Potential of wall rocket (*Diplotaxis erucoides*) as a new crop: influence of the growing conditions on the visual quality of the final product

Carla Guijarro-Real, Jaime Prohens, Adrián Rodríguez-Burruezo, Ana Fita*

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Camino de Vera s/n. 46022 Valencia, Spain

*Corresponding author: Ana Fita. Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Camino de Vera s/n. 46022 Valencia, Spain. *E-mail address:* anfifer@btc.upv.es, *telephone number* +34 96 3879418, *fax number* +34 96 3879422

E-mail addresses

Carla Guijarro-Real: carguire@etsia.upv.es

Jaime Prohens: jprohens@btc.upv.es

Adrián Rodríguez-Burruezo: adrodbur@upvnet.upv.es

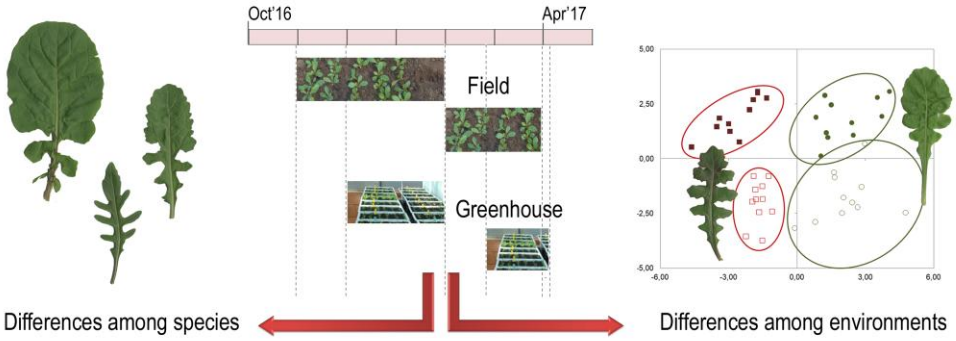
Ana Fita: anfifer@btc.upv.es

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Highlights

- Wall rocket leaf morphology is different from other rocket crops.
- Morphological differentiation can be used to enhance and promote it as new crop.
- Significant inter- and intra-population variation was detected among accessions.
- High environmental effects resulted in low heritabilities.
- Field cultivation promotes lobated, dark green leaves, commercially appreciated.

Graphical abstract



Abstract

Wild edible plants can be used for developing new crops and diversifying food markets. Wall rocket (*Diplotaxis erucoides*) is an annual weed with potential as a new crop. The present study aims at evaluating the effects of different growing conditions in the visual quality of this potential new crop. We evaluated eleven accessions of wall rocket, together with commercial rocket accessions (*Eruca sativa* and *D. tenuifolia*). Experiments were simultaneously conducted under field and greenhouse systems, and performed during two seasons. Fifteen descriptors related to leaf size, colour and shape were evaluated. Analysis of variance detected significant differences in size and shape among the three species studied, revealing the distinctiveness of wall rocket from the other rocket crops. This distinctiveness may enhance its establishment as a new crop. Comparison between the wall rocket accessions was also performed. There was relatively low morphological diversity among them. By contrast, the growing conditions had a high effect on the visual quality, especially for colour related traits and intensity of lobation, and also in the flowering time. As a consequence, the heritability estimates were low to moderate. The principal component analysis (PCA) clustered accessions according to the growing conditions, thus reinforcing the importance of environment in the morphology of wall rocket. The most promising quality of the leaves was obtained under field conditions, where the bright green colour and intensity of lobation were enhanced. In particular, accession DER006-1 was identified as a good candidate for developing a new cultivar. These results establish a basis for the management of wall rocket as a new crop. At the same time, results regarding the low diversity registered for morphology in the accessions evaluated have important implications for future breeding programmes of wall rocket.

Keywords

Diplotaxis erucoides; Field cultivation; Greenhouse cultivation; Leaf colour; Leaf morphology; New crops

1. Introduction

Rocket crops are minor vegetables from the family *Brassicaceae* characterized by the distinctive pungent taste and aroma of their leaves (Bell and Wagstaff, 2014). This common name includes different species, from which only two are economically important as crops: *Diplotaxis tenuifolia* (L.) DC. (wild rocket), and *Eruca vesicaria* (L.) Cav. subsp. *sativa* (Miller) Thell., also known as *E. sativa* Mill. (salad rocket) (Tripodi et al., 2017). Although known since Antiquity, these two species are a perfect model of modern domestication for becoming cultivated crops (D'Antuono et al., 2009; Molina et al., 2016). Salad rocket is appreciated and widely cultivated in the Middle East and Southern Asia, while wild rocket has gained much popularity in European countries (Cavaiuolo and Ferrante, 2014). However, other related species from these genera are also edible and have the potential of becoming new crops, although nowadays they remain underutilized (D'Antuono et al., 2009; Di Gioia et al., 2018). Among them, wall rocket (*Diplotaxis erucoides* (L.) DC. subsp. *erucoides*) is an edible species of potential interest.

Wall rocket is an annual wild and weedy plant widespread around the Mediterranean regions of Europe and Africa, Central Europe and Western Asia, but also naturalized in America (Martínez-Laborde, 1990; Pignone and Martínez-Laborde, 2011). As a wild vegetable, wall rocket has been traditionally gathered in different countries such as Italy, Spain or France, for being consumed raw in salads, or added to other dishes like pasta, soups and omelettes (Couplan, 2015; D'Antuono et al., 2009; Guarrera and Savo, 2016). The edible part of this species is represented by the tender leaves, which are

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

mainly gathered during the vegetative stage of the plant. They are appreciated by the pungent, slightly bitter flavour which resembles the characteristic spicy, even burning flavour of some *Brassicaceae* crops such as mustard or wasabi. The flowers can be used as an edible, decorating component as well (Bianco et al., 1998), and present the same characteristic flavour of leaves, but at lighter intensity. On the contrary, the flavour clearly differs from the common rocket crops (D'Antuono et al., 2009). This distinctive character can be a key feature for promoting its exploitation as a new crop.Wall rocket can reach the flowering stage in a short period, which varies depending on the season and region. The species should be harvested prior to the appearance of the floral bud, as it is common in rocket crops (Bell et al., 2015; Caruso et al., 2018; D'Antuono et al., 2009). This condition, together with the staggered sowing commonly used in the management of rocket crops, allows the establishment of several commercial cycles during the year. This means that the crop would have to be grown in different seasons. However, there is a lack of information regarding the influence of season on the leaf morphological traits of wall rocket. Environmental conditions such as the quantity or quality of light received, together with the temperature ranges, determine the duration of the vegetative cycle and can also affect different morphological traits related to the quality of the final product (Hatfield and Prueger, 2015; Stagnari et al., 2018). Moreover, although these conditions are dependent on the season, the use of protective systems such as greenhouses can modify them. For this reason, in the current study we have evaluated the field system and an alternative protected system under heated greenhouse. The aim of this study is to establish a base for the establishment of wall rocket as a new crop. Two independent experiments were carried out in two consecutive growing cycles in which environmental conditions differed, so that an indirect effect of the time of sowing could be also considered. We consider that a better understanding on

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

the management of wall rocket as a crop may contribute to its enhancement. Other studies have analyzed the effect of cultivation practices (e.g., soilless cultivation) on other species with potential as crops (e.g., Egea-Gilabert et al., 2013; Egea-Gilabert et al., 2014); even for wall rocket, the effect of cultivation management on nutritional traits have been tested (Di Gioia et al., 2018). However, we have not found works analyzing the effect on visual quality. On the other hand, the study was developed with pre-selected germplasm of wall rocket from the domestication programme that is being developed at the Universitat Politècnica de València (UPV, Valencia, Spain), in order to evaluate the effect of season and growing system in the visual quality of the crop. The use of local germplasm adapted to Mediterranean conditions may be more adequate for its future establishment as a new crop in countries from this region.

2. Material and methods

2.1. Plant material and cultivation

Ten pre-selected accessions of wall rocket, corresponding to the first generation seedlings of ten different wild populations collected in the Valencian Community (Spain), were used in the current study (Table S1). These local populations are conserved at the UPV. In addition, four commercial cultivars of rocket species from Shamrock Seed Co. (Salinas, CA, USA) were included: var. SSC2402, and var. Wild rocket, both belonging to *D. tenuifolia*; var. S. Rocket SSC2965 from *E. sativa*; and var. Wasabi corresponding to *D. erucoides*. The latter is, to our knowledge, the only commercial variety available of this species.

Plants were simultaneously grown in two different conditions at the UPV experimental stations: in a heated glass greenhouse with plants growing in trays (39º 29' 0" N, 0º 20' 26" W), and in field under anti-pest mesh (39º 28' 56" N, 0º 20' 11" W). Two independent experiments were carried out during two consecutive growing cycles, the late autumn-winter season (from now on, "winter season") and late winter-early spring season (from now on, "spring season"), with the same experimental design followed in the four conditions. Thus, for each condition the fourteen accessions (with independence of the species) were distributed according to a randomised block design, consisting of five blocks, one replicate per accession and block, and thirty plants per replicate (Fig. 1).

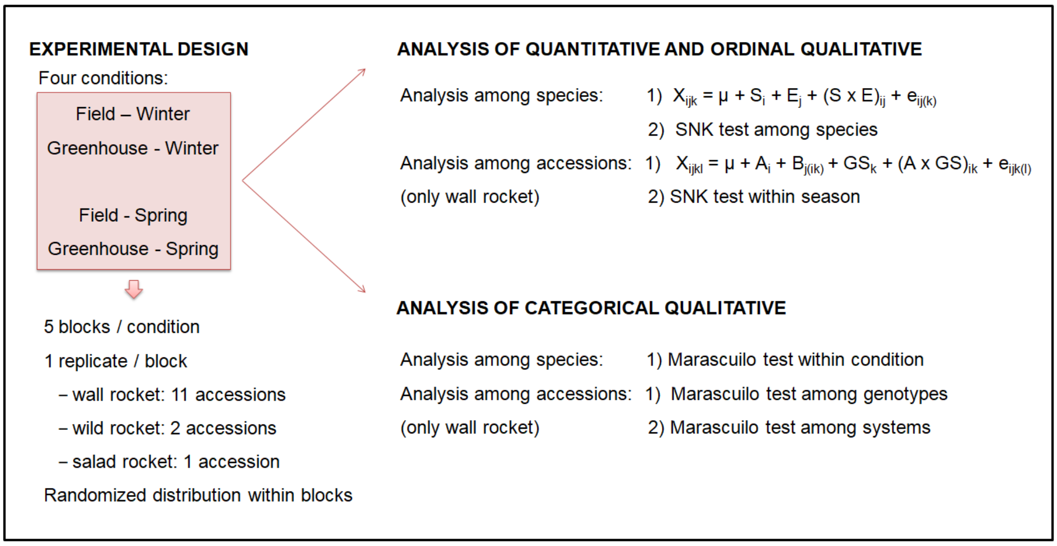


Fig. 1. Experimental layout and statistical treatment of data.

Seeds were treated with 2.5% sodium hypochlorite for 5 min followed by 100 ppm gibberellic acid solution for 24 h. The treatment was applied in order to break possible secondary dormancy and ensure a high, synchronised germination (Martínez-Laborde et al., 2007). Treated seeds were sown in commercial Neuhaus Humin-substrat N3 substrate (Klasmann-Deilmann Gmbh, Geeste, Germany), placed for two days in a growing chamber with long day conditions (16/8 h, 25 ºC) to promote the fast germination of seeds, and then moved to a heated greenhouse. Thirty plants per replicate were used. Plants used for the greenhouse system were directly sown in 40 x 25 cm^2 trays and remained in the heated greenhouse during all the experiment. Plants for the field system were firstly sown in seedling trays with the commercial substrate,

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

placed into a greenhouse until the second true leaf appeared and then thirty plants per replicate were transplanted to the field, using the same plant density as in the greenhouse system.

2.2. Morphological and agronomic traits

A total of eleven quantitative and four qualitative traits were evaluated. Many traits were adapted from the normalized descriptors for salad rocket (*Eruca spp.*) (IPGRI, 1999), considering the diversity among the three species. In addition, other traits that we considered of relevance were also included (Table 1). The fourth leaf of five plants per accession and replicate were analyzed when fully expanded and before the elongation of the floral stem. The relative chlorophyll content was measured with a chlorophyll meter (SPAD-502 Plus, Konica-Minolta, Tokio, Japan), and results were expressed as SPAD units. The rest of quantitative traits were measured using the Tomato Analyzer v3.0 software (Rodríguez et al., 2010). Qualitative traits were measured using predetermined values (categorical) or scales (ordinal), as indicated in Table 1. Finally, the days to flowering were calculated as days after sowing needed to ensure that the floral bud was visible in at least five plants per accession and replicate, before the floral stem elongation. This trait was only measured in the accessions of wall rocket.

Table 1. Descriptors used for the leaf characterization of the wall rocket, wild rocket and salad rocket accessions.

Descriptor	**Code**	**Units/scale**
Quantitative		
Days to flowering[a]	FLW-Time	days
Leaf length[b]	LL	cm
Leaf width	LW	cm

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

LL/LW ratio[b]	LL/LW	$cm \cdot cm^{-1}$
Leaf perimeter[b]	LP	cm
Leaf area[b]	LA	cm^2
Lamina colour lightness	L*	0 = black; 100 = white
Lamina colour hue angle	HUE	0º = red; 90º = yellow; 180º = green; 270º = blue
Lamina colour chroma	CHROMA	0 = completely unsaturated; 100 = fully saturated
Relative chlorophyll content	SPAD	SPAD units
Number of lobes	LOB-Num	-
Qualitative categorical		
Leaf blade shape	SHAPE	1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate
Terminal lobe shape	T-SHAPE	1 = lanceolate, wild rocket type; 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type
Margin shape	M-SHAPE	1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate
Qualitative ordinal		
Intensity of lobation	LOB-Int	0-5 (0 absent, 5 deep lobation)

[a]Trait measured for wall rocket accession, considered as days after sowing needed for developing a visible floral bud in at least five plants per accession, block and system

[b]Trait measured including petiole

2.3. Data analysis

Statistical treatment of data was different depending on the nature of the traits (i.e., quantitative and ordinal qualitative traits, or categorical qualitative traits). Two analyses were performed in both cases: 1) for comparison among species; and 2) for comparison among accessions of wall rocket (Fig. 1).

For quantitative and ordinal qualitative data, data were subjected to fixed effects model analysis of variance (Gomez and Gomez, 1984). The analysis among species was performed using the average values for each accession across the five blocks as data. Average data were submitted to a multivariate analysis of variance (ANOVA) in order to test the effects of species (S, with three levels: wall rocket, wild rocket and salad rocket), environment (E, with four environments: field-winter, greenhouse-winter, field-spring, greenhouse-spring) and S x E interaction. The linear model used 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 of the three species were obtained and significant differences were analyzed using the Student-Newman-Keuls multiple range test ($P = 0.05$). The second analysis only included the accessions of wall rocket. The effects of accession (A, eleven accessions), growing system (GS, field or greenhouse) and A x GS interaction for each season were tested by means of a multivariate ANOVA, using the values of the five replicates (blocks) for each accession. The linear model adopted in this case was: $X_{ijkl} = \mu + A_i + B_{j(ik)} + GS_k + (A \times GS)_{ik} + e_{ijk(l)}$, 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

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

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. Study of the differences was performed using a Student-Newman-Keuls test ($P = 0.05$). Broad-sense heritabilities (H^2) were calculated according to Wrickle and Weber (1986). H^2 for each specific condition was calculated by the formula: $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2)$, and for each system was calculated by the formula: $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GE}^2 + \sigma_E^2)$, where σ_G^2, σ_E^2 and σ_{GE}^2 are the estimates of genotype, environment, and genotype x environment variances, respectively.

Categorical qualitative data were expressed as percentage of each category against the total for each descriptor. Signification of differences were studied by means of the Marascuilo test ($P = 0.05$). A first analysis was performed among the three species for eahc specific environment. The second analysis compared traits among the eleven accessions of wall rocket and also among environments.

Finally, a Principal Component Analysis (PCA) was performed using the Clustvis tool (Metsalu and Vilo, 2015) for the accessions of wall rocket. Both quantitative and qualitative data were used in the PCA. Data were ln-transformed, centred and vector scaling was applied to rows prior to analysis. The category corresponding to "entire margin shape" was not included in the analysis since the category was only present in one accession and specific condition.

3. Results

3.1. Variation among the three species

The contribution of the species, environment and S x E interaction effects to the total sum of squares varied among traits. The species had great effect in most parameters related to size (up to 67.8%, for leaf area), and in the number of lobes and intensity of lobation (51.4% and 39.5%, respectively), while the effect of the environment was

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

mainly no significant (Table S2). In addition, there was a significant effect of the S x E interaction for all traits except for the leaf length/width ratio; the contribution to the total sum of squares for those traits ranged between 8.1% (lightness) and 46.0% (perimeter).

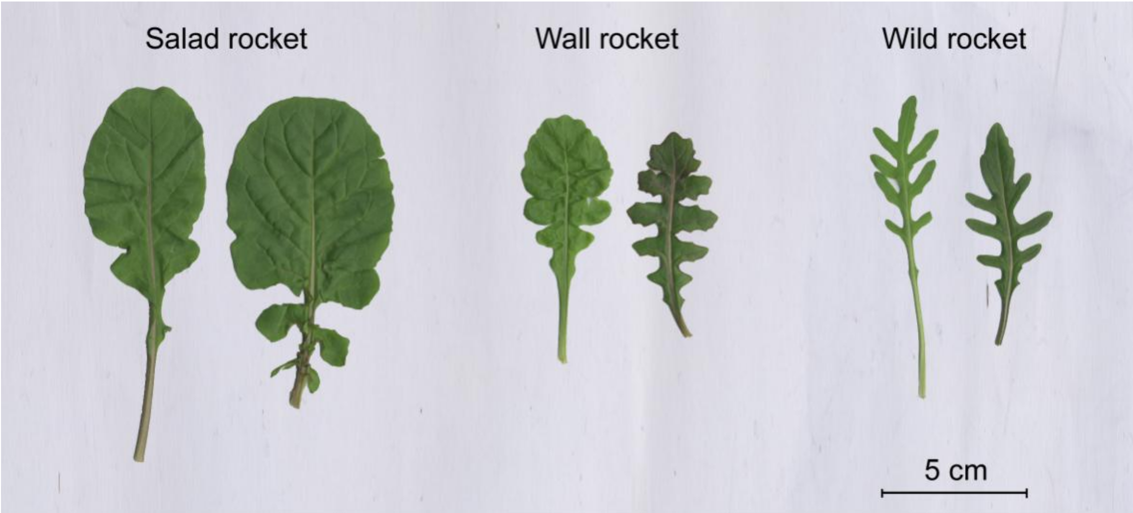


Fig. 2. Leaves of salad rocket, wall and wild rocket derived from plants cultivated under greenhouse (left) or field (right) systems.

Table 2 shows the mean values for the three species for the different traits. Compared to the other rocket crops, leaves of wall rocket were short in length and had a medium width value (Fig. 2);the leaf area was intermediate between wild and salad rocket. In addition, this species developed an intermediate lobation considering both number of lobes and intensity (Table 2); wild rocket displayed the greatest lobation characters, while salad rocket developed leaves more entire.

Table 2. Mean values of wall rocket (WallR), wild rocket (WildR) and salad rocket (SaladR) for the quantitative and ordinal qualitative traits evaluated.

Descriptor[a]	**WallR**	**WildR**	**SaladR**

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

LL	8.24^{a}	8.66^{b}	10.74^{c}
LW	3.01^{b}	2.80^{a}	3.87^{c}
LL/LW	2.78^{a}	3.14^{b}	2.83^{a}
LP	27.07^{a}	29.41^{ab}	30.44^{b}
LA	11.86^{b}	8.02^{a}	21.40^{c}
L*	44.13^{b}	44.57^{b}	40.96^{a}
Hue	128.60^{b}	127.74^{a}	128.80^{b}
Chroma	31.75^{a}	31.46^{a}	30.97^{a}
SPAD	38.60^{a}	39.85^{a}	40.02^{a}
LOB-Num	6.57^{b}	8.29^{c}	3.10^{a}
LOB-Intens	2.92^{b}	3.99^{c}	1.91^{a}

Means within traits with different letters indicate significant differences among species (P = 0.05) according to the Student-Newman-Keuls multiple range test

[a]LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Wall rocket also presented differences for qualitative traits (Fig. 2). On average, 61% of leaves presented spatulated shape both in field and greenhouse, together with obovate in greenhouse (33.8%) or obovate and elliptic in field (21.0% and 17.3%, respectively) (Fig. 3, Table S3). By contrast, wild rocket developed leaves mainly lanceolated but also elliptic (60.1% and 35.4% on average, respectively), while leaves of salad rocket were mainly obovate (77.5% on average). According to the shape of the terminal lobe, both wall rocket and salad rocket displayed salad rocket type, mainly rounded, but also acute in the former and broadly rounded in the latter (Fig. 3, Table S3). Finally, the margin shape of salad rocket and wild rocket was mainly entire, while in wall rocket the main shape was crenate-dentate.

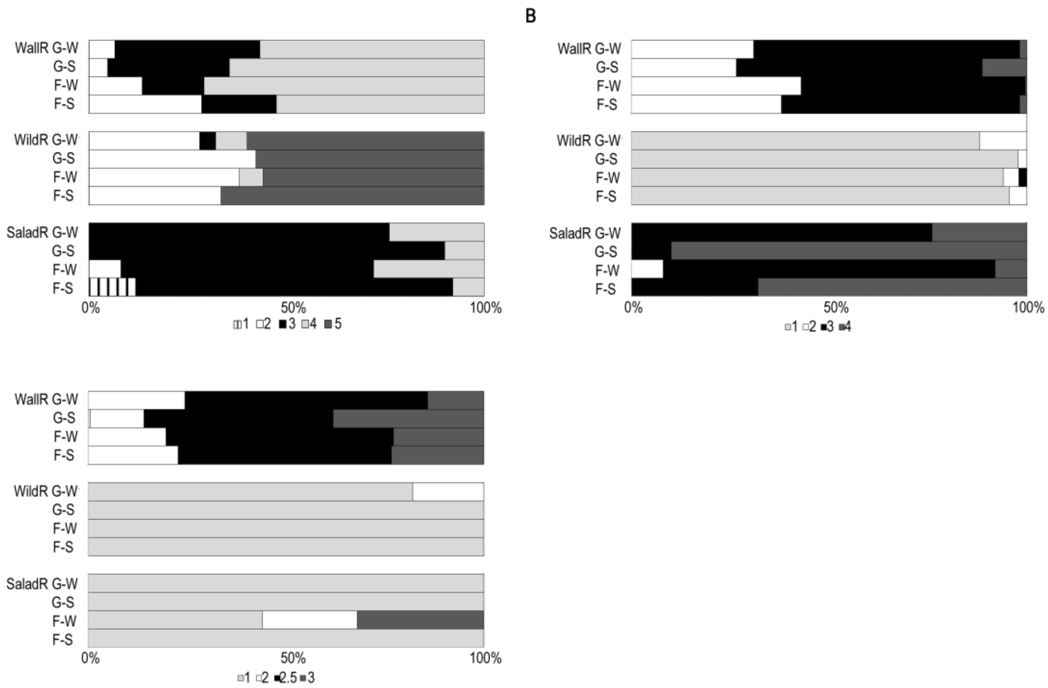


Fig. 3. Percentage of categorical descriptors analyzed in leaves of wall rocket (WallR), wild rocket (WildR) and salad rocket (SaladR), in the four environments described: greenhouse-winter season (G-W), greenhouse-spring season (G-S), field-winter season (F-W), field-spring season (F-S). A) Categories for the leaf shape: 1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate. B) Categories for the shape of terminal lobe: 1 = lanceolate to acute, wild rocket type; 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type. C) Categories for the shape of margin: 1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate.

3.2. Variation among wall rocket accessions

3.2.1. Effect of accession and environment in the quantitative traits

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Significant differences among the eleven accessions were determined for most quantitative traits and intensity of lobation, in both seasons (Table 3). However, differences were no significant for leaf length and area, but also for leaf width and colour hue angle in winter, and relative chlorophyll content in spring. In any case, the contribution of the accession effect to the total sum of squares was low, ranging between 1.7% (flowering time) and 25.0% (leaf length/width ratio) in winter, and between 5.2% (flowering time) and 28.1% (lightness) in spring (Table 3).

The effect of the growing system was also highly significant for most traits, specially during the winter season (Table 3). The contribution to the total sum of squares ranged from 0.0% (leaf width) to 90.4% (flowering time) in winter. Eight of the twelve traits presented percentages > 35%. Specifically, the system was the greatest contributor for the flowering time, leaf colour lightness and chroma, relative chlorophyll content and intensity of lobation (Table 3). In spring, this contribution was commonly lower, and ranged from 0.2% (flowering time) to 58.6% (number of lobes). Only three traits presented values > 35%: colour hue angle, number of lobes and intensity of lobation, being the greatest contributor in the latter two. Surprisingly, effects on the flowering time, leaf colour lightness and chroma were no significant and accounted for < 7% (Table 3).

On the other hand, A x GS interaction was only significant for three traits in spring (flowering time, leaf length/width ratio and intensity of lobation), and five in winter (leaf length/width ratio, colour lightness and chroma, relative chlorophyll content and intensity of lobation) (Table 3). Nevertheless, the contributions to the total sum of squares were, in any case, < 17%.

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Table 3. Sum of squares (in percentage, %) for effects of accession (A), growing system (GS), A x GS interaction, block (B), and residuals (R) for the quantitative and qualitative ordinal descriptors evaluated in the eleven accessions of wall rocket during the winter and spring seasons.

	Winter					**Spring**				
Descriptor[a]	**A**	**GS**	**A x G**	**B**	**R**	**A**	**GS**	**A x G**	**B**	**R**
FLW-Time	1.7^{**}	90.4^{***}	0.4^{ns}	3.3	4.1	5.2^{**}	0.2^{ns}	4.2^{*}	77.6	12.8
LL	6.0^{ns}	37.4^{***}	3.5^{ns}	6.8	46.3	8.3^{ns}	0.6^{ns}	12.6^{ns}	20.6	57.9
LW	15.1^{ns}	0.0^{ns}	8.5^{ns}	8.1	68.3	11.9^{*}	19.5^{**}	7.3^{ns}	17.2	44.1
LL/LW	25.0^{***}	43.4^{***}	5.7^{*}	3.6	22.2	21.7^{***}	34.4^{***}	16.4^{***}	3.3	24.2
LP	23.3^{**}	0.8^{ns}	5.9^{ns}	9.0	60.9	18.0^{***}	31.0^{**}	4.5^{ns}	17.8	28.8
LA	4.0^{ns}	11.5^{**}	5.7^{ns}	7.1	71.7	10.6^{ns}	10.0^{ns}	8.5^{ns}	18.7	52.2
L*	8.5^{***}	70.3^{***}	3.8^{**}	6.5	10.9	28.1^{***}	6.5^{ns}	4.5^{ns}	22.7	38.1
HUE	10.0^{ns}	21.7^{**}	14.5^{*}	6.8	47.0	6.3^{*}	37.6^{*}	2.5^{ns}	30.1	23.5
CHROMA	6.9^{***}	64.2^{***}	3.4^{ns}	9.9	15.5	13.5^{**}	5.3^{ns}	5.2^{ns}	38.1	37.9
SPAD	4.7^{**}	73.0^{***}	3.2^{*}	5.9	13.1	5.5^{ns}	27.9^{*}	3.7^{ns}	36.0	26.9
LOB-Num	23.2^{***}	43.9^{***}	2.4^{ns}	7.6	23.0	10.1^{***}	58.6^{***}	5.1^{ns}	4.2	22.0

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

LOB-Intens	16.8^{***}	54.1^{***}	4.3^{*}	8.2	16.6	14.8^{***}	50.9^{***}	7.2^{**}	6.3	20.7

^{ns}, *, ** and *** mean no significant, or significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively[a]LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

3.2.2. Heritability of quantitative traits

Heritability was low (< 30%) to moderate (< 70%) in all cases (Table 4). Moreover, heritability estimates of 0 were obtained for some traits under specific environments, like for some size related traits in greenhouse-winter condition, or colour related traits in field-spring condition.

In the greenhouse system, traits including leaf length/width ratio, colour lightness, number of lobes and intensity of lobation had moderate values in each specific season and in the system (Table 4). Moderate values were maintained under field conditions for leaf length/width ratio and colour lightness, but also for leaf width and area. Surprisingly, flowering time, colour related traits, chlorophyll content and lobation traits presented great differences between seasons in the field (Table 4). Thus, values were moderate or almost moderate for winter (0.21-0.59), while they decrease to very low (< 0.16) or even 0.0 during spring.

Table 4. Broad sense heritability (H^2) of the quantitative and ordinal qualitative descriptors evaluated in the eleven accessions of wall rocket under each growing condition, and global heritability in each system.

	Greenhouse			Field		
Descriptor[a]	**Winter**	**Spring**	**Total**	**Winter**	**Spring**	**Total**
FLW-Time	0.12	0.35	0.22	0.22	0.05	0.18
LL	0.00	0.05	0.06	0.20	0.11	0.05
LW	0.00	0.02	0.01	0.35	0.25	0.30
LL/LW	0.54	0.52	0.43	0.37	0.42	0.23
LP	0.04	0.16	0.17	0.41	0.39	0.36
LA	0.00	0.00	0.00	0.16	0.28	0.15
L*	0.31	0.40	0.36	0.59	0.16	0.39

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

HUE	0.06	0.20	0.17	0.31	0.00	0.13
CHROMA	0.15	0.22	0.31	0.42	0.00	0.19
SPAD	0.23	0.12	0.14	0.21	0.00	0.18
LOB-Num	0.44	0.27	0.36	0.33	0.13	0.18
LOB-Intens	0.45	0.43	0.43	0.45	0.03	0.24

[a]FLW-Time: flowering time (days), L: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

3.2.3. Variation registered for the qualitative traits

No great differences were determined among accessions for the qualitative traits (data not shown). Thus, all the accessions were analyzed together and only the effect of the environment was considered (Fig. 3, Table S4). The spatulate shape was predominant in both systems and seasons (> 52%), representing up to 70.9% of total under field-winter conditions. Obovate shape represented between 30.9% (spring) and 36.7% (winter) of leaves in the greenhouse system, while this percentage decreased to 17.3% on average in the field. By contrast, percentage of elliptic leaves in the latter increased up to 28.5% (spring) (Fig. 4, Table S4).

The terminal lobe was mainly rounded, with values between 56.7% (field-winter) and 67.3% (greenhouse-winter). The second main category corresponded to the acute shape, especially in the field, representing up to 42.9% of total leaves in winter (Fig. 4, Table S4). Finally, the margin was mainly an intermediate crenate-dentate shape. Interestingly, greenhouse-spring conditions increased the percentage of dentate margins to 38.3%.

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

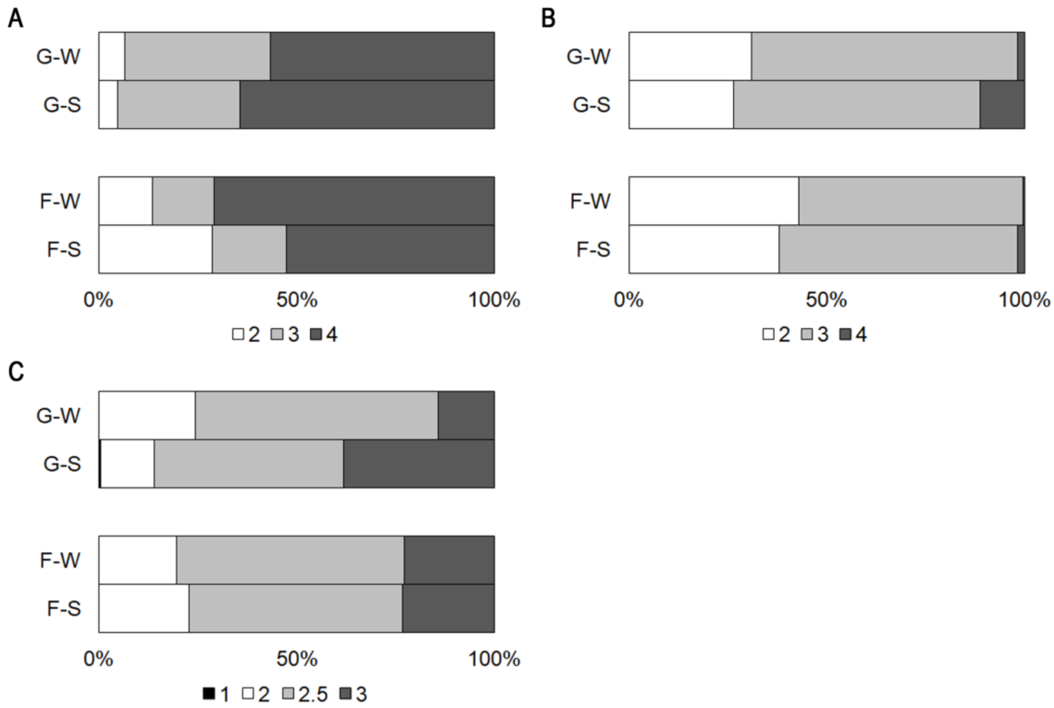


Fig. 4. Percentage of categorical descriptors analyzed in the eleven accessions of wall grown under the four environments described: greenhouse-winter season (G-W), greenhouse-spring season (G-S), field-winter season (F-W), field-spring season (F-S). A) Categories for the leaf shape: 2 = elliptic; 3 = obovate; 4 = spatulate. B) Categories for the shape of terminal lobe: 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type. C) Categories for the shape of margin: 1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate.

3.2.4. Principal Component Analysis

A PCA was performed using both quantitative and qualitative descriptors. The first and second component explained, respectively, 27.4% and 21.5% of the total variance (Fig. 5), which increased to 61.8% when the third component was considered. The first component had strong, positive correlation with colour related traits, leaf length and length/width ratio, obovate shape of leaves and rounded terminal lobe (Fig. 5a). It was negatively correlated with leaf perimeter, relative chlorophyll content, number of lobes and intensity of lobation, elliptic shape of leaves and acute terminal lobe, and flowering time. The second component had positive correlations with flowering time, leaf length/width ratio, colour lightness and chroma, and crenate-dentate margin shape (Fig. 5a). It was negative correlated with leaf width, perimeter and area, colour hue angle and broadly rounded terminal lobe.

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

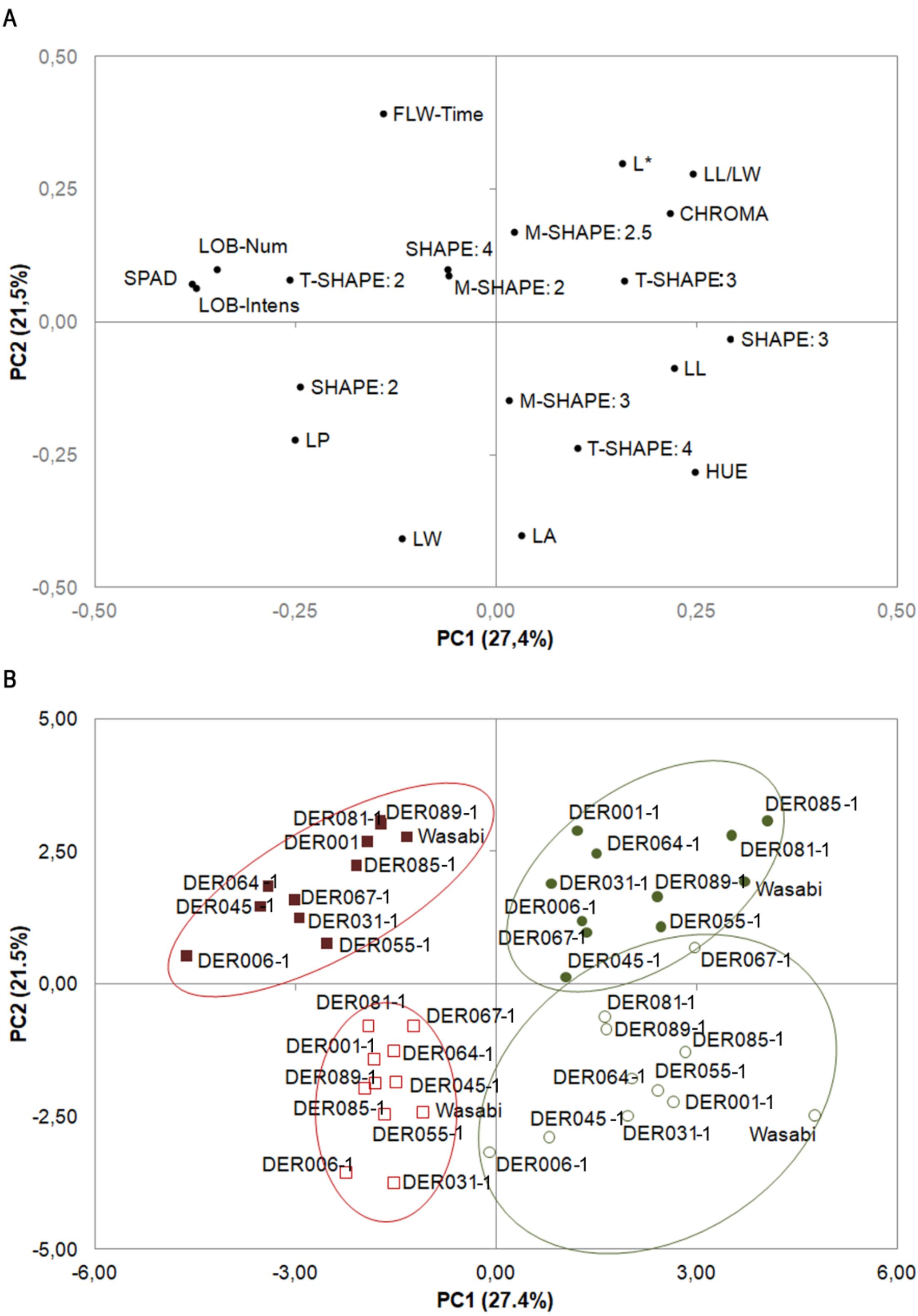


Fig. 4. Principal Component Analysis of the eleven accessions of wall rocket evaluated under the four growing conditions. A) PCA loading plot for the first (PC1) and second (PC2)

component analysis. Descriptors correspond to: LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio (cm·cm^{-1}), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation, SHAPE: leaf blade shape (2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate), T-SHAPE: terminal lobe shape (2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type), M-SHAPE: margin shape (2 = crenate; 2.5 = crenate-dentate; 3 = dentate). B) PCA score plot for the first (PC1) and second (PC2) components, with identification of the specific environmental conditions in which plants were grown: greenhouse-winter season (*coloured circle*), greenhouse-spring season (*open circle*), field-winter season (*coloured square*), and field-spring season (*open square*).

The projection of accessions in the PCA score plot confirmed that samples were mainly separated according to the specific conditions in which they were grown (Fig. 5b). The first component separated between samples from the greenhouse system, with positive values, and the field system, which had negative values. The second component separated samples between seasons (Fig. 5b). Samples grown in the winter season had positive values in this axis, while material from the spring season generally presented negative values. This distribution corresponded to differences in the mean values among conditions (Fig. 4, Table 5). For instance, plants in spring needed on average 34 days to reach the flowering stage. However, this time increased from 16 to 33 days in winter (greenhouse and field, respectively). Plants growing in the field increased the relative content in chlorophyll, especially in winter, as well as the number of lobes and intensity of lobation (Table 5). This system also increased the percentage of elliptic leaves (Fig. 4). By contrast, the greatest values of leaf colour lightness and chroma were found in plants grown in the greenhouse, winter condition (Table 5).

Finally, some accessions plotted in similar positions within each PCA graph (Fig. 5b). Thus, DER006-1 was mainly placed in the lower left extreme of the plot, opposite to the

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

commercial var. Wasabi. By contrast, accessions such as DER001-1, DER081-1, DER085-1 or DER089-1 mainly plotted close between them, but also close the commercial variety. Accessions from the greenhouse-spring season, however, formed a more compact plot that affected comparisons.

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Table 5. Mean values and range of the quantitative and ordinal qualitative descriptors evaluated in the eleven accessions of wall rocket in the greenhouse and field systems, during the winter and spring season.

	Winter				**Spring**			
	Greenhouse		**Field**		**Greenhouse**		**Field**	
Descriptor[a]	**Mean**	***Range***	**Mean**	***Range***	**Mean**	***Range***	**Mean**	***Range***
FLW-Time	50.47^{a}	(48.60; 52.60)	66.98^{b}	(65.40; 70.00)	34.18^{a}	(32.40; 36.60)	34.45^{b}	(33.80; 35.75)
LL	8.87^{b}	(7.92; 9.25)	7.45^{a}	(6.90; 8.03)	8.41^{a}	(7.57; 9.13)	8.25^{a}	(7.55; 8.93)
LW	2.82^{a}	(2.54; 3.13)	2.83^{a}	(2.56; 3.12)	3.04^{a}	(2.78; 3.26)	3.37^{b}	(3.17; 3.70)
LL/LW	3.20^{b}	(2.75; 3.70)	2.68^{a}	(2.50; 3.01)	2.78^{b}	(2.39; 3.19)	2.46^{a}	(2.20; 2.65)
LP	25.83^{a}	(21.82; 29.77)	26.50^{a}	(22.73; 30.71)	25.13^{a}	(21.85; 27.91)	30.84^{b}	(26.79; 37.40)
LA	11.23^{b}	(10.04; 12.45)	9.76^{a}	(8.58; 10.82)	12.39^{a}	(10.76; 14.01)	14.06^{a}	(12.56; 16.51)
L*	48.24^{b}	(46.90; 50.46)	43.19^{a}	(40.88; 44.20)	42.10^{a}	(40.35; 45.05)	43.00^{a}	(41.76; 44.52)
HUE	128.52^{b}	(127.54; 129.13)	127.49^{a}	(126.27; 128.77)	129.89^{b}	(129.15; 130.57)	128.50^{a}	(128.06; 128.99)
CHROMA	37.02^{b}	(34.79; 40.19)	29.41^{a}	(26.31; 31.72)	29.61^{a}	(26.32; 32.32)	30.94^{a}	(29.71; 31.98)
SPAD	34.43^{a}	(31.14; 37.24)	45.04^{b}	(42.58; 48.13)	34.82^{a}	(32.00; 37.76)	40.09^{b}	(37.87; 42.74)

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

LOB-Num	6.00[a]	(4.44; 7.52)	8.28[b]	(7.40; 9.20)	4.21[a]	(1.60; 6.48)	7.80[b]	(7.08; 8.52)
LOB-Intens	2.43[a]	(1.68 - 3.16	3.83[b]	(2.96 - 4.40	1.97[a]	(0.67 - 3.28	3.45[b]	(3.05 - 3.72)

Means within rows for each cycle with different letters are significantly different at *P* = 0.05 according to the Student-Newman-Keuls multiple range test

[a]FLW-Time: flowering time (days), L: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

4. Discussion

Wall rocket is broadly considered as a weed (e.g., Araj and Wratten, 2015; Martínez-Laborde et al., 2007; Pignone and Martínez-Laborde, 2011). However, our research is pioneering on the study of this species as a crop, with the aim of developing new commercial cultivars. The development of materials adapted to cultivated conditions and with distinctive traits increases the chances of the establishment of wall rocket as a new crop, by encouraging the acceptance of producers and consumers.

In particular, the present work was focused on analyzing the effect that greenhouse and field cultivations have on morphological traits of interest in the final product. These two systems present great differences in terms of temperature, light intensity, wind, or air humidity, among others, factors that can affect growth and development of plants (Figàs et al., 2018b). Moreover, for vegetable crops with short cycle, differences in the month of sowing determine the environmental conditions during the growth period, and this seasonal climate variability can affect visual quality as well (Bonasia et al., 2017). Commercial materials of salad and wild rocket were included in the analyses in order to compare with our materials. The three species have been previously compared in terms of nutritional characteristics (e.g., D'Antuono et al., 2008; Di Gioia et al., 2018). However, there is little information regarding parameters of visual quality in these commercial crops (e.g., Bonasia et al., 2017; Egea-Gilabert et al., 2009; Taranto et al., 2016). Our results showed that the three species were clearly differentiated in leaf size and shape. This indicates that the leaf traits chosen give a good result for comparing and describing the three species. Thus, they can be used as a basis for the future development of wild and wall rocket descriptors, since current normalized descriptors are specifically developed for *Eruca* spp (IPGRI, 1999). Furthermore, our results indicate that, even when different species can be considered together as rocket crops, they are distinct enough to be presented as different commercial products. In the particular case of wall rocket, this distinctiveness can play a key role in the commercial success of the new crop. In fact, food markets are continually looking for new products for diversification, and in some cases the opportunity derives from the domestication of wild edible plants (Egea-Gilabert et al., 2013).

When the eleven accessions of wall rocket were compared, results indicated the presence of genotypic differences. However, this variation was not of big magnitude, and its effect was low compared to the effect of the growing system. The lack of wide variation has been previously reported in other species from the same genus, in particular for wild rocket, in contrast to the greater variation registered for the salad rocket (Taranto et al., 2016). This relatively low diversity must be considered in breeding programmes, since breeders exploit genetic variability for developing materials of interest (Voss-Fels and Snowdon, 2016). Thus, our results suggest that a limit number of morphological different varieties may be developed with our materials. Nevertheless, according to the wide distribution described for the species (Pignone and Martínez-Laborde, 2011), we do not discard that materials from other regions may increase the morphological diversity.

By contrast, we found a great influence of the environmental conditions in most morphological traits. Modification of morphology by plants is a common adaptation to environmental conditions (Stagnari et al., 2018). Thus, our results support the idea that the evaluation of genotypes across different environments should be a critical determinant when new crop cultivars are being developed (Stommel et al., 2015). In addition, when the crop management to be implemented is unclear, as it happens with new crops, the evaluation of materials among different environments may help to identify the best environmental conditions in order to obtain a desirable quality.

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

The combined inter-population low genetic diversity and high influence of the environmental conditions, together with the intra-population diversity registered, affected heritability estimates. Unlike our results, in an ideal situation, heritability of morphological traits should be high (Figàs et al., 2018a), thus increasing the success of the breeding selection. In addition, estimates varied among growing conditions for many traits, which is described as a general behaviour for broad-sense heritability (Hoffmann and Merilä, 1999). Thus, estimates should be performed as independent for different conditions, and materials selected for those specific conditions (Rodríguez-Burruezo et al., 2002).

The PCA did not cluster materials by genotype or origin. Figàs et al. (2018b) found similar results when clustering materials of tomato corresponding to the same varietal type that are genotypically close among them. On another study, Egea-Gilabert et al. (2009) found that, even for accessions of *Eruca* sp. genotypically different, leaf morphological traits were not strong enough to group materials by origin. Thus, our clustering results reinforce the hypothesis that the materials had relative poor morphological variation among them. On the contrary, leaf morphology was strongly conditioned, not only by the growing environment but also the season. In this way, field conditions increased the number and intensity of lobation. Bell et al. (2017) found that marked leaf lobation in salad rocket increased acceptance in European consumers as it is the expected shape of rocket crops. In a similar way, growing wall rocket under conditions that increase the lobation may promote the acceptance of the crop. Colour of leafy vegetables plays also a decisive role in the acceptance by consumers (Colonna et al., 2016). In this respect, plants growing under field increased the relative chlorophyll content, main determinant of the colour in green vegetables (Roshanak et al., 2016), while the greenhouse-winter combination increased the chroma and lightness. Since

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

bright green colour of leaves is desirable for markets, our results indicate that field conditions will provide a final product with better colour-related traits. However, winter in the field promoted the accumulation of anthocyanins, visible as purple spots along the surface (data not shown). This accumulation, induced by low temperatures and related to stress tolerance (D'Amelia et al., 2018), implied loss of quality in the final product, and may indicate that the harsh winter is not adequate for the crop. However, the use of climate crop cover nets may reduce this negative aspect and other related to the very cold conditions, but new experiments should be conducted for assessing its effect.

On the other hand, leaves were smaller in winter, especially under field conditions. Reducing size of leaves is common when plants grow under cold temperatures (Buitrago Acevedo et al., 2017). During this season, plants also increased the vegetative stage period, especially under field conditions. This result matches expectations, since increasing the day length and temperature results in a higher development of rocket crops (Bonasia et al., 2017).

Finally, despite the low effect of genotype, distribution of accessions within each graph may be an indicator of the presence of some global similarities among specific materials. As a promising result, the pre-selected accession DER006-1 seems a good candidate for the breeding programme, with the goal of obtaining a new, distinct variety from the existing commercial Wasabi.

5. Conclusions

This work is a basis for the determination of the proper conditions for growing wall rocket as a new crop. In agreement with our findings, field conditions would be an adequate option for its development. Under these conditions, plants developed leaves with a higher number of lobes and also a great intensity of lobation, which may increase

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

its acceptance in markets due to similarity with wild rocket. However, wall rocket was distinct enough to be considered as a new vegetable. Field conditions also positively affected the colour quality of the product. Nevertheless, the presence of anthocyanins in the late autumn-winter season may damage this visual quality. Thus, the harsh winter conditions should be presumably avoided for growing this new crop in the field, unless protected.

On the other hand, results indicated the presence of low morphological variation among materials, and also low-moderate heritability of the traits evaluated. This lack of diversity must be considered for future breeding programmes. Nevertheless, multivariate principal component analysis was useful for defining the accession DER006-1 as a good candidate for the development of a new variety, distinct from the commercial var. Wasabi.

Declarations of interest

None.

Acknowledgements

C. Guijarro-Real is grateful to the Ministerio de Educación, Cultura y Deporte of Spain (MECD) for the predoctoral FPU grant (FPU14-06798). Authors also thank Dr. A. M. Adalid-Martínez, Ms. K. Aguirre, and Ms. S. Benická for helping in the field tasks.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Araj, S.E., Wratten, S.D., 2015. Comparing existing weeds and commonly used insectary plants as floral resources for a parasitoid. Biol. Control 81, 15–20. https://doi.org/10.1016/j.biocontrol.2014.11.003

Bell, L., Methven, L., Wagstaff, C., 2017. The influence of phytochemical composition and resulting sensory attributes on preference for salad rocket (*Eruca sativa*) accessions by consumers of varying TAS2R38 diplotype. Food Chem. 222, 6–17. https://doi.org/10.1016/j.foodchem.2016.11.153

Bell, L., Oruna-Concha, M.J., Wagstaff, C., 2015. Identification and quantification of glucosinolate and flavonol compounds in rocket salad (*Eruca sativa*, *Eruca vesicaria* and *Diplotaxis tenuifolia*) by LC–MS: Highlighting the potential for improving nutritional value of rocket crops. Food Chem. 172, 852–861. https://doi.org/10.1016/j.foodchem.2014.09.116

Bell, L., Wagstaff, C., 2014. Glucosinolates, myrosinase hydrolysis products, and flavonols found in rocket (*Eruca sativa* and *Diplotaxis tenuifolia*). J. Agric. Food Chem. 62, 4481–4492. https://doi.org/10.1021/jf501096x

Bianco, V.V., Santamaria, P., Elia, A., 1998. Nutritional value and nitrate content in edible wild species used in Southern Italy. Acta Hortic. 467, 71–90. https://doi.org/10.17660/ActaHortic.1998.467.7

Bonasia, A., Lazzizera, C., Elia, A., Conversa, G., 2017. Nutritional, biophysical and physiological characteristics of wild rocket genotypes as affected by soilless cultivation system, salinity level of nutrient solution and growing period. Front. Plant Sci. 8, e300. https://doi.org/10.3389/fpls.2017.00300

Buitrago Acevedo, M.F., Groen, T.A., Hecker, C.A., Skidmore, A.K., 2017. Identifying leaf traits that signal stress in TIR spectra. ISPRS J. Photogramm. Remote Sens. 125,

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

132–145. https://doi.org/10.1016/j.isprsjprs.2017.01.014

Caruso, G., Parrella, G., Giorgini, M., Nicoletti, R., 2018. Crop systems, quality and protection of *Diplotaxis tenuifolia*. Agriculture 8, e55. https://doi.org/10.3390/agriculture8040055

Cavaiuolo, M., Ferrante, A., 2014. Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. Nutrients 6, 1519–1538. https://doi.org/10.3390/nu6041519

Colonna, E., Rouphael, Y., Barbieri, G., De Pascale, S., 2016. Nutritional quality of ten leafy vegetables harvested at two light intensities. Food Chem. 199, 702–710. https://doi.org/10.1016/j.foodchem.2015.12.068

Couplan, F., 2015. Le régal végétal. Reconnaître et cuisiner les plantes comestibles, Sang de la Terre, Paris.

D'Amelia, V., Aversano, R., Ruggiero, A., Batelli, G., Appelhagen, I., Dinacci, C., Hill, L., Martin, C., Carputo, D., 2018. Subfunctionalization of duplicate MYB genes in *Solanum commersonii* generated the cold-induced *ScAN2* and the anthocyanin regulator *ScAN1*. Plant Cell Environ. 41, 1038–1051. https://doi.org/10.1111/pce.12966

D'Antuono, L.F., Elementi, S., Neri, R., 2008. Glucosinolates in *Diplotaxis* and *Eruca* leaves: Diversity, taxonomic relations and applied aspects. Phytochemistry 69, 187–199. https://doi.org/10.1016/j.phytochem.2007.06.019

D'Antuono, L.F., Elementi, S., Neri, R., 2009. Exploring new potential health-promoting vegetables: Glucosinolates and sensory attributes of rocket salads and related Diplotaxis and Eruca species. J. Sci. Food Agric. 89, 713–722. https://doi.org/10.1002/jsfa.3507

Di Gioia, F., Avato, P., Serio, F., Argentieri, M.P., 2018. Glucosinolate profile of *Eruca sativa*, *Diplotaxis tenuifolia* and *Diplotaxis erucoides* grown in soil and soilless systems. J. Food Compos. Anal. 69, 197–204. https://doi.org/10.1016/j.jfca.2018.01.022

Egea-Gilabert, C., Fernández, J.A., Migliaro, D., Martínez-Sánchez, J.J., Vicente, M.J., 2009. Genetic variability in wild vs. cultivated *Eruca vesicaria* populations as assessed by morphological, agronomical and molecular analyses. Sci. Hortic. 121, 260–266. https://doi.org/10.1016/j.scienta.2009.02.020

Egea-Gilabert, C., Niñirola, D., Conesa, E., Candela, M.E., Fernández, J.A., 2013. Agronomical use as baby leaf salad of *Silene vulgaris* based on morphological, biochemical and molecular traits. Sci. Hortic. 152, 35–43. https://doi.org/10.1016/j.scienta.2013.01.018

Egea-Gilabert, C., Ruiz-Hernández, M.V., Parra, M.A., Fernández, J.A., 2014. Characterization of purslane (*Portulaca oleracea* L.) accessions: Suitability as ready-to-eat product. Sci. Hortic. 172, 73–81. https://doi.org/10.1016/j.scienta.2014.03.051

Figàs, M.R., Prohens, J., Casanova, C., Fernández-de-Córdova, P., Soler, S., 2018a. Variation of morphological descriptors for the evaluation of tomato germplasm and their stability across different growing conditions. Sci. Hortic. 238, 107–115. https://doi.org/10.1016/j.scienta.2018.04.039

Figàs, M.R., Prohens, J., Raigón, M.D., Pereira-Dias, L., Casanova, C., García-Martínez, M.D., Rosa, E., Soler, E., Plazas, M., Soler, S., 2018b. Insights into the adaptation to greenhouse cultivation of the traditional Mediterranean long shelf-life tomato carrying the *alc* mutation: A multi-trait comparison of landraces, selections, and hybrids in open field and greenhouse. Front. Plant Sci. 9, e1774. https://doi.org/10.3389/fpls.2018.01774

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. 2nd edition. Philippines, John Wiley & Sons, Inc.

Guarrera, P.M., Savo, V., 2016. Wild food plants used in traditional vegetable mixtures

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

in Italy. J. Ethnopharmacol. 185, 202–234. https://doi.org/10.1016/j.jep.2016.02.050

Hatfield, J.L., Prueger, J.H., 2015. Temperature extremes: Effect on plant growth and development. Weather Clim. Extrem. 10, 4–10. https://doi.org/10.1016/j.wace.2015.08.001

Hoffmann, A.A., Merilä, J., 1999. Heritable variation and evolution under favourable and unfavourable conditions. TREE 14, 96–101. https://doi.org/10.1016/S0169-5347-(99)01595-5

IPGRI, 1999. Descriptors for rocket (*Eruca* spp.). International Plant Genetic Resources Institute, Rome, Italy.

Martínez-Laborde, J.B., 1990. *Diplotaxis* DC., in: Nieto, G. (Ed.), Flora Ibérica: Plantas vasculares de La Península Ibérica e Islas Baleares. Real Jardín Botánico, CSIC, Madrid, pp. 346–362.

Martínez-Laborde, J.B., Pita-Villamil, J.M., Pérez-García, F., 2007. Short communication. Secondary dormancy in *Diplotaxis erucoides*: A possible adaptative strategy as an annual weed. Spanish J. Agric. Res. 5, 402–406. https://doi.org/10.5424/sjar/2007053-265

Metsalu, T., Vilo, J., 2015. ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 43, W566–W570. https://doi.org/10.1093/nar/gkv468

Molina, M., Pardo-de-Santayana, M., Tardío, J., 2016. Natural production and cultivation of Mediterranean wild edibles, in: Sánchez-Mata, M., Tardío, J. (Eds.), Mediterranean Wild Edible Plants: Ethnobotany and Food Composition Tables. Springer New York, New York, pp. 81–107.

Pignone, D., Martínez-Laborde, J.B., 2011. *Diplotaxis*, in: Kole, C. (Ed.), Wild crop relatives: Genomic and breeding resources. Oilseeds. Springer, Berlin, pp. 137–147.

Rodríguez, G.R., Moyseenko, J.B., Robbins, M.D., Huarachi Morejón, N., Francis, D.M., van der Knaap, E., 2010. Tomato Analyzer: A useful software application to collect accurate and detailed morphological and colorimetric data from two-dimensional objects. J. Vis. Exp. 37, e1856. https://doi.org/10.3791/1856

Rodríguez-Burruezo, A., Prohens, J., Nuez, F., 2002. Genetic analysis of quantitative traits in pepino (*Solanum muricatum*) in two growing seasons. J. Am. Soc. Hort. Sci. 127, 271–278.

Roshanak, S., Rahimmalek, M., Goli, S.A.H., 2016. Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and color of green tea (*Camellia sinensis* or *C. assamica*) leaves. J. Food Sci. Technol. 53, 721–729. https://doi.org/10.1007/s13197-015-2030-x

Stagnari, F., Di Mattia, C., Galieni, A., Santarelli, V., D'Egidio, S., Pagnani, G., Pisante, M., 2018. Light quantity and quality supplies sharply affect growth, morphological, physiological and quality traits of basil. Ind. Crops Prod. 122, 277–289. https://doi.org/10.1016/j.indcrop.2018.05.073

Stommel, J.R., Whitaker, B.D., Haynes, K.G., Prohens, J., 2015. Genotype × environment interactions in eggplant for fruit phenolic acid content. Euphytica 205, 823–836. https://doi.org/10.1007/s10681-015-1415-2

Taranto, F., Francese, G., Di Dato, F., D'Alessandro, A., Greco, B., Onofaro Sanajà, V., Pentangelo, A., Mennella, G., Tripodi, P., 2016. Leaf metabolic, genetic, and morphophysiological profiles of cultivated and wild rocket salad (*Eruca* and *Diplotaxis* spp.). J. Agric. Food Chem. 64, 5824–5836. https://doi.org/10.1021/acs.jafc.6b01737

Tripodi, P., Francese, G., Mennella, G., 2017. Rocket salad: Crop description, bioactive compounds and breeding perspectives. Adv. Hortic. Sci. 31, 107–113. https://doi.org/10.13128/ahs-21087

Voss-Fels, K., Snowdon, R.J., 2016. Understanding and utilizing crop genome diversity via high-resolution genotyping. Plant Biotechnol. J. 14, 1086–1094. https://doi.org/10.1111/pbi.12456

Wrickle, G., Weber, W.E., 1986. Quantitative genetics and selection in plant breeding. De Gruyter, Berlin.

Table S1. Geographical location of the original ten wild populations of wall rocket from which the pre-selected accessions derive.

Accession	Location	Province	Coordinates	
			Latitude	Longitude
DER089-1	Cabanes	Castellón	40º 11' 06" N	0º 10' 17" E
DER085-1	Castellón de la Plana	Castellón	39º 59' 42" N	0º 03' 36" W
DER001-1	Alfara del Patriarca	Valencia	39º 32' 21" N	0º 23' 06" W
DER006-1	Oliva	Valencia	38º 54' 42" N	0º 06' 49" W
DER055-1	San Isidro de Benagéber	Valencia	39º 34' 03" N	0º 23' 49" W
DER064-1	Casinos	Valencia	39º 41' 49" N	0º 42' 49" W
DER067-1	Losa del Obispo	Valencia	39º 41' 48" N	0º 53' 18" W
DER081-1	Benavites	Valencia	39º 43' 49" N	0º 14' 25" W
DER031-1	Montroy	Alicante	39º 21' 02" N	0º 38' 05" W
DER045-1	Jijona	Alicante	38º 38' 30" N	0º 28' 37" W

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Table S2. Sum of squares (in percentage, %) for the effects of species (S, wall rocket, wild rocket and salad rocket), environment (E), S x E interaction and residuals (R) for the quantitative and qualitative ordinal descriptors evaluated in the three species.

Descriptor[a]	**S**	**E**[b]	**S x E interaction**	**R**
LL	39.9***	19.5^{ns}	20.6***	19.9
LW	43.3***	24.1^{ns}	12.9***	19.7
LL/LW	15.9***	41.2**	8.2^{ns}	34.6
LP	9.9***	8.4^{ns}	46.0***	35.7
LA	67.8***	15.4^{ns}	9.5***	7.3
L*	17.5***	52.5**	8.1*	21.9
HUE	18.3***	10.7^{ns}	39.2***	31.8
CHROMA	0.8^{ns}	45.4^{ns}	22.1***	31.7
SPAD	1.8^{ns}	54.9^{ns}	28.7***	14.5
LOB-Num	51.4***	9.3^{ns}	12.6**	26.8
LOB-Intens	39.5***	9.8^{ns}	15.9***	34.8

^{ns}, *, ** and *** mean no significant, or significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

[a]LL: Leaf length (cm), LW: Leaf width (cm), LL/LW: Leaf length/Leaf width ratio ($cm \cdot cm^{-1}$), LP: Leaf perimeter (cm) LA: Leaf area (cm^2), L*: lamina colour lightness, HUE: Lamina colour hue angle, CHROMA: Lamina colour chroma, SPAD: Relative chlorophyll content (SPAD units), LOB-Num: number of lobes, LOB-Intens: Intensity of lobation

[b]Four environments were considered in the analysis, corresponding to: 1) greenhouse system in winter cycle; 2) field system in winter cycle; 3) greenhouse system in spring cycle; and 4) field system in spring cycle

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Table S3. Percentage of categorical descriptors (%) analyzed in leaves of wall rocket (WallR), wild rocket (WildR) and salad rocket (SaladR), in the four environments described: greenhouse-winter season (G-W), greenhouse-spring season (G-S), field-winter season (F-W), field-spring season (F-S).

	G-W			**G-S**			**F-W**			**F-S**		
Descriptor[a]	**WallR**	**WildR**	**SaladR**	**WallR**	**WildR**	**SaladR**	**WallR**	**WildR**	**SaladR**	**WallR**	**WildR**	**SaladR**
SHAPE												
1	-	-	-	-	-	-	-	-	-	-	-	12.0[a]
2	6.5[a]	28.0[b]	-	4.7[a]	42.2[b]	-	13.5[a]	38.0[b]	8.0[a]	28.5[a]	33.3[a]	-
3	36.7[b]	4.0[a]	76.0[c]	30.9[a]	-	90.0[b]	15.6[a]	-	64.0[b]	18.9[a]	-	80.0[b]
4	56.7[b]	8.0[a]	24.0[a]	64.5[b]	-	10.0[a]	70.9[b]	6.0[a]	28.0[a]	52.6[b]	-	8.0[a]
5	-	60.0[a]	-	-	57.8[a]	-	-	56.0[a]	-	-	66.7[a]	-
T-SHAPE												
1	-	88.0[a]	-	-	97.8[a]	-	-	94.0[a]	-	-	95.6[a]	-
2	30.9[b]	12.0[a]	-	26.5[b]	2.2[a]	-	42.9[b]	4.0[a]	8.0[a]	37.9[b]	4.4[a]	-
3	67.3[a]	-	76.0[b]	62.3[b]	-	10.0[a]	56.7[b]	2.0[a]	84.0[c]	60.2[b]	-	32.0[a]
4	1.8[a]	-	24.0[b]	11.3[a]	-	90.0[b]	0.4[a]	-	8.0[a]	1.9[a]	-	68.0[b]
M-SHAPE												
1	-	82.0[a]	100.0[b]	0.4[a]	100.0[b]	100.0[b]	-	100.0[b]	44.0[a]	-	100.0[a]	100.0[a]
2	24.4[a]	18.0[a]	-	13.6[a]	-	-	19.6[a]	-	22.4[a]	22.7[a]	-	-
2.5	61.5[a]	-	-	47.9[a]	-	-	57.5[a]	-	-	53.9[a]	-	-

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

3	14.2[a]	-	-	38.1[a]	-	-	22.9[a]	-	32.0[a]	23.4[a]	-	-

Different letters within row and environment indicate significant differences (*P* = 0.05) according to the Marascuilo procedure

[a]SHAPE: leaf blade shape (1 = orbicular; 2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate); T-SHAPE: terminal lobe shape (1 = lanceolate, wild rocket type; 2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type); M-SHAPE: margin shape (1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate)

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

Table S4. Percentage of categorical descriptors (%) analyzed in the eleven accessions of wall rocket grown under greenhouse or field conditions, during the winter and spring seasons.

	Winter		Spring	
Descriptor[a]	**Greenhouse**	**Field**	**Greenhouse**	**Field**
SHAPE				
2	6.5[a]	13.5[b]	4.7[a]	28.5[b]
3	36.7[b]	15.6[a]	30.9[b]	18.9[a]
4	56.7[a]	70.9[b]	64.5[b]	52.6[a]
T-SHAPE				
2	30.9[a]	42.9[b]	26.5[a]	37.9[b]
3	67.3[b]	56.7[a]	62.3[a]	60.2[a]
4	1.8[a]	0.4[a]	11.3[b]	1.9[a]
M-SHAPE				
1	-	-	0.4[a]	-
2	24.4[a]	19.6[a]	13.6[a]	22.7[b]
2.5	61.5[a]	57.5[a]	47.9[a]	53.9[a]
3	14.2[a]	22.9[b]	38.1[b]	23.4[a]

Different letters within row and environment indicate significant differences (P = 0.05) according to the Marascuilo procedure

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778

[a]SHAPE: leaf blade shape (2 = elliptic; 3 = obovate; 4 = spatulate; 5 = lanceolate); T-SHAPE: terminal lobe shape (2 = acute, salad rocket type; 3 = rounded, salad rocket type; 4 = broadly rounded, salad rocket type); M-SHAPE: margin shape (1 = entire; 2 = crenate; 2.5 = crenate-dentate; 3 = dentate)

Scientia Horticulturae 258 (2019) 108778 DOI: 10.1016/j.scienta.2019.108778