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Morphological Diversity and Bioactive Compounds in Wall Rocket (*Diplotaxis erucoides* (L.) DC.)

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Abstract: Wall rocket is a wild vegetable with interest to become a crop. However, the information regarding morphological variability in the species is scarce, despite the interest it has received for breeding programs. In addition, evaluating the phytochemical composition can also be useful for developing materials of a high quality. In this study, forty-four populations were evaluated for selected morphoagronomic traits and contents in ascorbic acid (AA), total phenolics (TP), and nitrates (NO_3^-). Wall rocket plants had, on average, an intermediate growth habit and a good response to transplant. Moderate variability, mainly for size-related traits, was found, with low to moderate heritability estimates ($H^2 < 0.35$). a Principal Component Analysis revealed that some materials may be selected for differenced traits. On the other hand, wall rocket materials had, on average, high contents in AA (53 mg 100 g^{-1}) and TP (116 mg CAE 100 g^{-1}) but also accumulated high levels of NO_3^- (891 mg 100 g^{-1}). Significant positive correlations were found for AA and TP, which could be exploited for increasing the antioxidant activity and properties of the final product. We provide new information on the variation of wall rocket for traits of morphological and phytochemical interest, which together with other traits, such as the profile of glucosinolates, can be useful for the selection of materials in future breeding programs.

Keywords: antioxidants; *Diplotaxis erucoides*; morphology; new crops; nitrates; phenotypic variability; wall rocket

1. Introduction

It is estimated that more than 7000 plant species have been used as food throughout history [1]. These edible species include established crops, neglected and underutilized crops, and wild edible plants (WEPs) directly collected in the wild or in modified systems where they can be found as weeds [2]. Changes in lifestyle, detachment from the nature or large-scale cultivation, among other reasons, gradually decreased the use of WEPs in the past [3,4]. However, Pinela et al. [4] highlighted a recent phenomenon of the revalorization of WEPs emerging in modern societies. Such renewed interest offers an opportunity for the development of new crops due to the establishment of domestication and adaptation programs.

Wall rocket (*Diplotaxis erucoides* (L.) DC.) represents an example of a WEP traditionally consumed in Mediterranean countries [4–7]. The leaves of this wild vegetable are eaten, along with their tender shoots, raw or cooked, in different dishes, such as salads, soups, omelettes, or pasta [7,8]. The species has a characteristic, little pungent flavor resembling other *Brassicaceae*, such as mustard seeds, horseradish, or wasabi. Regarding the nutritional quality, wall rocket may accumulate high levels of vitamin C and phenolic compounds, such as the cultivated rocket crops [9]. However, these species accumulate high amounts of nitrates as well [10–12]. Nitrates have been considered for decades as antinutrients with

potential negative effects for health [13,14]; thus, the accumulation in foodstuff must be controlled, especially for leafy vegetables.

The appreciation of wall rocket flavor by consumers, together with the accumulation of bioactive compounds, makes it a good candidate for being established as a crop. As far as we know, there is one unique commercial cultivar of wall rocket, but it is not cultivated extensively. Domestication and adaptation programs for establishing WEPs as crops require, in a first step, the collection and evaluation of materials searching for characters of interest. However, it is common to find a lack of information regarding these two key points in emerging crops, which represents a weakness for breeding programs [15]. In the case of wall rocket, the number of accessions that are currently available in germplasm banks is very low [16]. Thus, collecting materials is an imperative step for starting the domestication process. In addition, we have not found previous studies that analyze the morphoagronomic diversity present in the species. By contrast, previous studies have been developed in these terms for the taxonomically related salad rocket (*Eruca sativa*) and wild rocket (*Diplotaxis tenuifolia*), demonstrating a high degree of variation, especially in the former [17,18]. Characterization can be used to identify whether there is high degree of variation among the materials or not, to identify, if present, accessions with specific characters of interest, and to establish adequate selection or breeding strategies. The use of standardized descriptors allows for the effective characterization needed for breeding programs and related tasks and for the comparison of experimental data [19]. However, standardized descriptors are not usually found for underutilized crops and wild vegetables. In fact, no standardized descriptors have been described for wall rocket, although they can be found for *Eruca* spp. [20]. Due to the taxonomic relationship between both species, IPGRI (International Plant Genetic Resources Institute) decriptors may be used as a basis for characterizing wall rocket germplasm.

We have started a domestication and crop-adaptation program at the Universitat Politècnica de València (UPV, Valencia, Spain), in which this study is involved. The program is addressed to the release of commercial cultivars of wall rocket that is well adapted to our climatic conditions. In this context, the present study was focused on the phenotypic characterization of the local germplasm collected, mainly leaf characterization as this is the product of commercial interest, as well as in evaluating key composition traits. *Eruca* spp. descriptors [20] were selected for this task and were adapted when needed. This work offers a starting point for selection in breeding programs, and it is also key for developing descriptors for wall rocket.

2. Materials and Methods

2.1. Plant Material and Cultivation Conditions

Forty-four populations of wall rocket were evaluated in the current study. Forty-two represented seedlings from wild populations collected in the Valencian Community (CV, Spain) during the spring of 2015 (Figure S1). Two other populations (BGV-UPM 1235, Alicante, CV, Spain; and BGV-UPM 1549, Teruel, Aragon, Spain) were provided by the "Banco de Germoplasma Vegetal-UPM César Gómez Campo" (Madrid, Spain). All materials and the related origin information are conserved at the UPV.

The experiment was performed during the months of May to July 2016, at the UPV (39° 29′ 00″ N; 0° 20′ 27″ W). Seeds were treated with 2.5% commercial sodium hypochlorite plus 100 ppm gibberellic acid (Duchefa Biochemie, Haarlem, The Netherlands) solution [21]. Treated seeds were sown in seedling trays (112 cells, 22 mL each) using commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany), placed for one week in a growing chamber with long-day conditions (16/8 h, 25 °C), and then moved to a glasshouse. No additional light was provided in the glasshouse, which was equipped with a cooler system that turned on when the temperature was above 25 °C. Three weeks after sowing, the seedlings were transplanted to larger pots (15 L) filled with a mixture of commercial N3 substrate and coconut fiber (Horticoco, Valimex, Valencia, Spain) (1:1). For each population, three pots with 25 plants each were filled. Populations were placed following a randomized design. a drip irrigation system was used for watering and fertilizing the pots. The final

concentration of the main anions and cations added with the irrigation was: 11.47 mM NO_3^-, 1.00 mM NH_4^+, 1.50 mM $H_2PO_4^-$, 6.75 mM K^+, 3.25 mM Ca^{2+}, 2.50 mM Mg^{2+}, 2.82 mM SO_4^{2-}. For micromineral supply, the following salts were added to the system: 50 µM H_3BO_3, 10 µM Fe-EDTA, 4.5 µM $MnCl_2$, 3.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 0.1 µM $(NH_4)_6Mo_7O_{24}$. Leaves were harvested with the appearance of the first flower bud.

2.2. Morphoagronomic Characterization

Morphoagronomic traits for characterization were selected from normalized descriptors for *Eruca* spp. [20] and adapted when needed. Selected traits related to the whole plant as well as traits used for describing the leaves were used, as summarized in Table 1.

Table 1. Morphological and agronomic descriptors used for the characterization of wall rocket populations.

Descriptor[a]	Code	Type[e]	Scale/Units
Plant habit-dependent traits			
Plant growth rate	Growth	Qual ord	3 = slow; 7 = fast
Adaptation level[b]	Adaptation	Qual ord	3 = low; 7 = high
Leaf growth attitude	Attitude	Qual categ	1 = semi-prostrate; 2 = horizontal; 3 = semi-erect
Whole plant traits			
Size-related traits			
Plant height	$Height_{Plant}$	Quantit	cm
Plant width	$Width_{Plant}$	Quantit	cm
Stem height	$Height_{Stem}$	Quantit	cm
Internode length	$Length_{Internode}$	Quantit	cm
Descriptive traits			
Stem thickening	Thickening	Qual ord	3 = thin; 7 = thick
Stem color	$Color_{Stem}$	Qual categ	1 = light green; 2 = green, 3 = dark green; 4 = red/purple green; 5 = red/purple
Stem hairiness	Hairiness	Qual ord	3 = sparse; 7 = dense
Foliage[c]	Foliage	Quantit	
Leaf traits			
Size-related traits			
Leaf length[d]	Length	Quantit	cm
Leaf width	Width	Quantit	cm
Petiole length	$Length_{Petiole}$	Quantit	cm
Leaf perimeter[d]	Perimeter	Quantit	cm
Leaf area[d]	Area	Quantit	cm^2
Descriptive traits			
Leaf margin shape	Margin	Qual categ	1 = entire; 2 = crenate; 3 = dentate
Leaf blade shape	Shape	Qual categ	1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate
Leaf apex shape	Apex	Qual categ	1 = acute; 2 = rounded; 3 = broadly rounded
Leaf lobation intensity	Lobation	Qual ord	0 = absent; 5 = deep lobation
Petiole and midvein color	$Color_{Petiole}$	Qual categ	1 = white; 2 = light green; 3 = green; 4 = purple; 5 = red

[a]Traits were measured in the pre-flowering stage, which is when the first flower bud became visible but was not fully developed. [b]Refers to the adaptation of plants to greenhouse conditions after transplant. [c]Refers to the number of leaves developed. [d]Traits measured, including the petiole. [e]Descriptor type: qualitative categorical (Qual categ), qualitative ordinal (Qual ord) or quantitative (Quantit).

Plant habit-dependent traits were described considering the whole set of plants in each population, and included growth rate (Growth), adaptation level to transplant and greenhouse conditions (Adaptation), and leaf growth attitude (Attitude). In addition, five plants per population were used to measure plant height ($Height_{Plant}$) and width ($Width_{Plant}$), stem height ($Height_{Stem}$), and the length of the longest internode ($Length_{Internode}$). These plants were also used for describing the stem thickening (Thickening) and color ($Color_{Stem}$).

Finally, ten descriptors were evaluated in the leaves. Five descriptors were size-related traits, including leaf length (Length), and width (Width), petiole length ($Length_{Petiole}$), and total perimeter

(Perimeter) and area (Area). The measurements were performed using the Tomato Analyzer v 3.0 software [22]. On the other hand, the descriptive traits included the shape of blade (Shape), the leaf apex (Apex) and margin (Margin), the intensity of lobation (Lobation), and the petiole/midvein color ($Color_{Petiole}$). All traits were measured in ten leaves per population corresponding to second (lower) and fourth (upper) true leaves.

2.3. Chemical Analysis

When the floral bud appeared, the leaves were harvested for the chemical analyses. Fresh material was used for the determination of ascorbic acid (AA) while frozen (−80 °C), and freeze-dried material was destined to be analyzed for the total phenolics (TP) and nitrates (NO_3^-) contents. Analyses were performed in triplicate. The AA content was measured from fresh material by using the high performance liquid chromatography (HPLC) technique, according to Guijarro-Real et al. [21]. Briefly, 1.0 g was homogenated with 5 mL 3.0% (w/v) cold *meta*-phosphoric acid solution and filtered through a 0.22 µm PVDF filter (Teknokroma, San Cugat del Vallès, Spain). The determinations were performed with a 1220 Infinity HPLC system (Agilent Technologies; Santa Clara, CA, USA) using a Brisa C_{18} column (150 mm × 4.6 mm id, 3 µm particle size; Teknokroma, San Cugat del Vallès, Spain). The conditions were as follows: an isocratic phase of methanol: 1.0% acetic acid (5:95) during 15 min; an injection volume of 5 µL; a flow rate of 1 mL min^{-1}. The quantification was performed at 254 nm using *L*-ascorbic acid for external standard calibration. The results were expressed as mg AA 100 g^{-1} of fresh weight (FW).

The total phenolics (TP) content was evaluated using the Folin–Ciocalteu procedure [23] according to Guijarro-Real et al. [24]. Briefly, 0.125 g of freeze-dried material was extracted with 70% acetone containing 0.5% acetic acid for 24 h. Aliquots of 65 µl reacted with 500 µl of diluted Folin–Ciocalteu (1:10) for 5 min, plus 500 µl of 60 g L^{-1} sodium carbonate for 90 min. The absorbance was read at 765 nm and an external standard of chlorogenic acid was used for quantification. The results were expressed as mg of chlorogenic acid equivalents in 100 g of dry weight (mg CAE 100 g^{-1} FW).

The content in NO_3^- was determined with a nitrate-selective ion (Crison Instruments S.A., Alella, Barcelona, Spain). The extraction protocol was adapted from Egea-Gilabert et al. [25]. Thus, 0.1 g was homogenated with 50 mL of distilled water for 15 min, in continuous stirring. The measurement was obtained after adding 1 mL of 2 M diammonium sulfate [$(NH_4)_2SO_4$] buffer, and results expressed as mg NO_3^- 100 g^{-1} FW.

The Folin–Ciocalteu reagent, sodium carbonate, glacial acetic acid, and acetone were purchased from Scharlab S.L. (Mas d'En Cisa, Spain). *L*-ascorbic acid, chlorogenic acid, *meta*-phosphoric acid and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade reagents were used for HPLC analyses, and ACS-grade reagents were used for the rest of the analyses.

2.4. Statistical Analysis

The mean and ranges of each population were obtained for quantitative data. Data were subjected to a one-way analysis of variance (ANOVA). Negligible differences were obtained when comparing original and transformed data; thus, the original data were used for the analysis and calculation of heritabilities, as they provide information on actual data [26]. The total sum of the squares was partitioned in the sum of the squares of accession and residual effects [27], and were expressed as percentages. Broad sense heritability (H^2) was calculated as:

$$H^2 = \sigma_G{}^2 / (\sigma_G{}^2 + \sigma_E{}^2) \quad (1)$$

where $\sigma_G{}^2$ and $\sigma_E{}^2$ were the estimates of genotypic and residual variance, respectively [28]. In addition, Pearson linear correlations (r) among traits were studied. For qualitative data, percentages for each category were obtained and compared using the Marascuilo procedure ($p = 0.05$).

Finally, a Principal Component Analysis (PCA) was performed using the Clustvis tool [29], including quantitative and ordinal qualitative traits. Prior to the analysis, data were $\log_2$-transformed and centered, and vector scaling was applied to rows.

3. Results

3.1. Plant Habit-Dependent Traits and Whole Plant Characterization

The plant growth was mainly intermediate, with 66.2% of populations displaying a 4.5 to 5.5 score in a 3–7 scale (Figure 1). Populations DER040, DER051, DER055, and DER073 had the fastest growth, while DER069 had the lowest growth rate (Table S1). In addition, all populations had a good response to transplant and subsequent greenhouse growing conditions, determined as intermediate only for DER017 (Figure 1, Table S1). Moreover, the leaves growth attitude was mainly semi-erect, as described in 84.1% of populations (Figure 1). Only DER041 displayed a semi-prostrate growth attitude of leaves (Table S1).

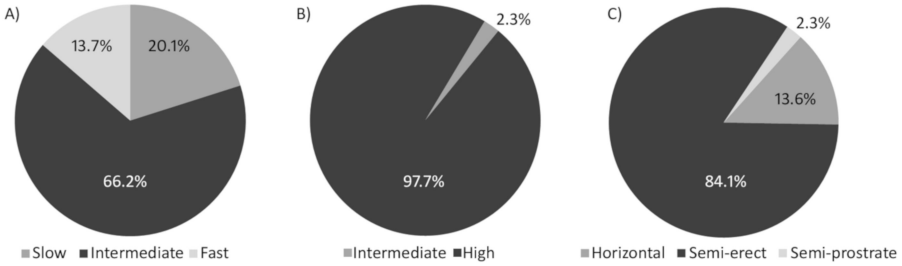

Figure 1. Percentage of populations included in the different categories described for the qualitative categorical descriptors (n = 44). (**A**) Plant growth rate with three categories ranging from slow rate (< 4 in the scale) to fast rate (> 6 in the scale). (**B**) Plant adaptation with two categories: intermediate (5 in the scale) and high adaptation (> 6 in the scale). (**C**) Leaf growth attitude with three defined categories: semi-prostrate, horizontal, and semi-erect. For more details refer to Table 1.

The mean values and ranges for the plant size-related traits are shown in Table 2 (for individual values of each population, refer to Table S1). Significant differences ($p < 0.05$) were observed among populations for all traits. The contribution of the population effect to the total sum of squares ranged between 26.6% ($Width_{Plant}$) and 47.5% ($Height_{Stem}$) (Table 2). Therefore, the broad-sense heritabilities were moderate in the case of $Height_{Plant}$, $Height_{Stem}$, and $Lengh_{Internode}$ (0.28, 0.35, and 0.28, respectively) and were low for Foliage and $Width_{Plant}$ (0.13 and 0.09, respectively) (Table 2). On the other hand, positive correlations were established among these traits (Table 3). All Pearson correlations were significant ($p < 0.05$). The greatest correlation coefficient was found for $Height_{Stem}$/$Length_{Internode}$ (r = 0.926), and relatively high correlations ($r > 0.65$) were also established between these traits and $Height_{Plant}$.

Table 2. Mean value, range, percentage of the total sum of squares for the effects of population and residuals, and broad sense heritability (H^2) for plant quantitative traits (n = 44).

			Sum of squares (%)		
Descriptor[a]	Mean	Range	Population	Residual	H^2
$Height_{Plant}$ (cm)	8.92	(6.48–10.74)	41.6***	58.4	0.28
$Width_{Plant}$ (cm)	13.33	(10.56–16.60)	26.6*	73.4	0.09
$Height_{Stem}$ (cm)	3.99	(2.38–6.30)	47.5***	52.5	0.35
$Length_{Internode}$ (cm)	1.04	(0.56–1.56)	42.3***	57.7	0.28
Foliage (number of leaves)	8.33	(7.40–9.40)	30.1**	69.9	0.13

*, ** and *** indicate no significant or significant at $p < 0.05$, 0.01 and 0.001, respectively. [a]For details, refer to Table 1.

Table 3. Pearson linear correlations between plant size-related traits (n = 44).

	$\mathbf{Height_{Plant}}$	$\mathbf{Height_{Stem}}$	$\mathbf{Length_{Internode}}$
$Width_{Plant}$ [a]	0.431**	0.399**	0.303*
$Height_{Plant}$		0.707***	0.672***
$Height_{Stem}$			0.926***

*, ** and *** indicate no significant or significant at $p < 0.05$, 0.01 and 0.001, respectively. [a]For details, refer to Table 1.

Finally, traits such as stem thickening, color and hairiness, and foliage, were evaluated. Overall, populations developed a thin to intermediate stem, with a mean value of 3.9 on a 3–7 scale, and a sparse to intermediate degree of hairiness (4.06 on average on a 3–7 scale) (Table S1). The stem color was mainly described as green considering the total set of plants in each population. And the foliage developed at the moment of the flower bud emergence was similar in all populations, between seven and nine leaves on average (Table 2, Table S1).

3.2. Leaf Related Traits

A first analysis was performed in order to compare the lower and upper leaves developed in wall rocket materials (Figure 2). Significant differences ($p < 0.05$) were found for quantitative traits, except for leaf length, which displayed a mean value of 8.94 cm (Figure 3). Compared to lower leaves, the upper ones were on average wider (3.56 cm vs. 3.27 cm), with a longer perimeter (25.55 cm vs. 22.35 cm), and a larger area (16.30 cm^2 vs. 14.14 cm^2). By contrast, the lower leaves displayed longer petioles, on average 1.6 cm longer than those of the upper leaves (Figure 3).

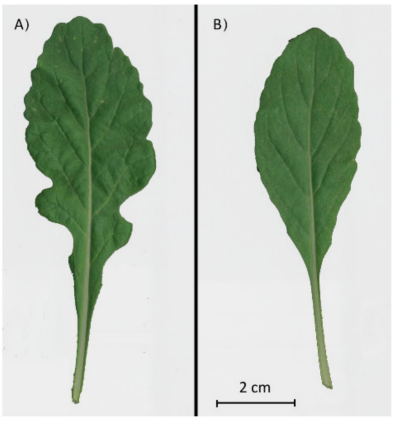

Figure 2. Differences in leaf traits between the upper (**A**) and lower (**B**) leaves.

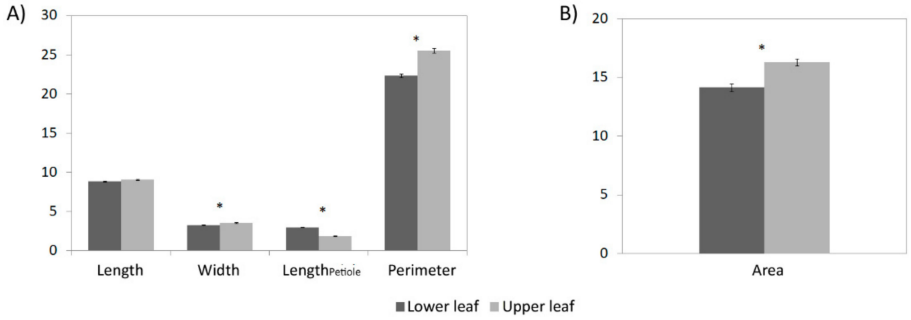

Figure 3. Mean values ± SE (n = 220) for size-related traits measured in the lower and upper leaves. (**A**) Comparison between lower and upper leaves for length, width, and the length of the petiole and perimeter (cm). (**B**) Comparison between the lower and upper leaves for area (cm^2).

Upper and lower leaves also differed in qualitative traits (Figure 3, Table 4). As an exception, the petiole/midvein color was light green to green in all leaves. The lower leaves were mainly obovate

(83.2%) to orbicular (15.0%), with a rounded (87.7%) to broadly rounded (10.0%) apex. On the contrary, the upper leaves combined obovate shape (76.8%) with remarkable percentages of elliptic (9.5%) and spatulate leaves (11.4%). The percentage of upper leaves displaying a rounded apex (62.7%) decreased compared to the lower leaves, while the development of an acute shape significantly increased, from 2.3% in the lower leaves to 29.6% in the upper ones (Table 4). The margin shape also displayed differences. While the lower leaves had a soft margin, mainly entire-crenate (47.7%) or crenate (45.4%), the upper leaves had sharper margins, with 50.9% developing a crenate–dentate margin and 17.3% displaying dentate margins. The intensity of lobation also increased in the upper leaves. Thus, the lower leaves were entire (0 in a 0–5 scale), while 46.8% of upper leaves displayed a score of 2 in the 0–5 scale (Table 4).

Table 4. Percentage of leaf-related categorical traits analyzed in the populations of wall rocket, for the lower and upper leaves (n = 220).

Descriptor[a]	Lower Leaf	Upper Leaf
Shape	1: 15.0%^{b}	1: 2.3%^{a}
	-	2: 9.5%
	3: 83.2%^{ns}	3: 76.8%^{ns}
	4: 1.8%^{a}	4: 11.4%^{b}
Apex	1: 2.3%^{a}	1: 29.6%^{b}
	2: 87.7%^{b}	2: 62.7%^{a}
	3: 10.0%^{ns}	3: 7.7%^{ns}
Margin	1: 5.5%	-
	1.5: 47.7%^{b}	1.5: 0.9%^{a}
	2: 45.4%^{b}	2: 30.9%^{a}
	2.5: 1.4%^{a}	2.5: 50.9%^{b}
	-	3: 17.3%
Lobation	0: 99.6%^{b}	0: 6.8%^{a}
	1: 0.4%^{a}	1: 25.9%^{b}
	-	2: 46.8%
	-	3: 20.5%

Different letters among rows correspond to significant differences according to the Marascuilo procedure. ^{ns} indicates non-significant differences. [a]For details, refer to Table 1.

Considering that the upper leaves were more representative as a commercial product, these were the ones for analyzing size-related differences among populations (Table 5). As for plant traits, the contribution of the population effect to the total sum of squares was below 50.0% in all traits. $Length_{Petiole}$ displayed the lowest contribution (29.7%), while Width, Perimeter, and Area had a population effect close to 50.0% (43.0%, 43.4% and 43.2%, respectively). Thus, broad-sense heritability estimates for these traits were also low (for Length and $Length_{Petiole}$) to moderate (for Width, Perimeter and Area) (Table 5). All Pearson correlations among these traits were significant ($p < 0.05$) and positive (Table 6). $Length_{Petiole}$ was greatly correlated to Length (r = 0.70). On the other hand, Length and Width were highly correlated to both Perimeter and Area ($r > 0.75$).

Table 5. Mean value, range, percentage of the total sum of squares for the effects of population and residuals, and broad sense heritability (H^2) for leaf quantitative traits (n = 44).

Descriptor[a]	Mean	Range	Sum of squares (%)		H^2
			Population	Residual	
Length (cm)	9.05	(7.27–10.47)	32.4***	67.56	0.16
Width (cm)	3.56	(2.71–4.37)	43.0***	57.0	0.30
Perimeter (cm)	25.42	(20.52–32.02)	43.4***	56.6	0.30
Area (cm^2)	16.37	(10.68–24.17)	43.2***	56.8	0.30
$Length_{Petiole}$ (cm)	1.84	(0.75–2.95)	29.7*	70.4	0.13

* and *** indicate no significant or significant at $p < 0.05$ and 0.001, respectively. [a]For details, refer to Table 1.

Table 6. Pearson linear correlations between leaf size-related traits (n = 44).

	$Length_{Petiole}$	Width	Perimeter	Area
Length[a]	0.700***	0.696***	0.941***	0.800***
$Length_{Petiole}$		0.309***	0.554***	0.371***
Width			0.759***	0.922***
Perimeter				0.784***

*** indicate no significant or significant at $p < 0.001$. [a]For details, refer to Table 1.

3.3. Chemical Composition

As bioactive compounds, the content in AA and TP were evaluated in the 44 populations of wall rocket. The average content in AA was 53.5 mg 100 g^{-1} FW, with values ranging between 31.0 (BGV-UPM 1235) and 86.7 (DER064) mg AA 100 g^{-1} FW (Table S2). The content in TP, measured by means of the Folin–Ciocalteu procedure, had a mean value of 116.3 mg CAE 100 g^{-1} FW. a 1.24-fold difference was established between the lowest (DER051) and the highest (DER045) value. Finally, the average value for the content in NO_3^- was 890.6 mg 100 g^{-1} FW, with a difference of 1.8-fold times between the lowest (DER069) and highest (BGV-UPM 1549) values.

Significant linear correlations were found between AA and TP, and between TP and NO_3^-, while no significant correlation was found between AA and NO_3^- (Figure 4). The correlation coefficients were moderate in both cases, and positive in the case of AA and TP (r = 0.321) but negative for TP and NO_3^- (r = −0.448).

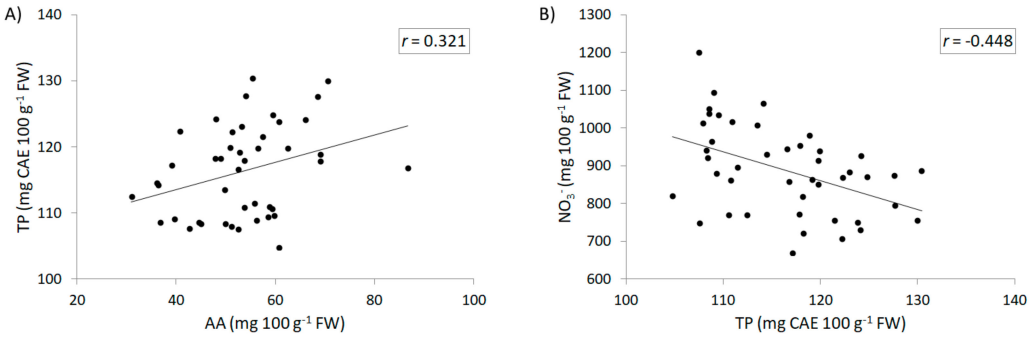

Figure 4. Correlation between nutritional parameters. (**A**) The correlation between the content in ascorbic acid (AA) and total phenolics (TP). (**B**) The correlation between total phenolics (TP) and the content in nitrates (NO_3^-).

3.4. Principal Component Analysis (PCA)

A PCA was performed with the quantitative and qualitative ordinal data (Figure 5). Only data from the upper leaves were included due to the high similarity of the lower leaves among the different populations. The first two principal components accounted for 41.4% of the total variability registered. The first component explained 28.6% of the variability registered, and it was positively correlated to most of the characters, with leaf-size descriptors as the traits displaying the strongest correlations (Figure 5a). Only the contents in AA, TP, and the plant growth attitude were negatively correlated to the first component, and from those, only the TP content had a correlation coefficient greater than 0.15 in absolute terms. On the other hand, the second principal component accounted for 12.7% of the total variability (Figure 5a). It was positively correlated to plant-size descriptors, especially height-related traits ($Height_{Plant}$, $Height_{Stem}$ and $Length_{Internode}$), although plant width had a correlation coefficient lower than 0.10 in absolute terms. The content in NO_3^- also had a positive, weak correlation (< −0.150) with this component. On the contrary, the strongest negative correlations were obtained for leaf size traits, leaf lobation, and TP content. Overall, the loading plot analysis grouped those descriptors, in the graphic, that displayed great correlations. Moreover, the analysis situated the leaf lobation trait close to leaf size-related descriptors in the graph, indicating a positive relationship among these characters.

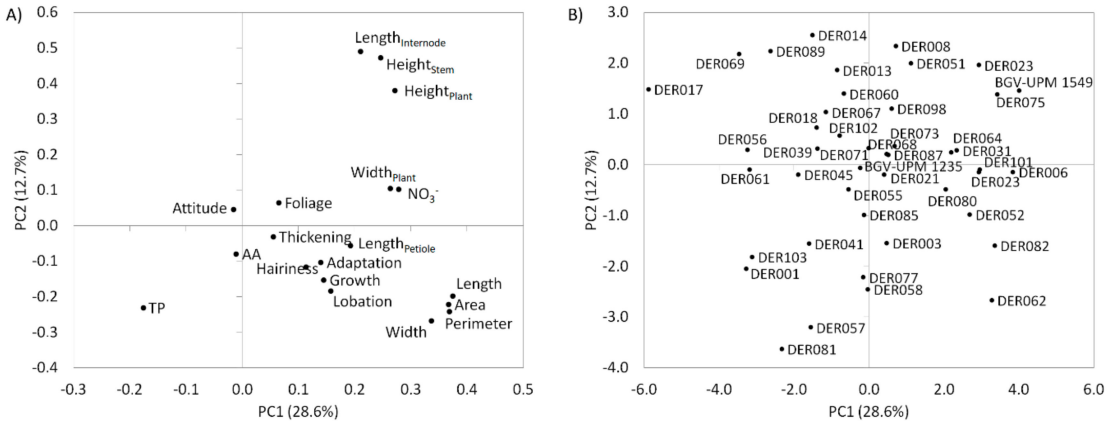

Figure 5. Principal Component Analysis (PCA) for the first and second principal components, performed with the quantitative plus qualitative ordinal data. (**A**) The PCA loading plot. (**B**) The PCA score plot.

The PCA score plot showed the distribution of populations in the first and second components (Figure 5b). The analysis clearly separated the population DER017 from the rest. This population displayed on average the lowest values for leaf-size traits and also had low values for plant-size traits and low transplant adaptation (Table S1). On the contrary, populations with similarities grouped together. Thus, populations on the right side of the PCA displayed greater values for leaf-size traits compared to other populations, especially in terms of Length and Perimeter (Figure 5b, Table S1). Considering the plant-size traits, the PCA grouped populations DER008, DER023, DER051, DER075, and BGV-UPM 1549 on the right-top of the graphic, with high and similar values for $Height_{Plant}$, $Height_{Stem}$ and $Length_{Internode}$. In contraposition, populations DER001, DER057, DER081, and DER103 had low values for those traits and were grouped on the left-bottom of the graphic (Figure 5b, Table S1). Finally, no correlations between the geographic origin and distribution of populations in the analysis were determined.

4. Discussion

The characterization of the germplasm available for target traits is an essential step for selection and breeding programs [10]. As far as we know, this is the first report to characterize and compare wall rocket germplasm. Some adaptations of IPGRI descriptors [20] were required. For instance,

the intensity of lobation for Eruca does not easily match with the lobation patterns found in wall rocket. Moreover, other descriptors, such as the petal color that in wall rocket is characterized by white flowers with no color variation, although not used in the current study, should also be revised and adapted [30]. Thus, specific descriptor lists should be established for the species in order to help in breeding programs and study comparisons.

Low to moderate differences were found among populations. The low diversity registered may be explained by genetic factors 1) related to the species or 2) to the material collected. On the one hand, it may be that wall rocket as a species does not display great morphological variability for the traits evaluated. a similar situation has been observed for wild rocket, which, in contrast to salad rocket, displays greater diversity as a species [17,18]. On the other hand, this low variability may also be related to the area prospected. The strategy followed for our breeding program is the Focused Identification of Germplasm Strategy (FIGS). It is based on the assumption that wild germplasm from a specific origin carry adaptative traits resulting from the natural selection pressures in that environment [31]. Thus, collecting local germplasm may increase success in breeding programs aimed at obtaining cultivars for our region and other similar Mediterranean regions. However, the FIGS strategy may have resulted in the collection of low variability, and using populations from other, farther regions may result in a diversity increase. Interestingly, the accessions transferred from the BGV-UPM, which were collected also in Spain five decades ago, did not greatly differ from the current populations either.

The PCA is considered a useful tool for screening germplasm in breeding programs [32] and is widely used when morphoagronomic traits are analyzed. It should be noticed in this point that only the upper leaves were included. The lower leaves were greatly different and should consequently be avoided during the harvest in order to increase the homogeneity of the final product. The PCA results suggest that DER017 may be a good candidate for obtaining small plants developing small leaves. By contrast, populations on the right of the graphic may be selected for increasing the leaf size of the future cultivar. However, broad-sense heritabilities for these descriptors were estimated as low to moderate. The moderate heritability estimates are a key point for current and future selection programs in wall rocket, and should be considered for adequate selections. Nevertheless, the high Pearson correlations determined between leaf length, width, perimeter and area indicated that leaf proportions are maintained despite leaf size. These correlations may be also considered as indicators of low variance among populations in terms of leaf shape and intensity of lobation, reinforcing the results obtained for these traits.

Regarding the composition traits, our results were promising for the classification of wall rocket as a crop with high accumulation of bioactive compounds. The species has been described as being rich in glucosinolates, mainly sinigrin [33,34], while the evaluation of other compounds with an antioxidant capacity are less studied. According to our study, wall rocket could be considered a leafy vegetable with relevant amounts of AA, like rocket crops and other *Brassicaceae* [9,35]. The contents found in our work were significantly greater than the levels previously determined by Salvatore et al. [36], although differences may correspond in part to the different treatment of materials (fresh vs. boiled). In addition, our results highlighted the content in total phenolics in concordance with the work of Disciglio et al. [11]. Phenolics are less studied in *Brassicaceae* than in other botanical families and are usually included in works focused on the study of glucosinolates as secondary metabolites of importance in this family [33,37,38]. However, our results suggest that these compounds may be considered to be a part of the antioxidant capacity of wall rocket as well. Therefore, we suggest that evaluating the diversity and concentrations of specialized metabolites in relation to the morphological diversity could provide further insight in the potential of wall rocket as a new crop with high added value. Such metabolites would also include, together with AA and TP, other compounds not described in the present work, such as the glucosinolates, which are of high importance in the biological activity and also for the taste of the species [39]. On the other hand, a high content in NO_3^- was also determined. Values were significantly greater than the ones previously established for the

species [11,40]. The accumulation of NO_3^- can vary among genotypes [41], but it is strongly affected by growing conditions and crop practices. Factors such as light intensity, air temperature and moisture, growth density, the duration of the growing period and fertilization, among others, can determine these values [42]. Our results suggest that the growing conditions used in this assay may not be adequate for wall rocket cultivation, while modifying the cultivation practices may result in a quality improvement.

5. Conclusions

This is the first study in analyzing the morphological aspects of wall rocket by comparing a large quantity of materials. The limited variation in terms of size, morphology, and development traits should be considered in breeding programs, as it will determine the amount of different materials that can be developed. Despite this moderate variation, some materials may be selected, as revealed by the PCA analysis, such as DER017 or those populations with bigger leaves. In addition, the morphological differences between lower and upper leaves suggest that it would be possible to increase the homogeneity of the final product by avoiding the harvest of the lower leaves.

Regarding the composition traits, wall rocket has been confirmed as a vegetable with high content in ascorbic acid and phenolic compounds. It is also a NO_3^- bioaccumulator. However, the establishment of negative correlations among these traits is a promising result to be exploited in terms of genetic selection and/or the selection of adequate cultivation practices. In summary, the information revealed in this study can be considered a tool for understanding the expected variation for wall rocket as a species. Nevertheless, including new materials from other regions, as well as other traits, such as glucosinolates, may increase the knowledge on variability in this potential new crop.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/2/306/s1, Table S1: the scores (for qualitative traits) or mean values (for quantitative traits) determined in the populations of wall rocket evaluated for plant habit-dependent traits, whole plant traits, and leaf traits. Table S2: the mean values and coefficients of variation (CV, %) for the content in ascorbic acid (mg 100 g^{-1} FW), total phenolics (mg CAE 100 g^{-1} FW), and nitrates (mg 100 g^{-1} FW). Figure S1: the geographical location of the wild populations of wall rocket used in the present study.

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