

**Original article****ASSERTION OF WILD ROCKET (*Diplotaxis tenuifolia*)  
VARIABILITY REGARDING SOME PHENOTYPIC  
CHARACTERS IN SEEDLING STAGE****LAZAR Simona-Laura\*, Mihai-Lucian LUNG, Mihai LAZAR, Oana CIUZAN,  
Doru PAMFIL***University of Agricultural Sciences and Veterinary Medicine Cluj - Napoca, Mănăştur St., No. 3 – 5,  
400327 Cluj-Napoca, Romania*Received 15 April 2015; received and revised form 10 August 2015; accepted 15 August 2015  
Available online 1 September 2015**Abstract**

Wild rocket (*Diplotaxis tenuifolia*) is a fourth generation vegetable with an increasing awareness of benefits in human alimentation. Such species should be conserved in seed banks in order to maintain their diversity. The phenotypic characterization of the accessions stored in such banks is mandatory but requires a high amount of resources. This study focusses on the assessment of the phenotypic variability in seedling stage in order to reduce the time and costs for the characterization of germplasm. Eight wild and cultivated accessions were used. The variability was average and high even in this early stage. All the parameters measured gave a wide picture of the differences between cultivars. The conclusion is that, unless seed is needed, it is less expensive and faster to assess the phenotypical differences between cultivars only in plantlet stage and good overview of the characters is obtained.

**Keywords:** grape, *Diplotaxis tenuifolia*, phenotype characterization, germplasm, quantitative traits.

**1. Introduction**

For over 10,000 years, man adapts cultivated plants to his requirements [1], often eliminating the varieties useless to him. This action leads to the disappearance of a large number of varieties of plants cultivated, as the local populations. This requires rational use of genetic resources leading to the creation of special areas for plant biodiversity conservation. The establishment of gene banks facilitated the conservation of plant genetic resources, the most new and modern form of germplasm storage.

The activities are very complex in gene banks [2] for scientific purpose but also for practical purposes, for the future food resources diversity. One of these activities is phenotypical germplasm evaluation. The phenotypic evaluation has some limitations such as costs with the personnel, difficulties in obtaining results in a relative short time and costs with the crop culture. The present research is focused in germplasm characterization in seeding phase, as a measure of asserting the variability of some accessions.

Wild rocket, like other minor species on which IPGRI [3], is a key crop selected for raising awareness about the world large agro-biodiversity potential and to show how little of this wealth is valued. It is estimated that of the 7,000 edible species worldwide, only a small fraction, around 150, are in fact grown and marketed. A greater attention to this neglected species, Cinderellas of

\* Corresponding author.  
Fax: +40-264-593792  
Tel: +40-264-596384  
e-mail: lazarsimonalaura@gmail.com

agriculture, is an important step both for agriculture and for diet diversification, which ultimately contributes to improve our life quality [4].

*Diplotaxis* genus is an important and well-known member of the family *Brassicaceae*.

This genus is widespread in the world, comprising 31 species [5], some of which are considered weeds[6], while others are important sources of food, herbal remedies, animal feed but also a source the gene for cytoplasmic male sterility in crops of *Brassicaceae*. In Romania, there are only three species found: *D.tenuifolia* [7], *D.erucoides* [8] and *D.viminea* [9].

The present study is focused in germplasm characterization in seeding phase, as a measure of

asserting the variability of some accessions.

## 2. Material and Methods

As biological material, different accessions were used with different proveniences as shown in Table 1. When assessing phenotypic quantitative characters, simple measuring tools (ruler and electronic caliper) were used.

In order to characterize the morphology of wild rocket accession, it was developed a list of phenotypic descriptors that are broadly general and with those proposed for rocket (*Eruca vesicaria*) [10] but also from UPOV regulations [11] in respect of varieties of *Diplotaxis* sp.

Table 1. The origin of the biological material

Species	Company/ Institution	Country of origin	Provenience	Simbol propriu	Year of seed harvest
<i>D. tenuifolia</i>	Tozer Seeds	UK	Cultivated	1T	2009
<i>D. tenuifolia</i>	Tozer Seeds	UK	Cultivated	2T	2009
<i>D. tenuifolia</i>	AvanSeed	Denmark	Cultivated	5T	2009
<i>D. tenuifolia</i>	AvanSeed	Denmark	Cultivated	12T	2009
<i>D. tenuifolia</i>	IPK Gatersleben	Italy	Spontaneous	15T	1984
<i>D. tenuifolia</i>	IPK Gatersleben	unknown	Spontaneous	16T	1965
<i>D. tenuifolia</i>	National natural history museum	France	Spontaneous	17T	-
<i>D. tenuifolia</i>	RostockUniversity	Germany	Spontaneous	18T	-

Statistic analysis. For each of the quantitative traits the following parameters were calculated: average, standard deviation (SD), maximum and minimum value, coefficient of variability.

Also, the measurements were subjected to statistical analysis ANOVA to reveal the existence of possible differences between accessions.

For all pairs of characters correlation coefficients were calculated to highlight a possible link between the phenotypic characters.

## 3. Results and Discussions

In the seedling stage four quantitative characters were considered, on which the measurements were made at one month after sowing.

These characters were the number of leaves, leaf length, leaf width and length. Table 2 summarizes the values of these characters for all accession used.

The total number of plants taken in the

study is 480 seedling phase, 60 plants per accession in three replicates. In terms of the number of leaves per plant, the mean for this character is 5.46, with a minimum of two and a maximum of nine leaves.

The coefficient of variation for this purpose generally is 25.39% which indicates an average variability.

Petiole length of all cultivated plants reached a maximum length of 12.50 cm.

The shortest length of stalks was 0.50 cm, with a mean of 3.26 cm.

As seen in terms of uniformity coefficient, this character presented a high variability.

Leaf length mean was 7.45 cm as shown in Table 2, with large amplitude. In this case, as in the case of the leaf width, and the ratio between the length and the width of the leaf, the coefficient of variation exceeds 30%, which reveals a great variety of these characters.

Mean leaf width had a value of 1.63 cm although in this case was very high amplitude.

The ratio between length and width of the leaf gives the leaf shape. The value is 4.85 resulting in an elongated shape.

Following the values of the coefficient of variability for each cultivar separated, in terms of

the characters mentioned above, it can be seen that they are lower than for all plants analyzed in Table 3. This is expected because all cultivars belonging to either commercial varieties or wild population category.

Table 2. Centralisation and dispersion values of the plant characters in the plantlet phase

Character	No of plants	Mean	Standard deviation	Error	Minimum value	Maximum value	Coefficient of variation(s%)
No of leaves per plant	480	5.46	1.39	0.06	2	9	25.39
Petiol length (cm)	480	3.26	2.44	0.11	0.50	12.50	74.77
Leaf length (cm)	480	7.45	3.74	0.17	0.90	21.00	50.22
Leaf width (cm)	480	1.63	1.01	0.05	0.50	16.00	62.37
Leaf length/leaf width ratio	480	4.85	1.70	0.08	0.51	12.00	35.05

Thus, in terms of number of leaves at the seedling stage, the uniformity coefficient is between 30.88 to accession 16T and 18.41 to accession 5T as shown in Table 3. Noteworthy is the average variability of the commercial varieties (1T, 2T, 5T and 12 T). For the wild cultivars, this character has a lower variability compared with the foregoing;

variability that still is considered an average over 20%.

In the petiole length the cultivar 2T is noted with the highest variability (57.35%) and 5T cultivar with the lowest value of this coefficient (22.37%), the remaining values are exceeding 30% in all cases apart from accession 15T (24.65%).

Table 3. Coefficient of variability for the quantitative characters in the plantlet phase

Character	Accession							
	1T	2T	5T	12T	15T	16T	17T	18T
No of leaves per plant	25.30	20.26	<u>18.41</u>	22.77	25.64	<b>30.88</b>	24.30	18.66
Petiol length(cm)	44.01	<b>57.35</b>	<u>22.37</u>	34.74	24.65	51.49	36.57	30.25
Leaf length(cm)	42.06	27.75	23.30	26.41	28.75	28.04	<u>18.76</u>	<b>56.68</b>
Leaf width(cm)	52.64	39.87	<u>27.22</u>	<b>72.22</b>	45.69	32.49	32.58	42.71
Leaf length/leaf width ratio	28.52	11.82	14.10	<b>32.76</b>	<u>11.00</u>	21.21	22.59	32.21

Like the other two characters above, the leaf length medium and high variability, the lowest and the highest being 18.76% (17T) and 56.68% (18T). The rest of the cultivars show an average variation for this character except 1T which has a value of 42.06%, close to that of 17T.

Leaf width shows the average variability only in the case 5T (27.22%), the rest of the accessions exceeding the threshold of 30% and thus falls within the high variability with the highest value in accession 12T - 77.22%.

Variability ratio between length and width of leaf, although it has two values exceeding 30%

(12T and 18T), remains average for most accessions.

After analyzing these characters in the seedling stage it can be concluded that, although four of accessions are commercial varieties, medium and large variability exists in all accession taken into observation, so all can be considered to be placed in a bank seed.

Analysis of variance (ANOVA) on plant characters in seedling stage for each accession was performed using Duncan's multiple comparisons test. Values of F are summarized in Table 4 between 12.15 and 152.11, and the significance (p) is at the

0.0001 for all characters of the plant, with a value of less than 0.05, which means that there are differences significant between variants, differences statistically proven.

After analyzing the collected data, the largest number of leaves per plant in seedling stage was accession 12T with a mean of 6.2 leaves per plant as shown in Table 5. Also, all commercial

cultivars had a higher number of leaves than those from wild flora. The average commercial varieties were over 5.38 leaves per plant, which is expected as the selection for this vegetable is towards a greater number of leaves in an early stage as part of productivity [12]. The biggest difference was between accession 12T and 15T who had 4.37 leaves per plant.

Table 4. Analysis of variance for the plant characters in the plantlet phase

Character		Sum of squares	DF	Variance $s^2$	F value	P value
No of leaves per plant	Between groups	140.69	7	20.10		
	Error	780.63	472	1.65	12.15	<0.0001***
	Total	921.33	479			
Petiol length (cm)	Between groups	1971.99	7	281.71		
	Error	874.15	472	1.85	152.11	<0.0001***
	Total	2846.13	479			
Leaf length	Between groups	4227.78	7	603.97		
	Error	2484.85	472	5.26	114.72	<0.0001***
	Total	6712.63	479			
Leaf width(cm)	Between groups	117.35	7	16.76		
	Error	375.27	472	0.80	21.09	<0.0001***
	Total	492.62	479			
Leaf length/leaf width ratio	Between Groups	787.07	7	112.44		
	Error	596.01	472	1.26	89.04	<0.0001***
	Total	1383.08	479			

\* Significant at  $P < 0,05$ ; \*\* Significant at  $P < 0,01$ ; \*\*\* Significant at  $P < 0,001$

Regarding the length of the petiole, the longest and the shortest were in the group of spontaneous accessions.

The highest value was registered with 7.04 cm for 18T and 15T lowest with 1.09 cm. Extremes are found in the wild accession group, and values closer to average can be found in the commercial varieties.

High values of leaves length are found, as expected, to a commercial variety -12T - 12,53cm. In a very short distance but statistically different is 5T with 11.17 cm.

The lowest values are found in 18T with an average length of 2.52 cm leaf. Accession 12T

presents the higher leaf width, 2.8 cm. The lowest value was in 17T - 1.21cm followed by 15T with 1,23cm. The accessions 1T, 2T and 18T are at small differences in statistical terms as underlined in table 5.

The ratio between length and width of the leaf, as mentioned above, form an overview regarding leaf shape.

The highest values of this report, with no significant differences, were encountered at 1T and 5T, with 6.03 and 5.9 respectively. The lowest value obtained was for accession 18T - 1.68.

Thus, it can be seen that the selection this time directed to obtain more balanced.

The correlations between plant characters in seedling stage were calculated using bivariate Pearson correlation in Table 6.

According to this table, number of ten pairs of characters was studied, finding nine correlation coefficients that were at the significant level of 0.01. Of these correlations, petiole length

and leaf length/width ratio and also leaf width the ratio mentioned previously are negatively correlated.

The only pair in which there was no significant correlation was leaf length / width ratio and the number of leaves per plant, and the value of  $r$  in this case was negative ( $r = -0.059$ ).

Table 5. Multiple comparison of the differences between the accessions means for plant characters in the plantlet phase

Accession	No of leaves per plant	Petiol length(cm)	Leaf length(cm)	Leaf width (cm)	Leaf length/leaf width ratio
1T	5.60 ± 1.42 b.c	3.05 ± 1.34 d	7.90 ± 3.32 c	1.43 ± 0.75 c.d	<b>6.03 ± 1.72 a</b>
2T	5.38 ± 1.09 c.d	1.72 ± 0.98 e. f	6.16 ± 1.71 d	1.32 ± 0.53 c.d	4.82 ± 0.57 c
5T	6.00 ± 1.10 a. b	4.71 ± 1.05 c	11.17 ± 2.60 b	1.92 ± 0.52 b	<b>5.90 ± 0.83 a</b>
12T	<b>6.20 ± 1.41 a</b>	5.21 ± 1.81 b	<b>12.53 ± 3.31 a</b>	<b>2.80 ± 2.02 a</b>	5.28 ± 1.73 b
15T	<u>4.37 ± 1.12 e</u>	<u>1.09 ± 1.36 g</u>	5.68 ± 1.63 d	<u>1.23 ± 0.56 d</u>	4.82 ± 0.53 c
16T	5.65 ± 1.74 b. c	2.01 ± 1.03 e	7.43 ± 2.08 c	1.61 ± 0.52 b.c	4.78 ± 1.01 c
17T	4.97 ± 1.21 d	1.26 ± 0.46 f.g	6.23 ± 1.17 d	<u>1.21 ± 0.39 d</u>	5.47 ± 1.24 b
18T	5.53 ± 1.03 b.c	<b>7.04 ± 2.13 a</b>	<u>2.52 ± 1.43 e</u>	1.48 ± 0.63 c.d	<u>1.68 ± 0.54 d</u>
Mean	5.46 ± 1.39	3.26 ± 2.44	7.45 ± 3.74	1.63 ± 1.01	4.85 ± 1.70

Observing the data in Table 6.11 it can be concluded that the longer the leaf, the width of the leaf increases. It is also obvious that the number of leaves at the seedling stage increases with the length of the leaf, leaf width and petiole length.

Heritability study is important in terms of phenotypic variability components knowledge especially for the characterization of accessions. Also, the study of the heritability is very important

for plant breeding and selection directed [13].

In order to see to in what degree the phenotypic variability is determined by genotypic variability, broad sense heritability coefficient was determined based on total variance, environment variance, genotypic and phenotypic variance in table 7. These values allow the determination of characters whose variability is more or less influenced by environmental factors [14].

Table 6. Pearson correlation for the plant characteristics of the accession grown in the greenhouse

	No of leaves per plant	Petiol length (cm)	Leaf length	Leaf width (cm)	Leaf length/leaf width ratio
No of leaves per plant	1				
Petiol length(cm)	0,447**	1			
Leaf length	0,539**	0,321**	1		
Leaf width(cm)	0,459**	0,439**	0,592**	1	
Leaf length/leaf width ratio	-0,059	-0,351**	0,358**	-0,250**	1

\*\* Correlation is significant at the 0,01 level (2-tailed); \*. Correlation is significant at the 0,05 level (2-tailed); n=480

All five descriptors showed a high and medium variability according to table 7. The plant leaf number and leaf width had a value of this indicator over 0.7. Regarding the higher heritability of

the number of leaves per plant, this is important because this character belongs to productivity indices [12] and the selection and existence in a seed bank of this type of accessions is salutary.

Table 7. Environmental, genotypic and phenotypic variance. Heritability in broad sense for the morphological characters evaluated on the accessions cultivated in the green house in the plantlet phase

Character	Phenotypic variance	Genotypic variance	Total variance	Heritability	Significance*
No of leaves per plant	140.69	780.63	921.33	0.85	High
Petiol length(cm)	1971.99	874.14	2846.13	0.31	Medium
Leaf length(cm)	4227.78	2484.85	6712.63	0.37	Medium
Leaf width(cm)	117.35	375.27	492.62	0.76	High
Leaf length/leaf width ratio	787.07	596.01	1383.08	0.43	Medium

\* Signification of the heritability values: low = 0 - 0.3; medium = 0.3 - 0.7; high = 0.7 - 1.0

For other descriptors, broad heritability has values between 0.31 for petiole length and 0.47 for the ratio between length and width of the leaf. Thus, we conclude that these characters were influenced by both environmental and genetic factors. Given the above, we can conclude that there is certainty that these characters will be found in the descent due to the high heritability coefficient.

#### 4. Conclusions

Regarding the number of leaves per plant in seedling stage, this character has higher value in commercial varieties as a result of pressure from plant breeding work towards this character. The number of leaves per plant is important for adaptation on different environments. Regarding the petiole length, the conclusion is that this not an important character for plant breeding this character, probably because it shortens the harvest. From the above it can be concluded regarding leaf length and width, they are characters of horticultural interest, being directly related to the productivity of the variety. For this reason it can be easily seen that the greatest length and width of leaves were found in commercial varieties. The same goes for the ratio between length and width of the leaf, the highest values meeting in commercial varieties. The conclusion of the analysis of this report is that commercial varieties trend is to have very long narrow leaves.

The analysis of the plants on the seedling stage can give a wide picture of a variability of accessions. Unless seed is needed, for germplasm variability characterization, it is less expensive and faster to assess the phenotypical differences between cultivars only in plantlet stage.

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