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Growing Conditions Affect the Phytochemical Composition of Edible Wall Rocket (*Diplotaxis erucoides*)

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Abstract: Wall rocket (*Diplotaxis erucoides*) is a wild vegetable with the potential to become a crop of high antioxidant quality. The main bioactive compounds include ascorbic acid (AA), sinigrin, and a high content of total phenolic compounds (TP). It also accumulates nitrates. Since these compounds are affected by environmental conditions, adequate crop management may enhance its quality. Eleven accessions of wall rocket were evaluated under field and greenhouse conditions during two cycles (winter and spring) and compared to *Eruca sativa* and *Diplotaxis tenuifolia* crops. The three species did not differ greatly. As an exception, sinigrin was only identified in wall rocket. For the within-species analysis, the results revealed a high effect of the growing system, but this was low among accessions. The highest contents of AA and TP were obtained under field conditions. In addition, the levels of nitrates were lower in this system. A negative correlation between nitrates and antioxidants was determined. As a counterpart, cultivation in the field–winter environment significantly decreased the percentage of humidity (87%). These results are of relevance for the adaptation of wall rocket to different growing conditions and suggest that the field system enhances its quality. The low genotypic differences suggest that intra-species selections in breeding programs may consider other aspects with greater variation.

Keywords: ascorbic acid; *Diplotaxis erucoides*; field; greenhouse; new crops; nitrates; sinigrin

1. Introduction

Modern societies have become increasingly aware of the importance of diet as part of a healthy lifestyle. Thus, many consumers look for additional health benefits to be obtained from specific foods, which are known as functional foods [1,2]. On the other hand, some consumers are demanding products with new and differentiated aromas and tastes to enrich daily dishes and increase the culinary experience [3]. These demands offer an opportunity for the enhancement of wild edible plants (WEPs). In fact, several WEPs have high bioactive properties and may be considered as potential functional foods [4,5], while they have also differentiated organoleptic characteristics that are highly appreciated [6]. Apart from the direct harvest from the wild, a promising strategy for such revalorization could be domestication and adaptation into cultivation systems; this is an alternative that offers several advantages such as better yields, uniformity and accessibility [3].

Mediterranean cultures have a rich ethnobotanic knowledge and tradition in the consumption of WEPs, as has been compiled in many works (e.g., [7–9]). These reports show a great diversity of WEPs

that have potential as new crops, including the edible *Diplotaxis erucoides* (L.) DC. (wall rocket). Wall rocket is an annual plant from the *Brassicaceae* family, broadly distributed along the Mediterranean areas of Europe and Africa to the Middle East [7]. Considered as a weed for many crops, the species is also appreciated as a wild vegetable for its tender leaves, with a characteristic pungent flavor, and also for its flowers as decorative elements. Wall rocket is eaten fresh or cooked, added to salads, soups, pasta dishes or even fried in omelettes [8,9]. One commercial variety of wall rocket is currently available (var. Wasabi, Shamrock Seed Company, Inc.), but as far as we know, its cultivation is negligible.

Wall rocket is taxonomically related to the popular rocket crops *Eruca sativa* Mill. (salad rocket) and *D. tenuifolia* (L.) DC. (wild rocket). These crops accumulate large amounts of phytochemicals including vitamin C, phenolic compounds and glucosinolates [10] and could therefore be considered for in vivo models and clinical assays addressed to test their potential bioactive properties. In fact, both vitamin C and phenolic compounds are potent antioxidants against plant oxidative stress, and such compounds could be also involved in reducing the risk of different illnesses such as cardiovascular diseases, hepathotoxicity and general inflammation risks [11–14]; in addition, vitamin C is an essential microelement with antiscorbutic activity [15]. Glucosinolates (GSLs) are secondary metabolites from *Brassicaceae* and other families within the *Brassicales* order [16]. The enzymatic hydrolysis of GSLs releases volatile compounds that are responsible of the bitter and pungent flavor of *Brassicaceae* species [17]. In addition, different compounds in this class have been analyzed in terms of their potential health benefits using in vivo models, as reviewed by Dinkova-Kostova and Kostov [18]. Together with the bioactive compounds, rocket crops also accumulate high amounts of nitrates [19,20], considered to be antinutrients with potential health risks [21]. Thus, maximum levels are established for the commercial production of rocket crops (*E. sativa* and *Diplotaxis* sp.) and other vegetables in Europe [22].

Rocket crops are cultivated under field and greenhouse conditions [23] and can be grown in the Mediterranean regions for most of the year. As a result, these crops are subjected to growth under variable agronomic and environmental factors that include temperature, length and incidence of sunlight, irrigation, soil type, or time of harvest, among others. These environmental changes can affect the accumulation of bioactive compounds [24]. For instance, an increase of light intensity and photoperiod can decrease the content of nitrates [25]. Stresses such as heat shock, chilling or high light conditions activate the accumulation of protective phytochemicals such as ascorbic acid or phenolic compounds [26]. Abiotic stresses such as growing under non-optimal temperature conditions can increase the content of glucosinolates as well [27].

Although these are general behaviors, information related to the effect of cultivation on wall rocket is scarce. Ceccanti et al. [4] suggested that, as part of the breeding programs of wild edible plants into new crops, it is important to study the proper cultivation practices to allow large-scale, high-yield production and, at the same time, ensure a good-quality product including nutritional quality. In this sense, testing different growing environments may lead to identifying the most adequate conditions for the development of wall rocket as a crop. In addition, the use of a local germplasm may help to establish the crop in the Mediterranean regions, as these materials would have been naturally selected for its adaptation to these conditions [28]. Thus, the current study aimed to analyze the effect of growing systems (greenhouse and field) and cycles (winter and spring) on selected compounds of relevance including ascorbic acid, sinigrin and nitrates, as well as the content of total phenolics, for pre-selected accessions of wall rocket derived from local germplasm. In addition, accessions of *D. tenuifolia* and *E. sativa* were used as reference materials with the aim of contextualizing the values obtained for wall rocket, as the acceptance of a new, differentiated crop will also depend on its recognisable differences from other crops. Overall, the study allows us to gain a general insight of the behaviour of wall rocket as a crop. Moreover, the current study can be useful for establishing a basis for the future exploitation of this emerging crop, which may have a high added value due to its content of bioactive compounds.

2. Materials and Methods

2.1. Plant Material and Cultivation

Ten pre-selected accessions of wall rocket and four commercial cultivars of rocket species were evaluated in the experiment. The pre-selected accessions corresponded to the second generation seedlings from wild populations collected in the Valencian Community (Spain) (Table S1). Seeds are conserved at the Universitat Politècnica de València (UPV, Valencia, Spain), where a domestication program is being developed. The commercial cultivars (from Shamrock Seed Co., Salinas, CA, USA) included the species *D. tenuifolia* (var. SSC2402 and var. Wild Rocket), *E. Sativa* (var. S. Rocket SSC2965), and the only commercial variety of *D. erucoides* that, to our knowledge, is currently available (var. Wasabi).

The experiments were performed at the UPV following the same experimental design as described in Guijarro-Real et al. [29]. Thus, two independent growing cycles were evaluated: the late autumn–winter season (hereafter called the winter season) and late winter–early spring season (hereafter called the spring season). In each cycle, assays were simultaneously carried out in two cultivation systems: a heated glasshouse (39°29′0″ N, 0°20′26″ W) and an experimental field under an anti-pest mesh (39°28′56″ N, 0°20′11″ W).

First of all, seeds were treated with a pre-germinative treatment in order to break the possibly secondary dormancy and increase the germination uniformity [30]. Thus, seeds were treated with commercial sodium hypochlorite 2.5% (v/v) for 5 min plus gibberellic acid 100 ppm (Duchefa Biochemie, Haarlem, The Netherlands) for 24 h. Treated seeds were sown in commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany) and placed in a growing chamber with long day conditions (16/8 h, 25 °C) for two days. For materials used in the greenhouse system, sowing was directly performed in 40 × 25 cm^2 trays, in which plants remained for the entire experiment; plants used for the field system were instead sown in seedling trays.

Two days after being sown, trays were moved to a greenhouse. Trays used for the greenhouse system remained in these conditions during the entire experiment. In contrast, plants used in the field system were allowed to grow in the greenhouse until the appearance of the second true leaf and then were transplanted to the field until the end of the experiment. In both the greenhouse and field systems, the same experimental design was followed: a complete randomized block design with five blocks, with each block including one replicate of 30 plants per accession. This totals 8400 plants used for the experiments performed.

2.2. Preparation of Samples

All plants in each replicate were harvested together as a pool, except for plants with visible growing damages (e.g., a very small size compared to the average of the block) that were discarded. Samples were processed on the same day as harvesting. One fresh sub-sample was used for the analysis of ascorbic acid, and the rest were frozen at −80 °C and then lyophilized. The difference between the weight before and after lyophilization was used to calculate the percentage of moisture. The lyophilized material was powdered with a commercial grinder and stored in darkness until being analyzed for total phenolics, sinigrin and nitrates. All results were expressed as contents per each 100 g of fresh weight (FW) using the percentage of moisture for conversion, as this result provides a more appropriate value considering that the product is eaten raw.

2.3. Traits Measured

The content of ascorbic acid (AA) was measured according to Cano and Bermejo [31] with slight modifications. Briefly, 1.0 g of fresh material was homogenized with 5 mL of cold meta-phosphoric acid 3.0% (v/v) for 1 min using a mortar. The aqueous phase was filtered through a 0.22 µm PVDF filter and analyzed on a HPLC 1220 Infinity LC System (Agilent Technologies; Santa Clara, CA, USA) using a BRISA C_{18} column (150 mm × 4.6 mm i.d., 3 µm particle size; Teknokroma; Barcelona, Spain). The mobile phase consisted of methanol: 1% acetic acid (5:95) for 15 min at a flow rate of 1 mL min^{-1}. The

injection volume was 5 µL, and quantification was performed at 254 nm using an external standard calibration of *L*-ascorbic acid (Sigma-Aldrich, Saint Louis, MO, USA).

The content of sinigrin (SIN) was determined as described by Grosser and van Dam [32] with slight modifications. Firstly, 0.1 g of powdered samples was heated for 2 min at 75 °C using a Termoblock TD150 P2 (Falc Instruments, Treviglio, Italy) for myrosinase inactivation [33]. Extraction was then performed using 1 mL of methanol 70% (v/v) for 15 min at 75 °C. After centrifugation, the supernatant was collected. The extraction step was repeated with 1 mL of methanol 70% (v/v) for another 15 min at 75 °C. Both supernatants were mixed and injected into an SPE column containing a DEAE Sephadex anion exchanger (A-25, Sigma-Aldrich, Saint Louis, MO, USA) activated with 20 mM sodium acetate buffer (pH 5.5) and incubated with 20 µL of diluted sulfatase (Sigma-Aldrich, Saint Louis, MO, USA) overnight. Desulphonated sinigrin was eluted with 500 µL plus 500 µL of milliQ water and analyzed using the same HPLC apparatus as for AA analysis and a Luna® Omega C_{18} column (150 mm × 4.6 mm i.d., 3 µm particle size; Phenomenex, Torrance, CA, USA). The mobile phases consisted of acetonitrile (A) and water (B), with the following gradient: from 98% A to 65% A in 35 min, then equilibrated for 5 min to the initial conditions. The injection volume was 10 µL and the flow rate was 0.75 mL min^{-1}. Quantification was performed at 229 nm using desulphoned sinigrin hydrate (PhytoPlan, Heidelberg, Germany) as an external standard.

The content of total phenolics (TP) was determined according to the Folin–Ciocalteu procedure [34] as in Guijarro-Real et al. [35]. For that, 0.125 g of lyophilised material was extracted with 5 mL of acetone 70% (v/v) containing acetic acid 0.5% (v/v) for 24 h under continuous stirring. Aliquots of 65 µL were incubated with 500 µL of diluted Folin-Ciocalteu (1:10; Scharlab S.L., Sentmenat, Spain) for 5 min; then, 500 µL of sodium carbonate 60 g L^{-1} was added and incubated for other 90 min. Quantification was performed at 765 nm in a iMark™ Microplate Reader spectrophotometer (Bio-Rad, Hercules, CA, USA). Chlorogenic acid (Sigma-Aldrich) was used as an external standard and the results were expressed as mg of chlorogenic acid equivalents (mg CAE 100 g^{-1} FW).

Finally, the content of nitrates was determined using a nitrate-selective ion (Crison Instruments S.A., Alella, Barcelona, Spain), with an extraction protocol adapted from Egea-Gilabert et al. [36]. Nitrates from 0.1 g were extracted with 50 mL of distilled water for 15 min under continuous stirring and stabilized with 1 mL of 2 M diammonium sulfate ($(NH_4)_2$ SO_4) buffer at the moment of measurement using the nitrate-selective ion.

2.4. Data Analysis

Data were subjected to a fixed effects model analysis of variance [37] using the Statgraphics Centurion XVII v.17.2 (Statpoint Technologies, Inc., Warrenton, VA, USA). Two different analyses were performed: (1) a comparison among materials from different species, and (2) a comparison among accessions of wall rocket. For the analysis of species, the average values for accession considering the five replicates per environment were used as data. Data were then submitted to a multivariate analysis of variance (ANOVA) and the effects of species (S, corresponding to three levels: wall rocket, wild rocket, salad rocket), environment (E, four levels: greenhouse in winter, field in winter, greenhouse in spring, field in spring) and the S × E interaction were tested. The linear model applied was

$$X_{ijk} = \mu + S_i + E_j + (S \times E)_{ij} + e_{ij(k)}$$

where X_{ijk} is the value for accession k of species i and environment j, µ is the general mean, S_i is the effect of the species i, E_j is the effect of the environment j, $(S \times E)_{ij}$ is the effect of the interaction between species i and environment j, and $e_{ij(k)}$ is the residual error of the accession k. Mean values and standard error were obtained for the three species and significant differences determined using the Student–Newman–Keuls multiple range test ($p = 0.05$).

The analysis of wall rocket aimed to study the presence of differences among accessions and/or among systems, considering each growing cycle independently [29]. Thus, individual data were submitted to a multivariate analysis of variance (ANOVA) and the effects of accession (A, eleven

accessions), growing system (GS, two levels: greenhouse, field) and the A × GS interaction were tested. The linear model applied was

$$X_{ijkl} = \mu + A_i + B_{j(ik)} + GS_j + (A \times GS)_{ik} + e_{ijk(l)}$$

where X_{ijkl} is the value for replicate l of accession i in block j and growing system k, μ is the general mean, A_i is the effect of the genotype i, $B_{j(ik)}$ is the effect of block j for accession i and system k, GS_j is the effect of the growing system j, $(A \times GS)_{ik}$ is the effect of the interaction between accession i and system k, and $e_{ijk(l)}$ is the residual error of the replicate l. Mean values and standard errors were obtained, and significant differences among environments were determined according to the LSD test ($p = 0.05$). Accessions were ranked for their average values of AA, TP, SIN and NO_3^- within each environment, where high levels of AA, TP and SIN and low levels of NO_3^- were a positive trait. These ranks were then used to obtain a global ranking table for the eleven accessions of wall rocket. Finally, the Spearman rank coefficients of correlation (ρ) were calculated for phenotypic ($n = 44$) and environmental ($n = 213$) correlations.

3. Results

3.1. Differences among Materials of Different Species

Wall rocket was compared to the reference materials including two accessions of *D. tenuifolia* and one accession of *E. sativa* in terms of the percentage of moisture and contents of AA, TP and NO_3^-. Differences in the contents of SIN were not analyzed because this compound was only present in wall rocket. A significant effect of the environment was determined in the four traits evaluated (Table 1). This factor was the main contributor to the total sum of squares in all cases, with values ranging between 52.8% (NO_3^-) and 72.6% (TP). On the contrary, the species factor was only significant for the percentage in moisture and the content in AA. Their contribution was, in any case, lower than 10.5%. Finally, a significant S × E interaction was determined for all traits except for the content of AA, and the effect of this interaction accounted for up to 17.7% of the total sum of squares (Table 1).

Table 1. Sum of squares (in percentage, %) and degrees of freedom (*d.f*) for the effects of species (S) with three levels: wall rocket ($n = 44$), wild rocket ($n = 8$) and salad rocket ($n = 4$); environment (E) with four levels: greenhouse–winter, field–winter, greenhouse–spring, and field–spring; S × E interaction and residuals for the percentage in moisture and the content in ascorbic acid (AA), total phenolics (TP) and nitrates (NO_3^-).

Trait	Sum of Squares (%)			
	S	**E**	**S × E**	**Residual**
d.f	*2*	*3*	*6*	*44*
Moisture	4.21 *	56.22 *	17.69 ***	21.88
AA	10.22 ***	59.10 **	5.57 ^{ns}	25.12
TP	0.83 ^{ns}	72.61 **	7.74 *	18.82
NO_3^-	3.86 ^{ns}	52.78 *	13.98 **	29.38

^{ns}, *, ** and *** indicate no significant or significant at $p < 0.05$, 0.01 and 0.001, respectively.

The average values for each trait are represented in Figure 1. The percentage of moisture was close to 90.0% for the three species, with salad rocket displaying the highest values on average. Both wild rocket and wall rocket significantly decreased the moisture of leaves under the field–winter environment, while the percentage in salad rocket was stable for the environments tested. Regarding the content of AA, wall rocket accumulated the highest value on average (70.02 mg AA 100 g^{-1}), at approximately 30% greater than wild rocket. The effect of the environment was similar in the three species, with the greenhouse environments providing the lowest values. As an exception, the accumulation of AA in salad rocket was not affected by the growing system (field or greenhouse) during the spring cycle (Figure 1).

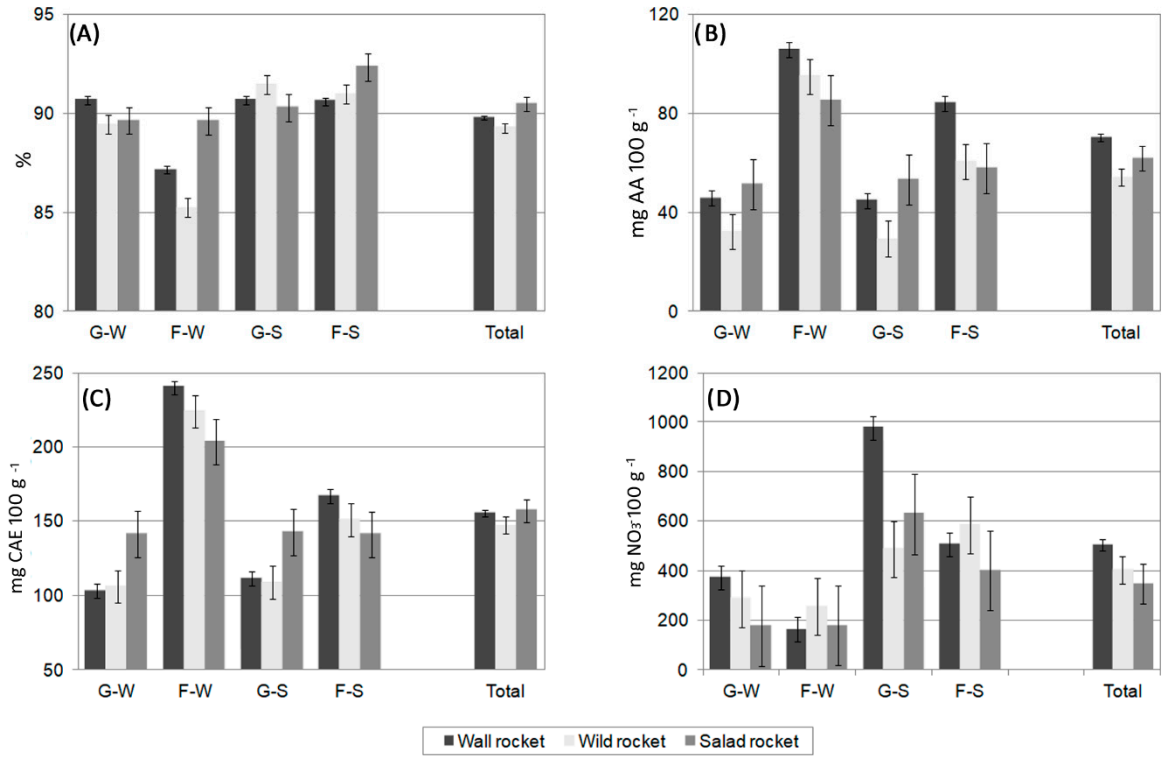

Figure 1. Mean values ± SE for the traits determined in each environment tested (greenhouse–winter, G-W; field–winter, F-W; greenhouse–spring, G-S; field–spring, F-S) for wall rocket (n = 11), wild rocket (n = 2) and salad rocket (n = 1), and global average values: (**A**) Percentage of moisture (%); (**B**) Content of ascorbic acid (AA, expressed as mg in 100 g^{-1} FW); (**C**) Content of total phenolics (TP, expressed as mg of chlorogenic acid equivalents, CAE, in 100 g^{-1} FW); (**D**) Content of nitrates (NO_3^-, expressed as mg in 100 g^{-1} FW).

Although no significant differences were established among species for the contents of TP and NO_3^-, both traits were affected by the S × E interaction (Table 1). The content of TP was not significantly affected by the environment for salad rocket, while the field environments increased the estimated TP in the other species (Figure 1). Finally, none of the three species showed total average values above 600 mg NO_3^- 100 g^{-1}. However, the spring environments significantly increased the accumulation of these ions, with the maximum value obtained for wall rocket growing in the greenhouse–spring environment.

3.2. Variation among Wall Rocket Accessions

3.2.1. Effects of Accession, Growing System and Interaction

The effects of accession (A), growing system (GS) and A × GS interaction were independently analyzed for each growing cycle (Table 2). The winter cycle was highly affected by the growing system for all traits except for the content of NO_3^-. The contribution of this factor to the total sum of squares ranged between 16.5% (NO_3^-) and 81.1% (TP); moreover, this factor was the greatest contributor to the percentage of moisture, AA and TP (>50%). On the contrary, the contribution of the growing system to the total sum of squares was lower during the spring cycle, with percentages significantly decreasing for all traits (Table 2). Moreover, during this cycle, its effect was only significant for the contents of AA and TP. As in the winter cycle, it remained the main contributor to the total sum of squares for both AA and TP, accounting for 52.8% and 57.3%, respectively.

Table 2. Sum of squares (in percentage, %) and degrees of freedom (*d.f*) for the effects of accession (A, $n = 11$), growing system (GS, field or greenhouse), A × GS interaction, block and residuals for the percentage in moisture, ascorbic acid (AA), total phenolics (TP), sinigrin (SIN) and nitrates (NO_3^-) evaluated in the eleven accessions of wall rocket during the winter and spring cycles.

Cycle	Trait	A	GS	A × GS	Block	Residual
d.f		*10*	*1*	*10*	*8*	*80*
Winter	Moisture	3.63 ^{ns}	60.62 ***	2.92 ^{ns}	6.36	26.47
	AA	3.81 *	63.37 ***	3.26 ^{ns}	14.92	14.64
	SIN	10.00 *	35.89 **	4.19 ^{ns}	11.44	38.48
	TP	1.21 ^{ns}	81.11 ***	1.33 ^{ns}	5.42	10.93
	NO_3^-	2.84 ^{ns}	16.55 ^{ns}	3.04 ^{ns}	34.22	43.35
Spring	Moisture	1.72 ^{ns}	0.07 ^{ns}	6.14 ^{ns}	47.30	44.76
	AA	2.75 ^{ns}	52.85 ***	7.00 *	13.63	23.77
	SIN	11.63 ^{ns}	5.10 ^{ns}	14.96 ^{ns}	9.06	59.25
	TP	2.97 ^{ns}	57.35 **	3.54 *	22.76	13.38
	NO_3^-	9.53 ^{ns}	5.04 ^{ns}	9.49 ^{ns}	32.99	42.96

^{ns}, *, ** and *** indicate no significance or significance at $p < 0.05$, 0.01 and 0.001, respectively.

On the other hand, the effect of accession was not significant for most traits in any of the cycles (Table 2). In fact, this factor only affected the contents of AA and SIN during the winter cycle, accounting for 3.8% and 10.0% of the total sum of squares, respectively. In a similar way, the A × GS interaction effects were mostly non-significant (Table 2). As an exception, an interaction effect was determined during the spring cycle for the contents in AA and TP.

3.2.2. Effects of Accession, Growing System and Interaction

The average values and dispersion for the different traits evaluated in each environment are summarized in Figure 2. Results were compared between systems for each cycle, while the indirect effect of the growing period was also evaluated by comparing within systems.

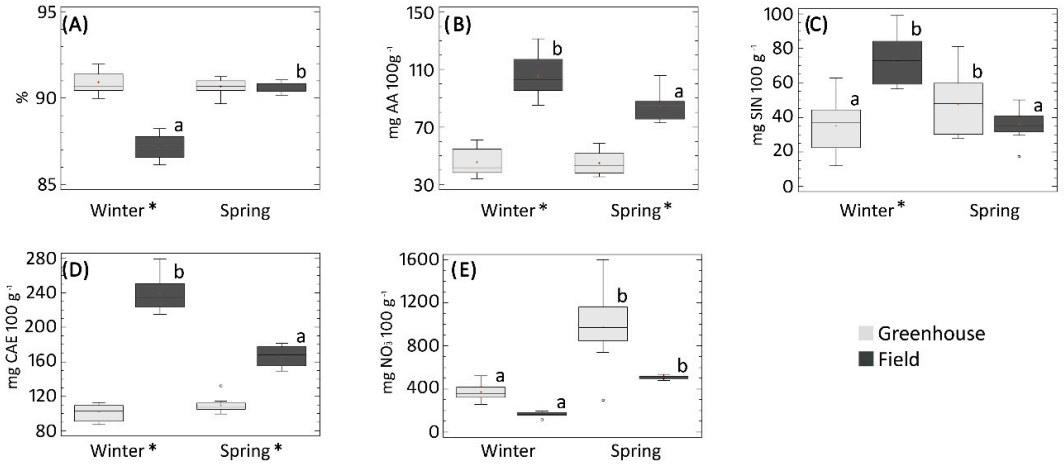

Figure 2. Box and whisker plot reflecting the average values (marked as +) and distribution for the traits evaluated in the accessions of wall rocket ($n = 11$) growing under greenhouse or field systems, during the winter and spring cycles. (**A**) Percentage of moisture (%). (**B**) Content of ascorbic acid (AA, expressed as mg in 100 g^{-1} FW). (**C**) Content of sinigrin (SIN, expressed as mg in 100 g^{-1} FW). (**D**) Content of total phenolics (TP, expressed as mg of chlorogenic acid equivalents, CAE, in 100 g^{-1} FW). (**E**) Content of nitrates (NO_3^-, expressed as mg in 100 g^{-1} FW). * indicates significant differences between systems within cycles according to the LSD test ($p = 0.05$). Different letters indicate significant differences between cycles within systems according to the LSD test ($p = 0.05$).

The percentage of moisture was only affected by the field–winter cycle. Plants growing under these conditions reduced the accumulation of water in leaf tissues in approximately 4% in comparison with the other environments tested, where the percentage in moisture was around 90.7%. On the contrary, the contents of AA, TP and SIN significantly increased when plants grew in the field-winter environment. Values under these conditions were more than two-fold greater with respect to the greenhouse system (Figure 2). A similar performance was found during the spring cycle for the contents of AA and TP but not for the content of SIN. Thus, the accumulation of AA and TP also increased for plants growing in the field system in the second cycle, but differences among systems were lower in this case.

An indirect effect of the growing period was also found (Figure 2). Plants growing in the greenhouse displayed the least differences between cycles. In this system, the cycle only affected the levels of SIN and NO_3^-, with plants growing in spring displaying the highest contents. Thus, the accumulation of NO_3^- displayed a 2.6-fold increase with respect to the winter cycle (Figure 2). In fact, this environment provided the maximum levels of NO_3^- considering the four environments (974 mg 100 g^{-1} FW). Regarding the field system, the results indicated that all traits were influenced by the growing cycle (Figure 2). Plants growing during the winter cycle had higher contents of AA, TP and SIN. The greatest increase was found for the levels of SIN, corresponding to a two-fold increase (35.4 mg 100 g^{-1} vs. 73.0 mg 100 g^{-1} FW). On the contrary, the winter cycle resulted in a reduction of the levels in NO_3^-. In fact, plants growing in this environment displayed the lowest accumulation (164 mg NO_3^- 100 g^{-1} FW) (Figure 2).

The accessions were ranked considering their bioactive properties as well as the levels of NO_3^- along the four environments tested (Table 3). The high content of bioactive compounds was considered as a positive trait, while the accumulation of NO_3^- was considered as negative. The commercial cv. Wasabi ranked second together with accession DER055-1. The first in rank was DER001-1, although it had a low rank position for the levels of NO_3^-. DER006-1was also very close to the commercial cultivar. On the contrary, accessions DER064-1 and DER085-1 had the lowest scores.

Table 3. Average values and coefficient of variation (CV, %) for the contents of ascorbic acid (AA), sinigrin (SIN), total phenolics (TP) and nitrates (NO_3^-) evaluated in the 11 accessions of wall rocket across the four environments tested, and overall rank. Contents are expressed as mg 100 g^{-1} FW, and the levels of total phenolics are expressed as equivalents of chlorogenic acid (CAE). n = 4.

Accession	n	AA		SIN		TP		NO_3^-		Rank
		Mean	CV	Mean	CV	Mean	CV	Mean	CV	
DER001-1	4	70.21	35.6	67.96	31.7	163.88	39.9	573.32	73.4	1
DER055-1	4	77.45	40.1	48.17	18.9	153.33	35.3	470.74	63.7	2.5
cv. Wasabi	4	73.61	54.5	47.93	51.1	168.10	48.7	319.33	49.8	2.5
DER006-1	4	68.91	40.9	55.29	47.2	158.84	39.3	494.37	76.0	4
DER031-1	4	67.70	32.9	59.76	38.8	149.84	39.3	527.23	87.2	5.5
DER089-1	4	64.21	54.3	44.01	48.9	143.00	39.5	542.74	61.3	5.5
DER081-1	4	64.50	41.2	48.52	38.2	152.74	40.8	656.95	97.8	7
DER045-1	4	62.30	44.9	43.10	52.4	141.96	39.3	493.40	55.1	8
DER067-1	4	69.55	55.2	33.99	55.9	159.66	51.1	438.57	54.2	9
DER064-1	4	79.71	47.5	37.40	56.6	155.16	46.8	522.47	75.2	10
DER085-1	4	71.82	39.6	39.62	29.8	148.97	38.2	502.18	66.6	11
Total	44	70.00	40.1	47.79	42.7	154.13	37.3	503.75	67.5	

3.2.3. Correlation between Nutritional Traits

All Spearman rank phenotypic correlations among traits were highly significant (Table 4). A positive correlation was found between the percentage of moisture and the levels of NO_3^- in the tissue (ρ = 0.498). On the contrary, both traits were negatively correlated with the content of bioactive compounds. Among them, the highest correlation coefficients were obtained among moisture and

bioactive compounds, while the correlations with the content in NO_3^- were $\rho < -0.56$. On the contrary, positive correlations were found among bioactive compounds, with the content of AA–TP having the highest coefficient ($\rho = 0.921$) (Table 4).

Table 4. Phenotypic (above the symmetry axis, $n = 44$) and environmental (below the symmetry axis, $n = 213$) Spearman rank correlations (ρ) between the percentage in moisture, ascorbic acid (AA), total phenolics (TP), sinigrin (SIN), and nitrates (NO_3^-) determined in the accessions of wall rocket.

	Moisture	**AA**	**TP**	**SIN**	**NO_3^-**
Moisture		−0.7032 ***	−0.7832 ***	−0.7820 ***	0.4981 ***
AA	−0.5712 ***		0.9209 ***	0.4554 **	−0.5549 ***
TP	−0.8546 ***	0.6488 ***		0.6211 ***	−0.4898 **
SIN	−0.5878 ***	0.3670 ***	0.5545 ***		−0.3489 *
NO_3^-	0.2374 **	−0.2149 **	−0.3252 ***	−0.2489 ***	

^{ns}, *, ** and *** indicate no significant or significant at $p < 0.05$, 0.01 and 0.001, respectively.

Similar results were obtained for the analysis of environmental correlations, with lower coefficients being generally found in this case (Table 4). The greatest decrease was found for the moisture –NO_3^- correlation ($\rho = 0.237$). A high reduction in the ρ coefficient comparing environmental vs. phenotypic correlations was also determined for the AA–NO_3^- content (−0.215 vs. −0.555, respectively). Finally, a moderate environmental correlation was found for the AA–TP ($\rho = 0.649$) (Table 4).

4. Discussion

Wall rocket is a common weed in the Mediterranean regions. However, it is also appreciated as a wild edible vegetable [38] and therefore has the potential to become a new crop. The present work aimed at studying the effect of different cultivation environments on the nutritional quality of pre-selected accessions of wall rocket. Due to the close phylogenetic relationships and similarities in terms of growth and commercial use, wall rocket may be potentially cultivated in similar environments as the already established rocket crops. Thus, it might be produced in field or greenhouse systems, although soil-less systems may also be available [23,39]. The greenhouse and field environments differ in several factors such as temperature, light intensity, air humidity or the effect of rains, among others [40]. These factors can also differ between growing cycles. Environmental factors have been proven to affect the leaf morphology of wall rocket [29]. In addition, environmental factors have been proven to influence the accumulation of secondary metabolites and nitrates in different crops [41–43]. Thus, the quality of wall rocket in terms of bioactive compounds and nitrates may be affected as well.

As phytochemicals, the contents of AA, TP, SIN and NO_3^- were evaluated. Only the reduced form of vitamin C was evaluated since we previously concluded that the AA form represented around 90% of the total vitamin C in wall rocket materials [44]. Differences between wall rocket and the reference materials were moderate and only significant for the content of AA and the percentage of moisture. Thus, the traits analyzed in the present work were not useful enough to clearly separate among them. These results are in contrast to our previous work evaluating the leaf morphology in the three species, where materials were clearly differentiated by the shape and size of leaves [29]. In consequence, the exploitation of distinctiveness in wall rocket with a commercial purpose could focus on other traits such as visual traits. As exception, wall rocket had as a distinctive trait the accumulation of SIN as main glucosinolate, since this compound was neither determined in *E. sativa* nor *D. tenuifolia* materials. These findings are in agreement with previous works comparing the glucosinolate profile of the three species [23,45]. Nevertheless, different profiles in other wall rocket materials, characterized for the absence of SIN, have been identified as well [45,46]. Discrepancies may correspond to differences related to the origin of materials, inter-subspecies differences—i.e., the analysis of *D. erucoides* subsp. *erucoides* or subsp. *longisiliqua* materials—as suggested by D'Antuono et al. [45], or they may even correspond to inter-specific crosses.

In a second analysis, the 11 accessions of wall rocket were compared among systems under two different growing periods. It has been previously observed that the growing period in a short-cycle species such as wall rocket can affect its morphology [29,47]. Moreover, the accumulation of different compounds such as nitrates can be affected by the growing period as well, as Bonasia et al. [48] described for wild rocket. Our results showed a low contribution of the accession effect to the total sum of squares, together with a general absence of significance. This result was an indicator of low nutritional variation among the accessions analyzed. The lack of variation may be due to the close geographic origin of materials, as original populations were collected in a relatively small territory. On the other hand, the low variation found may be due in part to a high intra-population variability considering that no homogenization efforts have been addressed, as suggested by the residual effect. However, a commercial cv. was included, which is assumed to be obtained from different populations, presumably with a different origin, and to show a high degree of uniformity. Thus, these low differences may also correspond to the low variation of wall rocket as a species in terms of bioactive compound contents and NO_3^- accumulation capacity. Nevertheless, some accessions could be considered in new programs as promising materials, including, for example, accessions DER055-1 and DER006-1. The latter was, in fact, also selected by its morphology as a promising material [29].

A comparison of different environments demonstrated a high effect on the final phytochemical composition of the product. Plants growing in the field during the winter cycle experienced the most extreme environment, both considering the two systems (greenhouse vs. field during the winter cycle) and the different growing periods (winter vs. spring in the field system). High adverse conditions took place during this growing cycle with remarkable low temperatures, so plants were subjected to high abiotic stresses. Abiotic stress increases the levels of reactive oxygen species, causing oxidative stress in plants [49]. As part of the defense response to this possible oxidative damage in plant tissues, the content of secondary metabolites such as AA and phenolic compounds can increase as well. Oh et al. [26] found that plants of lettuce exposed to cold stress increased the accumulation of protective metabolites by the activation of genes involved in their biosynthesis. In the same way, it has been observed among *Brassicaceae* that plants accumulate a greater content of glucosinolates when they grow under non-optimal temperatures [27], as our results suggest. In particular, it has been observed that a decrease in temperature can increase the accumulation of glucosinolates [50,51]. By contraposition, the filed-winter environment resulted in the accumulation of the lowest content in NO_3^-, which is also of interest for a commercial purpose. Light intensity has been positively correlated to nitrate reductase activity and a consequent lower accumulation of NO_3^- [48]. This season-dependence explains the different maximum limits established for lettuce and rocket crops in the European Union [22]. However, our experiment was conducted in two consecutive cycles with similar light exposure, and therefore light differences may be not be high enough to affect the reductase activity. Thus, other physiological processes may be related to this different accumulation.

The field–spring environment also provided a product accumulating high levels of pytochemicals of interest (AA, SIN, TP) and low levels of NO_3^-. The content of AA was significantly higher than previously described by Salvatore et al. [52], who found that mature, wild plants of wall rocket on average accumulated 13.9 mg AA 100 g^{-1}. Values were also comparable or even greater than levels of vitamin C (VC) previously described for wild rocket by Spadafora et al. [10] (22 mg 100 g^{-1} FW) or Durazzo et al. [24] (21–81 mg 100 g^{-1} FW). The accumulation of SIN, however, did not reach the levels previously described for wall rocket by Di Gioia et al. [23], with an average level of 11.6 mg g^{-1} DW. In addition, the content of NO_3^-, although greater in this cycle, was below the maximum limit of 7000 mg kg^{-1} established for the commercialization of rocket crops harvested before April [22]. Comparable or slightly lower levels have been previously found in cultivated rocket crops [43], usually ranging between 3500–4500 mg kg^{-1} but reaching 7349 mg kg^{-1}. Levels described for non-cultivated wall rocket would be between 2000–2500 mg kg^{-1} [53,54], suggesting that the species tends to increase this accumulation under cultivated conditions. Finally, the increased percentage

of moisture was reflected in a greater visual appearance and less coriaceous aspect—traits that are essential for consumer acceptance.

In contrast, the greenhouse system may not be adequate for the commercial production of wall rocket according to our results. Heated greenhouses are used to provide a more appropriate and stable temperature for plant growth compared to field conditions, but also affect other factors such as wind, air humidity, solar radiation, the effect of rains and storms or crop management [40]. Thus, our results suggest that growing wall rocket under greenhouse conditions would enhance the homogenization of most of the traits evaluated but provide a product of lower quality, especially in terms of AA and TP contents. Moreover, plants in this system accumulated high levels of NO_3^-, which in the spring cycle exceeded the limits established [22] and made the product obtained not commercially acceptable.

Finally, phenotypic coefficients of correlations were greater than the environmental ones. These results indicated that the different factors evaluated had a similar effect on materials on average; however, the residuals among those traits had a lower correlation. The high correlation between AA and TP may be explained by their antioxidant function in the plants and their accumulation in response to environmental stresses [55]. However, both AA and TP had lower correlations with the content of SIN. As with AA and phenolic phytochemicals, the accumulation of glucosinolates in plant tissues is part of the plant response mechanism against abiotic stress conditions and can therefore be affected by environmental factors such as light intensity, season or fertilization [16,27,41], but it is also highly related to the biotic stress by pests and pathogens [16,27]. The combined effect of both biotic and abiotic stress could be responsible for this lower correlation. On the other hand, a negative correlation between the antioxidant phytochemicals and the percentage of moisture was found. This may correspond to a concentration effect in the tissues [41], although it could be also related to the plant behavior and defense system against cold stress. Król et al. [56] found that leaves of grapevine developed under cold stress reduce their percentage of moisture, although the total phenolics were also decreased; on the contrary, Oh et al. [26] found that the exposure of lettuce to chilling conditions increased the total phenolics against oxidative damage. Moreover, the negative correlation with these compounds and the content of NO_3^- has been previously observed [48], in agreement with our results. Finally, a positive correlation between the percentage of moisture and the levels of NO_3^- was found, as extensively observed in many species [57]. This positive correlation between both traits is related to the osmotic effect of NO_3^- ions, meaning that its accumulation increases the capacity of tissues to retain water [20,57].

5. Conclusions

This work aimed to evaluate the most adequate conditions for the establishment of wall rocket as a new crop. Our results indicated that growing this vegetable under field conditions would enhance the accumulation of AA and TP in the final product. Moreover, the accumulation of NO_3^- was reduced in this environment compared to the greenhouse system. Among all environments, the field–winter system resulted in the lowest content in NO_3^-, which is a trait of high interest for a commercial purpose, but also the lowest percentage of moisture, with this reducing the visual quality and presumably consumer acceptance. Thus, our results suggest that stressful conditions such as low temperatures in winter may not be adequate for commercial production in an unprotected field. In this sense, the use of crop thermal blankets may reduce such stress.

The low variability of the phytochemicals among accessions of wall rocket may reflect the low genotypic differences among the selected materials or at a species level. Moreover, the levels found in wall rocket did not clearly differ from the reference crops. As an exception, wall rocket had, as a distinctive trait, the presence of sinigrin as its main glucosinolate unlike the other species, as previously described [23,45]. These results increase the information available for the species and are of relevance for breeding programs and future commercial strategies, suggesting that the promotion of the distinctiveness of this new crop among the other rocket crops should focus on other aspects such as visual quality or flavor instead of bioactive traits.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/12/858/s1, Table S1: Geographical location of the original ten wild populations of wall rocket.

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