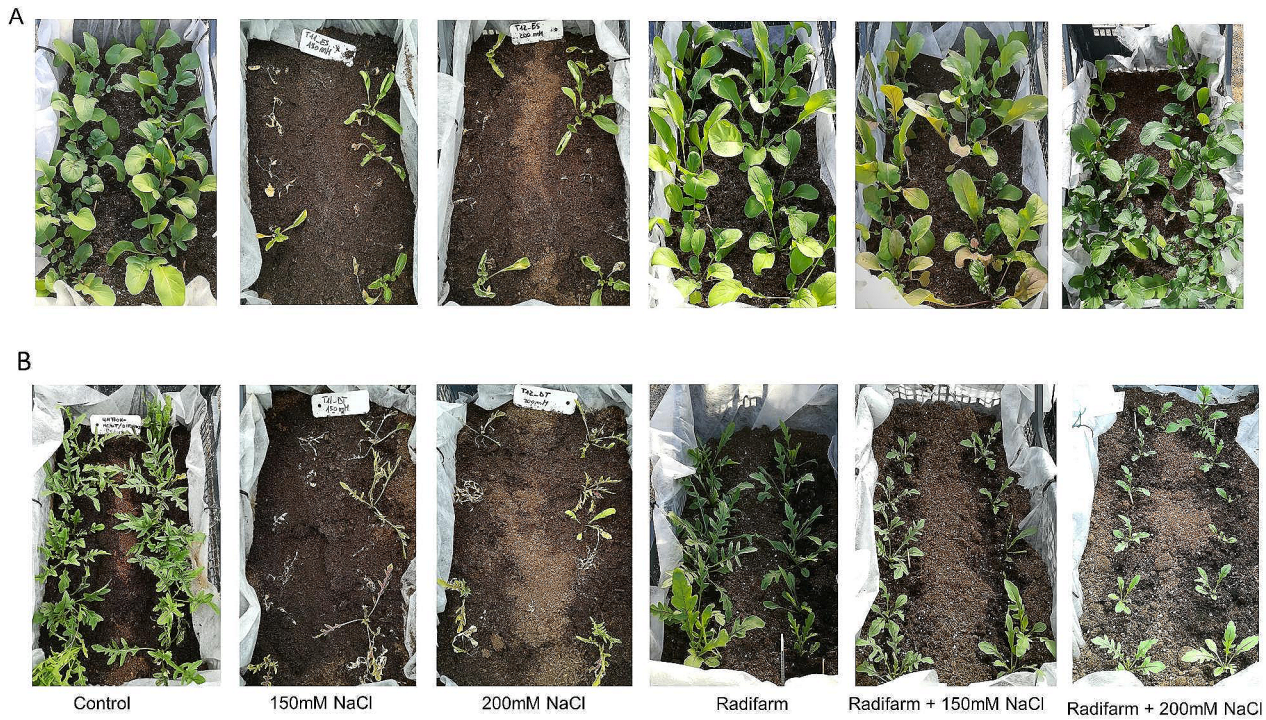

Fig. 3 Effect of different saline irrigations and biostimulant on *Eruca sativa* (ES) (**A**) and *Diplotaxis tenuifolia* (DT) (**B**) growth. Two saline levels (NaCl 150 mM and 200 mM) and water (control) solutions were used during the whole experiment. The effect of the addition of Radifarm® was tested in both species in presence and absence of salinity

to 0.08 and 0.16 g in T2 and T3, respectively. The addition of Radifarm® induced a significant increase of FW and DW under T6 compared to T2 and T3 treatments. Comparable results were observed in DT. The only difference between the two species was the response to salt. Under T2 and T3, DT showed a reduction in PH, LN, FW, DW and LA/LN compared to T1. The addition of Radifarm® contributed to a partial recovery of PH, LN and LA/LN, mainly under 150 mM NaCl (T5). In both species, the LA followed a very similar trend of LA/LN (supplementary Table 2). The application of Radifarm® improved DW in ES under T5 and T6 compared to T2 and T3, while in DT this increase was significant only between T2 and T5.

Table 3 Secondary metabolites result of *E. sativa* (ES) and *D. Tenuifolia* (DT) irrigated with three salt levels (NaCl 0, 150 mM and 200 mM), in presence and absence of biostumulant (Radifarm®). Each combination of salt and biostimulant was indicated by a code (T1 to T6). In the table are reported the average value and the standard deviation of Dualex indices: chlorophyll content (CHL), flavonoids (FL), nitrogen balance index (NBI), further details are indicated in material and methods section. Each species has been analyzed separately. Different letters show significant differences ($p < 0.05$) after the Tukey HSD test for post hoc comparisons for each species

Code	Specie	Salt	Biostimulant	CHL (Dualex units)	FL (Dualex units)	NBI (Dualex units)
T1	ES	Water	-	35.90 ± 3.28e	0.82 ± 0.01a	43.54 ± 4.08d
T2		150 mM_NaCl	-	22.50 ± 0.30b	1.05 ± 0.07b	21.50 ± 1.69b
T3		200 mM NaCl	-	12.40 ± 1.54a	1.46 ± 0.21c	8.77 ± 2.37a
T4		Water	Radifarm®	30.30 ± 1.10d	0.86 ± 0.09a	35.66 ± 3.81c
T5		150 mM NaCl	Radifarm®	27.00 ± 1.06 cd	0.86 ± 0.12a	31.85 ± 4.08c
T6		200 mMaCl	Radifarm®	26.50 ± 2.13c	1.08 ± 0.03b	24.56 ± 1.79b
T1	DT	Water	-	30.30 ± 2.47 cd	0.85 ± 0.01b	35.77 ± 3.34c
T2		150 mM NaCl	-	17.50 ± 0.41b	1.31 ± 0.18c	13.50 ± 1.43b
T3		200 mM NaCl	-	14.00 ± 0.37a	2.71 ± 0.03d	5.28 ± 0.33a
T4		Water	Radifarm®	32.80 ± 3.47d	0.79 ± 0.02ab	41.86 ± 4.40d
T5		150 mM NaCl	Radifarm®	28.80 ± 1.69c	0.85 ± 0.05b	34.26 ± 4.04c
T6		200 mM NaCl	Radifarm®	29.10 ± 1.74c	0.77 ± 0.01a	37.93 ± 2.31 cd

Table 4 Average value and standard deviation of protein, carbohydrates, lipids and total polyphenols in *Eruca Sativa* (ES) and *Diplotaxis tenuifolia* (DT) under three salt levels (water, 150 mM and 200 mM of NaCl) in the presence or in the absence of biostimulant (Radifarm®). Each combination of salt and biostimulant was indicated by a code (T1 to T6). Results were expressed as mg/kg of FW (mean + SD; $n = 3$), further details are indicated in material and methods section. Each species has been analyzed separately. Different letters show significant differences ($p < 0.05$) after the Tukey HSD test for post hoc comparisons for each species

Code	Specie	Salt	Biostimulant	Protein (mg/kg FW)	Carbohydrates (mg/kg FW)	Lipids (mg/kg FW)	Total polyphenols (mg/kg FW)
T1	ES	Water	-	37672.6 ± 3073.5d	4776.9 ± 119.7a	4000.1 ± 271.9 cd	3844.7 ± 402.1c
T2		150 mM NaCl	-	29936.6 ± 224.8bc	4894.4 ± 458.2a	3507.4 ± 148.2b	4043.7 ± 103.4c
T3		200 mM NaCl	-	-	-	-	-
T4		Water	Radifarm®	22091.4 ± 610.2a	23424.6 ± 144.1b	2998.7 ± 99.2a	2231.7 ± 34.3a
T5		150 mM NaCl	Radifarm®	26948.3 ± 1136.6b	23361.3 ± 481.5b	3812.0 ± 94.3bc	2790.1 ± 168.2b
T6		200 mM NaCl	Radifarm®	34467.5 ± 4471.4 cd	24714.5 ± 307.4c	4285.9 ± 322.8d	2590.1 ± 174.9ab
T1	DT	Water	-	23540.8 ± 2089.4a	3566.6 ± 147.3b	2756.3 ± 85.6b	1552.2 ± 72.1a
T2		150 mM NaCl	-	31236.9 ± 374.1b	4113.4 ± 44.5b	3213.1 ± 24.0c	2185.3 ± 189.0b
T3		200 mM NaCl	-	24567.3 ± 1305.3a	1779.3 ± 144.0a	2508.4 ± 27.4a	1457.2 ± 122.6a
T4		Water	Radifarm®	33394.9 ± 3457.4b	16272.8 ± 1232.4e	3226.8 ± 49.6c	4317.5 ± 427.7d
T5		150 mM NaCl	Radifarm®	59717.3 ± 502.6d	12933.7 ± 382.1d	4260.6 ± 41.7d	4770.6 ± 180.4d
T6		200 mM NaCl	Radifarm®	49812.4 ± 1313.6c	9711.4 ± 365.9c	3133.2 ± 259.4c	3592.5 ± 193.7c

3.2.2 Pigment Contents

In both species, chlorophyll content (CHL) was significantly affected by salinity, with a significant reduction (> 50%) under T3 in both species (Table 3). When Radifarm® was added under salt stress (T5 and T6), in both species an increase of CHL content was observed compared to T2 and T3 treatment. Furthermore, in DT, CHL under T5 and T6 did not show any difference compared to the control (T1).

Differences in leaf flavonol (FL) content were observed between control (T1) and salt treatments (T2 and T3) in both species. Indeed, FL increased when increasing salt stress was applied reaching the maximum value of 1.46 and 2.71 under 200 mM NaCl (T3) in ES and DT respectively. In ES, FL increased by 50% from 0.82 (T1) to 1.46 (T3), while in DT it shifted from 0.85 (T1) to 2.71 (T3). Overall, Radifarm® application contributed to a reduction of FL for both species between T2 and T5. In both species, FL under T5 was not statistically different from that registered under T1. The NBI value consistently decreased as a consequence of NaCl stress (T2 and T3) in both species. Radifarm® application in ES determined an increase of NB1 under T5 compared to T1. Radifarm® application to DT determined a NBI increase, so the value registered under T5 and T6 were similar to that registered under T1.

3.2.3 Nutritional Value of Leaves

Table 4 reported the most important nutrient values determined in the species analysed. Proteins, carbohydrates,

lipids and total polyphenols, were quantified in the leaves of both rocket species under all experiments except for *E. sativa* in the T3 treatment due to the plant stress symptoms that were so severe that the plant material was insufficient for their determination. Results show that differences between parameters are species- and treatment-specific. In fact, each parameter shows a different trend depending on the NaCl in the irrigation water and the presence or absence of the biostimulant Radifarm®. In ES case the results show that, in the absence of a biostimulant, salinity had a significant effect on the proteins and lipids, resulting in a lower concentration two parameters. For proteins, a decrease of approximately 20% was recorded under T2 treatment (29936.6 ± 224.8 mg/kg FW) if compared to the control T1 (37672.6 ± 3073.5 mg/kg FW). For lipids, a smaller reduction was recorded with an analyte concentration lower than 12% in the 150 mM NaCl treatment (3507.4 ± 148.2 mg/kg FW) compared to the control T1 (4000.1 ± 271.9 mg/kg FW). Whilst, under 150 mM salinity level, the carbohydrates and total polyphenols content remained unchanged. Interestingly, the combined effect of Radifarm® and salt led to an increase in protein and lipid content, even if the presence of the biostimulant determines a decrease in the initial concentration, as can be seen from the results obtained for T4 (22091.4 ± 610.2 mg/kg FW vs. 37672.6 ± 3073.5 mg/kg FW for protein and 2998.7 ± 99.2 mg/kg FW vs. 4000.1 ± 271.9 mg/kg FW for lipids). Only carbohydrates have a significant increase in their concentration recorded following treatment with Radifarm®. Carbohydrate content, in fact was increased by around 500% (23424.6 ± 144.1 mg/kg FW of T4 vs. 4776.9 ± 119.7 mg/kg FW of T1). Contrary to the other parameters, carbohydrates are not affected by the combination of NaCl and biostimulant, as their concentration remains almost unchanged. Only a moderate increase was recorded in a higher salinity level (200 mM), reaching a value of 24714.5 ± 307.4 mg/kg FW.

In contrast to macronutrients, Radifarm® affected negatively TP accumulation in the leaves, resulting in a significant decrease (over 30%) in all treatments. Among Radifarm® treatments, TP content increased under 150 mM and 200 mM NaCl, with a maximum at a moderate salinity level (21.1 ± 2.9 mg/kg FW). As the results show, DT plants showed a different behavior under salinity and Radifarm® treatment. Protein content increased when treated with the intermediate salinity level (150 mM), reaching 31236.9 ± 374.1 mg/kg FW, but didn't change in 200 mM NaCl treatment, where the amount was 24567.3 ± 1305.3 mg/kg FW, statistically similar whit that of the control (23540.8 ± 2089.4 mg/kg FW). The same trend was recorded for total polyphenols, whose concentration increased by 35% under 150 mM treatment (2185.3 ± 189.0 mg/kg FW) and was statistically similar to the Control (1552.2 ± 72.1 mg/kg FW) under higher salinity level (1457.2 ± 122.6 mg/kg FW). Carbohydrates amounts changed differently with the salinity treatment. Plants grown at 150 mM NaCl (4113.4 ± 44.5 mg/kg FW) showed similar concentrations of carbohydrates compared with those of control plants (3566.6 ± 147.3 mg/kg FW) and significantly decreased at the highest salinity concentration (1779.3 ± 144.0 mg/kg FW). Lipids content significantly increased in plants treated with 150 mM NaCl (3213.1 ± 24.0 mg/kg FW) and decreased in plants grown at 200 mM NaCl (2508.4 ± 27.4 mg/kg FW). For DT plants, treatment with Radifarm® resulted in an increase in the concentrations of all macronutrients, recording a 40% and 18% variation for proteins (33394.9 ± 3457.4 mg/kg FW) and lipids (3226.8 ± 49.6 mg/kg FW) respectively if compared to the experiment carried out in absence of Radifarm® (T1). For carbohydrates and TP, we recorded the greatest variation with increases of 180% and 350% for TP (4317.5 ± 427.7 mg/kg FW) and carbohydrates (16272.8 ± 1232.4 mg/kg FW), respectively compared to the experiment carried out in the absence of Radifarm® (T1) (1552.2 ± 72.1 mg/kg FW for TP and 3566.6 ± 147.3 mg/kg FW for carbohydrates). The combination of Radifarm® and NaCl resulted in a synergistic effect, leading to an increase in all macronutrient levels across all treatments. Notably, we observed an about twofold increase in protein levels in the samples treated with NaCl at 150 mM and 200 mM (T5-T6) compared to those without NaCl (T4) and without Radifarm® (T2-T3). For carbohydrates, although an increase in their concentration was recorded in all treatments conducted with Radifarm® compared to the corresponding experiments conducted in the absence of biostimulant, the results show that the combination with NaCl decreases their concentration in increasing salinity, reaching 12933.7 ± 382.1 mg/kg FW and 9711.4 ± 365.9 mg/kg FW at 150 mM and 200 mM respectively. The lipid and TP content in plants treated with Radifarm® was higher for all saline treatments, following a similar trend observed for the proteins and recording the highest concentration under 200 mM NaCl with 4260.6 ± 41.7 mg/kg FW and 4770.6 ± 180.4 mg/kg FW for lipids and TP, respectively.

4 Discussion

Salt stress is a major abiotic stress that significantly affects plant growth, inducing important reduction of crop yield (Yadav et al. 2020). In general, salinity tolerance is higher during the vegetative stage and lower during the germination/seedling and flowering phase (Bulgari et al. 2019a; Negrão et al. 2017). In the present study, ES and DT were exposed to three salinity levels from germination to harvest,

to evaluate their response from the early stage of development to the harvest.

In this study, *E. sativa* (ES) and *D. tenuifolia* (DT) were irrigated with NaCl and treated with Radifarm®, a biostimulant produced by Valagro s.p.a. that promotes root growth and helps plants overcome transplant stress (Dong et al. 2020). The analysis, conducted in two development stages of ES and DT, showed that the application of Radifarm®, a product commonly used to promote a successful transplant, affect the plant tolerance to salinity with different response based on the species and the biostimulant level (for Experiment 1). The two studied rocket salads exhibited different susceptibility to salt stress and recovery capability after Radifarm® application. DT showed a significant reduction in germination percentage (G) under salt150, with a dramatic effect under salt200, while ES showed a significant reduction in G only under salt200. Previous studies have reported significant intra and interspecific variation in salt tolerance within the Brassicaceae family (Kumar et al. 2009; Shariatinia et al. 2021). For example, Fallahi et al. (2015) reports a significant reduction in germination percentage even at lower salinity levels (50mM NaCl), while Miceli et al. (2003) observed higher tolerance in the early stages of cultivated rocket development at 10ds/m. At this stage, ES appeared to be the most responsive to the biostimulant treatment and more tolerant to salinity.

In the second experiment, the prolonged exposition to saline irrigation (150 and 200 mM NaCl) of the two species clearly showed an opposite trend compared to the germination experiment results. These findings agree to the research finding observed by Shariatinia et al. (2021) in ES collection. In our data the germination salinity tolerance alone is not a suitable stage to select salinity tolerance in rocket, therefore we also monitored physiological and biochemical parameters in Experiment 2.

The addition of Radifarm® in both species in combination with the two salt levels showed specific biometric and biomass responses in ES and DT (Experiment 2). In particular, in ES, Radifarm® increased some biometric parameters such as plant height (PH) and leaf number (LN) in all salt levels treatments, suggesting its role in supporting plant protection against the stress. Previous studies conducted by Zeljković et al. (2010) have already shown that the application of Radifarm® (after the transplantation) on basil, significantly improved its growth and development. In that study, the application of Radifarm® induced an increase of root growth that could be one of the major factors associated with the general improvement of the plant growth. The effect of Radifarm® was more evident in DT, clearly inducing a complete recovery of plant growth and leaves number. The enhancement of plant growth based on these two parameters could be associated to a more efficient DT nutrient uptake from soil. Campobenedetto et al. (2021) reported an increase in tomato growth as a consequence of biostimulant application under salt stress. Furthermore, Bulgari et al. (2019b) observed an improvement in plant growth after the application of protein hydrolyzed, suggesting a mitigation effect of the biostimulant on salt stress symptoms in lettuce. In the present study, the Radifarm® composition, rich in vitamins, amminoacids and proteins, polysaccharides, betaines, saponins, could have had two effects on rocket: species firstly, reducing plant salt sensitivity and secondly, improving the root development with a consequent increase of plant ability to catch macro and micronutrient from soil. ES showed a partial improvement of the biometric values without reaching the control condition status. In addition, a general decrease of fresh and dry weigh was observed in both species in all treatments observed compared to the management with fresh water, but Radifarm® application in presence of irrigation with salty water was able to protect from yield losses. Leaf area (LA) is a critical commercial parameter for vegetable crop species such as lettuce and rocket. ES and DT present different LA already in control conditions, with a higher value in ES than in DT. As reported by Urlić et al. 2017 in presence of Na and salinity stress, rocket reduce the leaf area in sensitive species to salinity. In our study, both species exhibited a significant reduction of LA and LA/LN proportionally to the salt stress level applied. Furthermore, the application of Radifarm®, in presence of the two salt levels, significantly increased the LA and LA/LN compared to 150 NaCl + 0 Radifarm (T2) and 200 NaCl + 0 Radifarm (T3) treatments. These data suggested that Radifarm® could contribute to the salt tolerance acquisition in the selected rocket species. Beside LA also the chlorophyll content plays a key role in plant tolerance to salt stress because it is associated to the leaf photosynthetic activity under stress conditions. We observed that both species presented a significant reduction of chlorophyll content because of salt level. These findings agree to previous studies conducted in several plant species (Nasrudin et al. 2022; ALKahtani et al. 2020). A general oxidative stress status and an increase of peroxidase and chlorophyllase activity are reported to be the two main events that occur in chlorophyll loss in salt-sensitive plant (Saxena et al. 2020). Furthermore, a stable content of chlorophyll could be a symptom of plant salt tolerance (Nounjan et al. 2020). The addition of Radifarm® induces a significant increase of the chlorophyll contents in both species. A similar trend was observed monitoring the Nitrogen balance index (NBI) parameter. In addition, a significant growing accumulation of flavonoid (FL) was observed under 150 and 200 mM NaCl compared with control, in both ES and DT. Several studies (Bibi et al. 2019; Hossain et al. 2022; Sarker et al. 2023), reported that secondary metabolites, such as polyphenols (including flavonols) increase as response of

salinity stress. As a consequence of the Radifarm® application also flavonols significantly decreased despite the salt level. These data were also confirmed by the NBI values. NBI, which is the ratio between the chlorophyll and epidermal flavonoids, also decreased as response to salt stress suggesting a lower N content under saline conditions (Sawariya et al. 2023) and increased as consequence of Radifarm® treatment.

Rocket is an important agricultural crop because of the nutritional and functional value of its leaves, which are an excellent source of macronutrients and antioxidant compounds. Due to its popularity as a green salad, retaining the amount of these compounds has important economic and health-related implications. Overall, the level of nutrients compound in plant can vary with cultural practices, genotype and pedoclimatic conditions (Afsar et al. 2020; Schiattone et al. 2017). Previous studies reported that salt stress induce multiple cellular disorder that range from cellular ions imbalance, ion toxicity, and osmotic stress that cause overproduction of reactive oxygen species (ROS) (Rady et al. 2018, 2019). This cellular state induces DNA damage and the degradation of lipid, protein, and chlorophyll content. In our study, almost all investigated nutritional parameters were significantly influenced by treatments with NaCl and Radifarm®. The effects of salt stress were more evident in ES, which did not survive under 200 mM of NaCl, than DT. The decrease of protein and lipid content of ES and DT, as effect of the highest salt level, agreed to previous study conducted by El Arroussi et al. (2018) in tomato. The protein content reduction under NaCl stress, could be related to the decrease of nitrate reductase enzyme activity (Meloni et al. 2004). In addition, considering that the nitrate assimilation is a key factor in the protein biosynthesis, the minor activity (under salt stress) of nitrogen assimilation it's probably one of the main causes of protein content reduction under severe salt stress.

Similarly to protein, also lipid content in both species showed a significant reduction under 200mM NaCl treatment. Lipid content is strongly influenced by the presence of ROS generated by the NaCl treatment (Taïbi et al. 2016). Under salt stress, lipid peroxidation occurs as a consequence of ROS in cell. Malondialdehyde (MDA), used as lipid peroxidation marker, have been shown to increase under salinity, indicating a general reduction of lipid content (Taïbiet al. 2016; Morales and Munné-Bosch 2019).

In term of carbohydrate in the present research, we observed no significant difference between control and moderate NaCl level (150mM NaCl). This data is compatible with a moderate tolerance to salinity of ES and DT species; on the other hand, an increase of carbohydrate content under NaCl 150mM followed by a significant reduction of this parameter was observed in DT under 200mM NaCl. As reported by Kerepesi and Galiba (2000), high level of carbohydrates, and in general their accumulation in plant, is associated with plant tolerance to salinity. In fact, the increase concentration of solutes in plant tissue (including sugars), can contribute to plant tolerance to stress conditions (Tester and Davenport 2003). Previous studies (Kerepesi and Galiba 2000; Almodares et al. 2008) showed that salt tolerant genotypes have the ability to accumulate more soluble carbohydrates compared to the susceptible one; this data will probably explain the increase level of carbohydrate under NaCl 150mM in DT. Obviously, the salinity tolerance in plant species has a threshold, above that level all plant tolerance mechanism collapse. This mechanism explains the reduction of carbohydrates at NaCl 200mM.

Finally in term of total polyphenols (TP) a similar trend to carbohydrate was found, with no significant difference in ES plant treated with NaCl and a moderate increase of TP at NaCl150mM in DT. Di Mola et al. (2023), reported that TP could largely varied based on genotype, species, harvest plant stage and level of salinity. In particular, our results confirm a reduction of TP at the highest salt level as reported by Kusvuran and Ellialtioglu 2021 in rocket.

Although ES and DT responded differently to Radifarm® treatment, overall, we observed that the Radifarm® addition induced a partial mitigation of salt stress symptoms associated to most of the nutritional parameters explored in both species. Proteins, carbohydrates lipids contents are in both species significantly higher to the corresponding saline treatment in absence of biostimulant. As suggested by Van Oosten et al. (2017), the acquisition of plant resistance to salinity under biostimulant treatment could be associated to the composition of the biostimulant itself. Radifarm® is rich in amminoacids, proteins, polysaccharides that will absolve different functions: increase the osmoprotection, scavaging the radicals (i.e. ROS), and improve the nutrient ability of plant from soil. These three factors in association with an improvement of chlorophyll content will significantly reduce protein and lipid degradation by ROS and improve the carbohydrate production. The recovery of these three nutritional factors might be associate to the TP at least in DT under NaCl and Radifarm® treatment. In plant the polyphenols compounds are commonly induced as response to abiotic stress such as salt stress (Navarro et al. 2006) and are involved in ROS-scavenging process (Navarro et al. 2006). In our experiment TP increase (mainly in DT) under salt and Radifarm® treatments, suggesting a possible increase, of antioxidant enzyme activities (SOD and CAT enzyme), that could reduce the ROS content and limit the typically unbalanced status of salinity stress conditions. The addition of the bioactive compounds of Radifarm may by responsible of the

improvement of antioxidant enzyme and consequently of a general major tolerance status revealed rocket.

5 Conclusion

In conclusion, this research confirmed our hypothesis that a biostimulant promoting root development could protect rocket salads growth under increasing concentration of salt in irrigation water. Radifarm® application contributed to protecting crop yield under moderate salinity (150 mM NaCl) supporting the plant growth and response to abiotic stress condition. In particular, the application of Radifarm® induced a positive effect on leaf number (LN) and plant height (PH) in *D. tenuifolia* (DT) plants treated with 150mM NaCl. These data suggest that the two species had a different response to salinity x Radifarm®. Nutritional value of wild and cultivated rocket under saline irrigation was also positively affected by the presence of Radifarm®, suggesting that the plant biostimulants can represent a valid strategy for enhancing crop quality and yield of vegetable crop species. The present study suggested that even one application of Radifarm® can affect the plant response to salinity at different levels (growth, pigments and nutritional aspects). Further studies are necessary to evaluate the potential salt tolerance effect of Radifarm® in other crop species and/or, the effect of multiple applications of the product during the entire plant development stages.

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Author Contributions Sara Melito: Conceptualization, methodology, investigation, data curation, original draft-writing; Giorgia Sarais: methodology, investigation, data curation, writing: review and editing; Debora Dessì: investigation, writing: review and editing; Antonietta Santaniello: methodology, writing: review and editing; Giovanni Povero: methodology, writing: review and editing; Giovanni Piga: investigation, writing: review and editing; Vittoria Giannini: Conceptualization, methodology, investigation, data curation, formal analysis, writing: review and editing. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict-of-interest.

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