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Evaluation of Two Wild Populations of Hedge Mustard (*Sisymbrium officinale* (L.) Scop.) as a Potential Leafy Vegetable

Marta Guarise [1,*], Gigliola Borgonovo [2], Angela Bassoli [2] and Antonio Ferrante [1]

[1] Department of Agriculture and Environmental Science—Production, Landscape, Agroenergy, Università degli Studi di Milano, 20133 Milano, Italy; antonio.ferrante@unimi.it

[2] Department of Food, Environmental and Nutritional Science, Università degli Studi di Milano, 20133 Milano, Italy; gigliola.borgonovo@unimi.it (G.B.); angela.bassoli@unimi.it (A.B.)

* Correspondence: marta.guarise@unimi.it; Tel.: +39-0250-316593

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Abstract: The minimally processed industry is always looking for produce innovation that can satisfy consumer needs. Wild leafy vegetables can be a good source of bioactive compounds and can be attractive for the consumer in term of visual appearance and taste. In this work, *Sisymbrium officinale* (L.) Scop., commonly called hedge mustard, was grown in a greenhouse and evaluated as a potential leafy vegetable. Two wild populations, Milano (MI) and Bergamo (BG), were grown in peat substrate and harvested at the commercial stage for the minimally processing industry. Leaf pigments such as chlorophyll and carotenoids were determined as well as chlorophyll *a* fluorescence parameters. Total sugars, antioxidant compounds such as ascorbic acid, phenolic index, total phenols, anthocyanins, and nitrate were determined at harvest. Significant differences between wild populations were found in April with higher nitrate content in BG, 2865 mg/kg FW than in MI, 1770 mg/kg FW. The nitrate levels of *S. officinale* measured in the present study are significantly lower than the maximum NO_3 level allowed in other fresh leafy vegetables. Ascorbic acid measured in November was higher in MI compared BG with values of 54.4 versus 34.6 mg/100 g FW, respectively. The chlorophyll *a* fluorescence data showed that BG reached optimal leaf functionality faster than MI. Overall results indicated that *Sisymbrium officinale* (L.) Scop. can be suggested as a potential leafy vegetable for the minimally processed industry.

Keywords: *Sisymbrium officinale*; *Brassicaceae*; hedge mustard; leafy vegetables

1. Introduction

Minimally processed leafy vegetable production has been evolving in recent years by providing new produce with beneficial effect on human health. There are several wild species that can be considered as potential leafy vegetables. The introduction of new species can be useful for diet enrichment and diversification. Moreover, wild plants can be highly adaptative to different environments.

Sisymbrium officinale (L.) Scop., synonym *Erysimum officinale*, commonly known as hedge mustard in English, *erísimo* in Spanish, *erisimo* or *erba cornacchia* in Italian, and *velar* in French, is a medicinal plant that belongs to the Brassicaceae family. This species could have potential for introduction into the leafy vegetable production for the minimally processed or fresh-cut industry.

S. officinale is a terophyte scapose plant with a reddish-violet erect trunk, that present a lot of trichomes and many branches. Basal leaves are different from the upper ones with a dentate shape. Hedge mustard has a linear racemose inflorescence; each flower has four small (1–2 mm) yellow petals;

the fruit is a tiny siliqua, close-fitting to the trunk. Flowering occurs in Spring–Summer, from May to July–August, depending on the climate. Siliqua pods usually are pubescent, once they reach maturity they release seeds. Seeds are very small, each siliqua can contain from 10 to 20 seeds. *S. officinale* is endemic in the Eurasian continent and widespread in all Italian regions from 0 to 1000 m. above sea level (a.s.l.), and rarely up to 2400 m a.s.l. [1]. This annual or biennial herbaceous plant is described as ruderal, growing on disturbed sites such as field margins and roadsides [2].

Flowers and leaves of hedge mustard are commonly used as a traditional medicinal herb for the treatment of sore throats, coughs, and hoarseness [3–5] under specific indication based upon long-standing use [6] and recent clinical studies [7]. For that reason, *S. officinale* is largely known as "singer's plant" and is used among singers, actors, and professionals who use the voice for working. The therapeutic activity of this plant is attributed to its sulfurated components. Dried flowering aerial parts contain: total glucosinolates (0.63–0.94%), mucilage (13.5–10.9%), total thiols (8.9–10.2%), and total flavonoids (0.50–0.56%). The main glucosinolate in *S. officinale* is glucoputranjivine [8]. It represents 58.3% of total glucosinolates on a fresh weight basis. This percentage declined to 32.5% after autolysis [6].

Brassicaceae is one of the most important botanical families in horticultural production in Mediterranean countries, due to their great diversity expressed both in spontaneous and cultivated species. In Italy, horticultural Brassicaceae are widespread on about 40,000 ha, in particular in the center-southern region [9].

In spite of its long, traditional therapeutic use for treating voice discomfort as dried plants (including leaves, stem, and flowers) for preparing decoctions, tinctures, or propolis, *S. officinale* has barely been investigated for its beneficial proprieties, and there are no data about its possible use and consumption as a fresh leafy vegetable. Its low agronomic requirements allow the cultivation in different Mediterranean environments.

In order to evaluate the possibility of recommending this species as a potential leafy vegetable, the aim of present study was to investigate production of two different wild populations, one collected in Milan and the other one in Bergamo, Italy. Cultivation was performed in pots containing fertilized substrate in a greenhouse. Total chlorophyll content and chlorophyll *a* fluorescence were measured for evaluating photosynthetic activity. The most common quality parameters that are usually considered for leafy vegetable evaluation were determined such as ascorbic acid, carotenoids, phenols, anthocyanins, nitrates, and total sugar. Furthermore, to evaluate the production of leaves, fresh and dry biomass were measured at the baby leaf stage which is usually the developmental stage for leafy vegetables destined to the fresh-cut industry.

2. Materials and Methods

2.1. Plant Material

Seeds of two wild populations of *Sisymbrium officinale* (L.) Scop., or hedge mustard, respectively named MI (Milan) and BG (Bergamo), obtained from controlled seed reproduction at Fondazione Minoprio (Como, Italy) during summer in 2017, were sown separately in polystyrene panels using common horticultural fertilized substrate under controlled conditions in a greenhouse at the Faculty of Agricultural and Food Science of Milan, 16 January 2018, for the first evaluation, and 8 October, for the second evaluation (Supplementary Materials Table S1). Cultivation was performed in the greenhouse of the Agricultural Faculty, which was a single gable covered with glass and provided with a cooling system and supplemental light only for the second growing cycle. The supplemental lighting was provided for 16 h from the 7:00 a.m. to 11:00 p.m. with 400 W/m^2 High Pressure Sodium lamps. The environmental parameters are reported in the Supplementary Materials Figure S1.

Plantlets were transplanted and grown in complete substrate (Vigorplant, Italy) containing the following components: 21% Baltic peat, 22% dark peat, 26% Irish peat, 13% volcanic peat,

18% calibrated peat at a pH of 6.5 in 10 cm diameter plastic pots. The plant density was 16–18 plants/pot or 80–90 plant/m^2.

Harvest was performed at the end of each cultivation cycle, on 3 May, and on 7 November, when the plant reached the commercial baby leaf stage, which corresponded to plants at a 15 cm height with 4–6 fully expanded true leaves. Plants were randomly chosen from each pot and sampled for the analyses. Plants were not supplied with extra-fertilizers in either experimental period and were watered every day to maintain optimal water availability.

2.2. Non-Destructive Analyses

Chlorophyll a Fluorescence

For the characterization of the two *S. officinale* wild populations, non-destructive analyses were conducted on fresh leaf tissue. Each week, starting from 18 of April to the first week of May 2018 and 7 November, chlorophyll *a* fluorescence was measured using a hand-portable fluorometer (Handy PEA, Hansatech, Kings Lynn, United Kingdom). Leaves were dark-adapted for 30 minutes using leaf clips. After this time, a rapid pulse of high-intensity light of 3000 μmol m^{-2} s^{-1} (600 W m^{-2}) was administered to the leaf inducing fluorescence. Fluorescence parameters were calculated automatically by the device: variable fluorescence to maximum fluorescence (Fv/Fm). From the fluorescence parameters, JIP analyses were performed to determine the following indices: Performance Index (PI), dissipation energy per active reaction center (DIo/RC), and density of reaction centers (RC/CSm).

2.3. Destructive Analyses

To evaluate qualitative characteristics of the two wild populations of *S. officinale*, small samples of fresh leaves, about 1 g for each sample, were sampled one month after transplanting and at the beginning of May for the first cultivation cycle, and on 7 November for the second cycle. Some leaf samples were immediately stored at −20 °C to prevent tissue degradation.

To evaluate the yield at the baby leaf stage, plant fresh weight and dry weight were recorded at the end of biological cycle, in May for the first cultivation cycle and in November for the second cycle.

2.3.1. Chlorophyll and Carotenoids

Chlorophyll and carotenoids were extracted from fresh leaves. Leaf disks of 5 mm diameter (or 20–30 mg) in 5 mL 99.9% methanol as solvent were kept in a dark cold room at 4 °C for 24 h. Quantitative chlorophyll determinations were carried out immediately after extraction. Absorbance readings were measured at 665.2 and 652.4 nm for chlorophyll pigments and 470 nm for total carotenoids. Chlorophylls and carotenoid concentrations were calculated by Lichtenthaler's formula [10].

2.3.2. Phenolic Index, Total Phenols, and Anthocyanins

For the following analyses, fresh leaf tissue (disks of 5 mm diameter, or 20–30 mg) was extracted in 3 mL 1% methanolic HCl. The Phenolic Index of leaf tissue was determined spectrophotometrically by direct measurement of leaf extract absorbance at 320 nm. After overnight incubation the supernatant was read at 320 nm. The values were expressed as ABS_{320nm}/g FW.

Total phenols were determined spectrophotometrically following the Folin-Ciocalteu reagent method [11] using 200 μL of each sample extract 7.8 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% Na_2CO_3. Samples were extracted for 2 h in the dark and then read at 760 nm. Total phenols were calculated using a standard curve performed with gallic acid.

Anthocyanin content was determined spectrophotometrically. Sample extracts were incubated overnight at 4 °C in darkness. The concentration of cyanidin-3-glucoside equivalents was determined spectrophotometrically at 535 nm using an extinction coefficient (ε) of 29,600 [12].

2.3.3. Ascorbic Acid Determination

Only the November samples were analyzed for ascorbic acid content. For analysis, about 1 g of frozen leaves (frozen at −80 °C) were homogenized in a mortar with 1.3 mL of cold 6% (*w/v*) metaphosphoric acid and centrifuged at 10.000 × *g* at 4 °C. The pellet obtained by centrifugation was washed with 1.06 mL of cold metaphosphoric acid solution and centrifuged again. The supernatants were combined and 6% metaphosphoric acid was added to make a final volume of 3.3 mL.

After filtration through nylon filter, a 10 µL sample aliquot was injected onto an Inertsil ODS-3 GL Science column at 20 °C attached to a Series 200 LC pump. Peaks were converted to concentrations by using the dilution of stock ascorbic acid to construct a standard curve. Chromatographic data were stored and processed with a PerkinElmer TotalChrom 6.3 data Processor (PerkinElmer, Norwalk, CT, USA) [13].

2.3.4. Nitrate Determination

Nitrate concentration was measured by the salicyl sulfuric acid method [14]. About 1 g of fresh leaves was ground in 5 mL of distilled water. The extracts were centrifuged at 4000 rpm for 15 min. After centrifugation, the supernatant was collected for colorimetric determinations. Twenty µL of sample were collected and 80 µL of 5% (*w/v*) salicylic acid in concentrated sulfuric acid were added. After the reaction, 3 mL of NaOH 1.5 N were added. Each sample was cooled, and absorbance was measured at 410 nm. Nitrate concentration was calculated referring to a KNO_3 standard calibration curve.

2.3.5. Total Sugar Determination

To determine total sugar levels, extracts were prepared as above for the determination of nitrate levels. Total sugars were determined using the anthrone assay [15] with slight modification. The anthrone reagent was prepared using 0.1 g of anthrone dissolved in 50 mL of 95% H_2SO_4. The reagent was left 40 min before use; then, 200 µL of extract was added to 1 mL of anthrone, put in ice for 5 min and vortexed. The reaction was heated at 95 °C for 5 min. Samples were cooled and absorbance was read at 620 nm. Total sugar concentration was calculated referring to a glucose standard calibration curve.

2.4. Statistical Analyses

Data from the first cultivation cycle were subjected to two-way ANOVA and differences among means were determined using Tukey's post-test ($P < 0.05$). Data from the second cultivation cycle were analyzed using a t-test ($P < 0.05$). The number of replicate samples used in each analysis or measurement is reported in the legend of the figures or tables.

3. Results

3.1. Total Chlorophylls, Carotenoids, Phenols, and Anthocyanins

The yield at the baby leaf stage, a 15 cm height and 4–6 leaves, for both wild populations ranged from 2.41 g to 4.00 g for the winter-spring season and from 2.26 g to 2.45 g for the autumn season. The dry weight percentage ranged from 10.64 to 10.76 in May and from 8.07 to 7.87 in November. No significant differences were found in FW or DW between values of MI and BG harvested in May and in November (Table 1).

The plants showed different leaf pigment contents at different sampling times. In May a higher chlorophyll content was observed in MI than in April, while no significant differences between MI and BG were found in either cycle (Table 2).

Table 1. Fresh weight and % of dry matter in MI and BG hedge mustard wild populations at the baby leaf stage after two production cycles. Data are expressed as means of five plants (n = 5).

Cycle		Wild Population	Fresh Weight (g/plant)		Dry Matter (%)	
I	**May**	MI	2.41	ns	10.64	ns
		BG	4.00		10.76	
II	**November**	MI	2.26	ns	8.07	ns
		BG	2.45		7.87	

Data were analyzed using a t-test ($P < 0.05$). ns means no statistical differences.

Table 2. Chlorophyll (a, b) and total carotenoid content in MI and BG hedge mustard wild populations after two production cycles. Data are reported as mg/g FW (n = 4).

Cycle		Wild Population	Chl a (mg/g FW)		Chl b (mg/g FW)		Total Carotenoids (mg/g FW)	
I	**April**	MI	0.63 b		0.19 b		0.120	
		BG	0.93 ab		0.37 ab		0.143	ns
	May	MI	1.22 a		0.42 a		0.215	
		BG	0.79 ab		0.27 ab		0.157	ns
II	**November**	MI	1.56	ns	0.51	ns	0.382	ns
		BG	1.44		0.47		0.379	

Data of cycle I were subjected to two-way ANOVA and differences among wild populations and dates within a cycle were determined using Tukey's test ($P < 0.05$). Data of cycle II were analysed using a t-test ($P < 0.05$). ns indicates no statistical differences.

In cycle I in April the total phenols and anthocyanins did not differ between populations. In May higher anthocyanins values were observed in BG compared to MI, while no significant differences were observed for total phenol among the two wild populations. Significant differences were found in phenolic index between the two wild populations (MI and BG) only in November with a higher concentration for BG. In November ascorbic acid determination revealed significant differences between MI and BG with a higher concentration for MI (Table 3). Among the antioxidant compounds, ASA and phenolics showed opposite differences between the two *S. officinale* populations cultivated in November, ASA higher and lower phenolic index in MI.

Table 3. Ascorbic acid (ASA), phenolic index, total phenols, and anthocyanin content in MI and BG hedge mustard wild populations (n = 4).

Cycle		Wild Population	ASA (mg/100 g FW)		Phenolic Index (ABS_{320nm}/g FW)		Total Phenols		Anthocyanins (mg/100 g FW)	
I	**April**	MI	-		27.45		1.13		32.13 ab	
		BG	-		26.72	ns	1.09	ns	26.38 b	
	May	MI	-		19.05		0.75		14.88 c	
		BG	-		32.60	ns	1.35	ns	38.79 a	
II	**November**	MI	54.45	*	42.51	*	1.62	ns	29.53	ns
		BG	34.57		55.44		2.37		31.51	

Data of cycle I were subjected to two-way ANOVA and differences among wild populations and dates within cycles were determined using Tukey's test ($P < 0.05$). Data of cycle II were analysed using a t-test ($P < 0.05$). An asterisk (*) indicates a significant difference and ns indicates no statistical differences.

3.2. Chlorophyll a Fluorescence Measurements

From the chlorophyll *a* fluorescence data, four parameters were considered: Fv/Fm (maximum quantum yield of PSII), PI (Performance Index), DI0/RC (rate of energy dissipated by PSII per reaction center), and RC/CSm (active RCs per excited cross-section). No significant differences were found

between the two wild populations for Fv/Fm and DIo/RC parameters. The Fv/Fm ratio after 26 April in both wild populations showed values higher than 0.83.

PI did not show significant differences between wild populations during cycle I. Significant differences were found between BG measured on 18 April and 2 May. PI index increased from 18 April to 2 May. Then, it decreased by the following measurement on 5 May but remained higher than the initial measurement.

Like the Fv/Fm ratio, the DIo/RC values did not show significant differences between wild populations and measurement times. However, this index declined in both wild populations during cultivation.

Significant differences were found for RC/CSm in MI between 18 April and 2 May and between 26 April and 2 May. In addition, significant differences were found in the BG wild population between 18 April and 26 April (Figure 1).

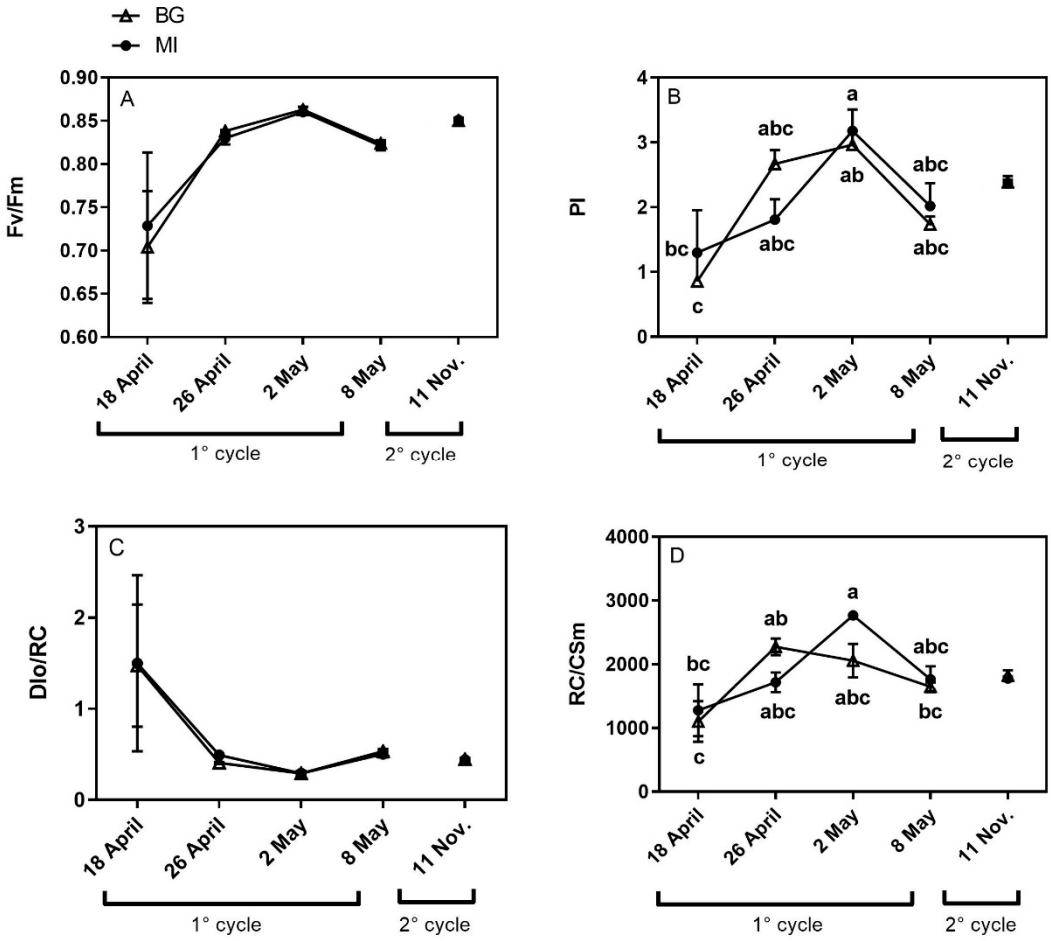

Figure 1. Chlorophyll *a* fluorescence parameters (Fv/Fm (**A**), PI (**B**), DIo/RC (**C**), and RC/CSm (**D**)) in leaves of two hedge mustard wild populations, MI and BG. Data are means with standard errors (n = 4 for April and May, n = 5 for November). Data of cycle I were subjected to two-way ANOVA and differences among wild populations and dates within cycles were determined using Tukey's test ($P < 0.05$). Data of cycle II were analysed using a t-test ($P < 0.05$). Different letters indicate statistical differences, and no letters indicate no significant differences.

3.3. Nitrate and Total Sugars

The quality of hedge mustard grown as baby leaf vegetables was also evaluated in terms of nitrate accumulation and total sugars. The nitrate content was statistically different between wild populations in April, with higher nitrate content in BG, 2865 mg/kg FW than in MI (Figure 2). In May, the two wild

populations showed lower and significantly different values compared with those measured in April, nitrate content ranged from 199 to 256 mg/kg, but there was no difference between them. In November, both wild populations showed a nitrate content that ranged from 1756 mg/kg FW to 1683 mg/kg FW, with no significant differences between them (Figure 2).

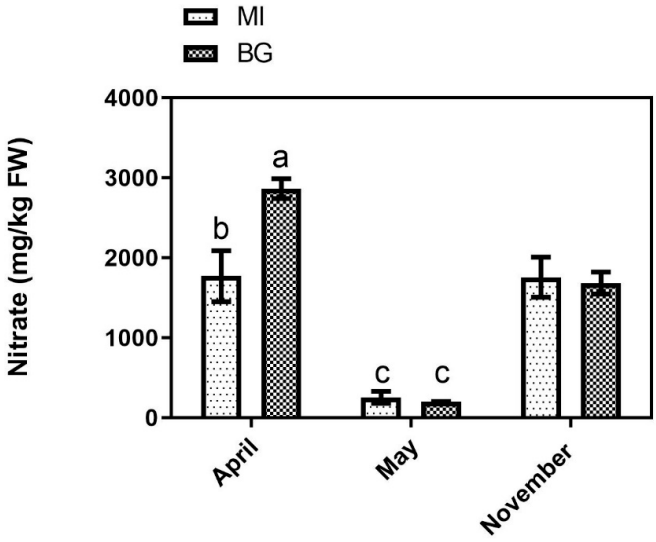

Figure 2. Nitrate content in leaves of two hedge mustard wild populations, BG and MI. Data are means with standard errors (n = 3 for April and May, n = 4 for November). Data of cycle I were subjected to two-way ANOVA and differences among wild populations and dates within cycles were determined using Tukey's test. Data of cycle II were analysed using a t-test ($P < 0.05$). Different letters indicate statistical differences for $P < 0.05$. Population means did not differ in November.

Total sugars were not statistically different between wild populations (MI and BG) or by dates within cycle I (April, May). The total sugar content ranged from to 5.84 mg Glu eq./g FW observed in MI in November, to 3.1 mg Glu eq./g FW in the April (Figure 3).

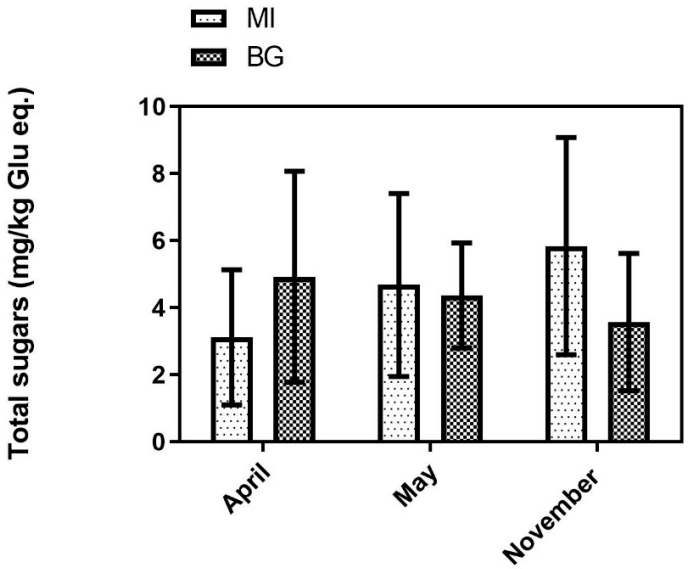

Figure 3. Total sugar content in leaves of two wild hedge mustard populations, BG and MI. Data are means with standard errors (n = 3 for April and May, n = 4 for November). Data of cycle I were subjected to two-way ANOVA. Data of cycle II were analysed using a t-test ($P < 0.05$). There were no significant differences between wild populations or times.

4. Discussion

The Brassicaeae family includes a wide number of species that can be used for vegetable production. Some of them have been described as potential vegetables and sources of antioxidant compounds for the Mediterranean area [16,17]. Hedge mustard is a wild Brassicaceae species widely dispersed and, therefore, it has been evaluated as a potential leafy vegetable for the minimally processed industry. The nutritional components were similar to other leafy vegetables and could provide a good quantity of ascorbic acid that is higher than lettuce, which shows values ranging from 10 to 30 mg/100 g [18]. The leaf pigments observed in hedge mustard were similar to other leafy vegetables such as rocket (*Eruca vesicaria* subsp. *sativa*) [19], lamb's lettuce (*Valerianella locusta*) [20], and lettuce (*Lactuca sativa*) [21]. It is well known that leaf pigments are important parameters because they contribute to leaf color and visual appearance [22]. The leaf color is very important in minimally processed leaf vegetables because it is the first quality parameter that consumers evaluate at purchase.

During the growing period lower chlorophyll concentrations were observed in spring than in autumn. Usually the leaf pigments are higher at lower light intensity; in our experimental conditions the higher values could be due to the low light conditions in the greenhouse during autumn, even though supplementary lighting was provided. This relationship between lower light availability and higher pigments have been found in different leafy vegetables [23].

Chlorophyll *a* fluorescence-derived parameters were used for evaluating the PSII activity of the two *S. officinale* wild populations (MI and BG). The Fv/Fm ratio indicated the maximal efficiency of PSII photochemistry, and it did not significantly change during the experiments. Values of Fv/Fm below 0.83 are usually considered as indicative of stressful conditions in plants [24]. The Fv/Fm ratio increased at the end of April, while the values were slightly lower at the earlier measurement. This result may be due to Fv/Fm increasing with leaf development until reaching the fully expanded stage, when the leaves are fully photosynthetically active. Values above 0.83 during May demonstrated that plants were under optimal growing conditions as was also observed in the second cycle performed in autumn. The higher values of PI in May also indicated higher light use efficiency and better performance of the plants [23]. The BG wild population seemed to have had faster adaptation and reached optimal leaf functionality earlier. In fact, BG also had higher RC/CSm values in April and these results were repeated in the second cycle performed in November

Nitrate plays a crucial role in the nutrition and function of plants and naturally occurs as a compound in the nitrogen cycle. In plants, nitrate levels are higher in leaves, whereas lower levels occur in tubers and seeds. In fact, leaf crops such as spinach (*Spinacea oleracea* L.), lettuce and rocket have high nitrate concentration [25]. Nitrate and nitrite are also commonly used as preservatives in food. Nitrate is non-toxic, but its metabolites and reaction products (nitrite, nitric oxide, and N-nitroso compounds) could be dangerous for human health inducing methaemoglobinaemia or carcinogenesis [26]. In addition to nitrate, leafy vegetables also provide several bioactive compounds with beneficial effects on health, and are widely recommended in the diet.

The European Union, in order to limit the nitrate supply in human nutrition, has defined the maximum nitrate levels permitted in some vegetables considered to have the highest levels of this compound. Nitrate concentrations are directly correlated with light availability. This may explain the lower values in May and higher values in April and November. However, the nitrate levels of *S. officinale* measured in the present study are significantly lower than the maximum NO_3 level allowed in rocket salad, another species from *Brassicacea* family, fixed at 6000 or 7000 mg NO_3/kg FW by the UE Commission [27], depending on the harvest time. Rocket plants grown in different cultivation systems in greenhouses such as soil, substrate or floating, have higher nitrate levels [28,29]. However, the higher ascorbic acid concentration compared to other leafy salad contributes a reduced risk of nitrosamine formation and carcinogenic effects of the nitrate in the diet. In fact, it has been reported that ascorbic acid is a nitrosation inhibitor and could inhibit nitrate reduction [30]. The low nitrate content of *S. officinale* is a good quality trait for possible use of this species as a leafy vegetable. Moreover,

under greenhouse cultivation, the level of ascorbic acid is similar or slightly higher than those found in plants grown in the wild [31].

The leaf sugar content is related to photosynthetic activity and biomass production. It is also an important parameter for the storage of the product. Higher sugar content can be potentially associated with higher shelf life, because sugars are used for the basal metabolism and maintaining quality of the product. The total sugar content measured in the present study (maximum level of 5.84 mg/g) is lower if compared to the average sugar content observed in rocket leaves, where the total sugar reached 6.30 mg/g in the first harvest and 7.61 mg/g in second harvest [32]. The total sugars were similar among the two wild populations which also corresponds to biomass production and dry matter percentage.

5. Conclusions

Sysimbrium officinale is a wild species from the *Brassicaceae* family, quite common in all temperate Euroasiatic areas. Our results indicated that this species can be successfully grown in a greenhouse with nutritional components as well as quality parameters such as nitrate, chlorophyll, and sugar content similar to the most common commercial leafy vegetables. It has a good concentration of ascorbic acid, higher than common leafy vegetables. Although these results suggest that *Sysimbrium officinale* can be grown as a leafy vegetable, further investigation will be required for evaluating quality during postharvest storage and handling.

Supplementary Materials: The following are available online at http://www.mdpi.com/2311-7524/5/1/13/s1, Figure S1: Temperature minimum and maximum and solar radiation (RG) during the cultivation period, Table S1: Cultivation period including sowing, transplanting, sampling, and harvest time.

Author Contributions: The authors contributed to the work as follows: conceptualization: A.F. and A.B.; methodology: M.G.; formal analysis: M.G.; investigation: M.G.; resources: G.B.; data curation: M.G.; writing—original draft preparation: M.G., A.F.; writing—review and editing: A.F., A.B.; supervision: A.F.; project administration: A.B.; funding acquisition: A.B.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pignatti, S. *Flora d'Italia*; Edizioni Edagricole: Bologna, Italy, 1982; Volume 2, p. 377.
2. Bouwmeester, H.J.; Karseen, C.M. Annual changes in dormancy and germination in seeds of Sisymbriumofficinale (L.) Scop. *New Phytol.* **1993**, *124*, 179–191. [CrossRef]
3. Politi, M.; Braca, A.; Altinier, G.; Sosa, S.; Ndjoko, K.; Wolfender, J.L.; Hostettmann, K.; Jimenez-Barbero, J. Different approaches to study the traditional remedy of "hierba del canto" Sisymbriumofficinale (L.) Scop. *Bol. Latinoam. Caribe Plantasmedicinales Aromàt.* **2008**, *7*, 30–37.
4. Blažević, I.; Radonić, A.; Mastelić, J.; Zekić, M.; Skočibusić, M.; Maravić, A. Hedge Mustard (Sisymbriumofficinale): Chemical diversity of volatiles and their antimicrobial activity. *Chem. Biodivers.* **2010**, *7*, 2023–2034. [CrossRef] [PubMed]
5. Di Sotto, A.; Vitalone, A.; Nicoletti, M.; Piccin, A.; Mazzanti, G. Pharmacological and phytochemicalstudy on *Sisymbriumofficinale* Scop. Extract. *J. Ethnopharmacol.* **2010**, *127*, 731–736. [CrossRef] [PubMed]
6. EMA—European Medicines Agency. *Assessment report on Sisymbriumofficinale (L.) Scop., Herba*; EMA: London, UK, 15 January 2014.
7. Calcinoni, O. Sisymbrium "Singers' Plant" Efficacy in Reducing Perceived Vocal Tract Disability. *J. Otolaryngol. Ent Res.* **2017**, *8*, 00243. [CrossRef]
8. Carnat, A.; Fraisse, D.; Carnat, A.P.; Groubert, A.; Lamaison, J.L. Normalization of hedge mustard, *Sisymbriumofficinale* L. *Ann. Pharm. Fr.* **1998**, *56*, 36–39.
9. Pardossi, A. *Orticoltura. Principi e Pratica*; Edizioni Edagricole: Bologna, Italy, 2018; pp. 261–276.
10. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigmentsof photosynthetic membranes. *Methods Enzymol.* **1987**, *148*, 350–382.

11. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
12. Giusti, M.M.; Rodríguez-Saona, L.E.; Wrolstad, R.E. Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. *J. Agric. Food Chem.* **1999**, *47*, 4631–4637. [CrossRef] [PubMed]
13. Rizzolo, A.; Brambilla, A.; Valsecchi, S.; Eccher-Zerbini, P. Evaluation of sampling and extraction procedures for the analysis of ascorbic acid from pear fruit tissue. *Food Chem.* **2002**, *77*, 257–262. [CrossRef]
14. Cataldo, C.A.; Maroon, M.; Schrader, L.E.; Youngs, V.L. Rapid colorimetric determination of nitrate in plant tissue by titration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. [CrossRef]
15. Yemm, E.W.; Willis, A.J. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **1954**, *57*, 508–514. [CrossRef] [PubMed]
16. Branca, F. Studies on some wild Brassicaceae species utilizable as vegetables in the Mediterranean areas. *Plant Genet. Resour. Newsl.* **1995**, *104*, 6–9.
17. Branca, F.; Li, G.; Goyal, S.; Quiros, C.F. Survey of aliphatic glucosinolates in Sicilian wild and cultivated Brassicaceae. *Phytochemistry* **2002**, *59*, 717–724. [CrossRef]
18. Albrecht, J. Ascorbic Acid Content And Retention In Lettuce1. *J. Food Qual.* **1993**, *16*, 311–316. [CrossRef]
19. Žnidarčič, D.; Ban, D.; Šircelj, H. Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. *Food Chem.* **2011**, *129*, 1164–1168. [CrossRef]
20. Ferrante, A.; Maggiore, T. Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. *Postharvest Biol. Technol.* **2007**, *45*, 73–80. [CrossRef]
21. Fallovo, C.; Rouphael, Y.; Rea, E.; Battistelli, A.; Colla, G. Nutrient solution concentration and growing season affect yield and quality of Lactuca sativa L. var. acephala in floating raft culture. *J. Sci. Food Agric.* **2009**, *89*, 1682–1689. [CrossRef]
22. Ferrante, A.; Incrocci, L.; Maggini, R.; Serra, G.; Tognoni, F. Colour changes of fresh-cut leafy vegetables during storage. *J. Food Agric. Environ.* **2004**, *2*, 40–44.
23. Colonna, E.; Rouphael, Y.; Barbieri, G.; De Pascale, S. Nutritional quality of ten leafy vegetables harvested at two light intensities. *Food Chem.* **2016**, *199*, 702–710. [CrossRef]
24. Bulgari, R.; Cola, G.; Ferrante, A.; Franzoni, G.; Mariani, L.; Martinetti, L. Micrometeorological environment in traditional and photovoltaic greenhouses and effects on growth and quality of tomato (*Solanum lycopersicum* L.). *Ital. J. Agrometeorol.* **2015**, *2*, 27–38.
25. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *EXBOTJ* **2000**, *51*, 659–668. [CrossRef]
26. Alberici, A.; Quattrini, E.; Penati, M.; Martinetti, L.; Gallina, P.M.; Ferrante, A.; Schiavi, M. Effect of the Reduction of Nutrient Solution Concentration on Leafy Vegetables Quality Grown in Floating System. *Acta Hortic.* **2008**, *801*, 1167–1176. [CrossRef]
27. EFSA—European Food Safety Authority. *Nitrate in Vegetables. Scientific Opinion of the Panel on Contaminants in the Food Chain*; Question N. EFSA-Q-2006-071; EFSA: Parma, Italy, 2008; Volume 689, pp. 1–79.
28. European Commission. *Maximum Levels for Nitrates in Foodstuffs*; Regulation (EU) N. 1258/2011 Amending Regulation (EC) N. 1881/2006; Official Journal European Union: Brussel, Belgium, 2011; pp. 15–17.
29. Hanafy Ahmed, A.H.; Khalil, M.K.; Farrag, A.M. Nitrate accumulation, growth, yield and chemical composition of Rocket (*Eruca vesicaria* subsp. sativa) plant as affected by NPK fertilization, kinetin and salicylic acid. *Ann. Agric. Sci. Cairo* **2002**, *47*, 1–26.
30. Ferrante, A.; Incrocci, L.; Maggini, R.; Serra, G.; Tognoni, F. Preharvest and postharvest strategies for reducing nitrate content in rocket (*Eruca sativa* L.). *Acta Hortic.* **2003**, *628*, 153–159. [CrossRef]
31. Tannenbaum, S.R.; Wishnok, J.S.; Leaf, C.D. Inhibition of nitrosamine formation by ascorbic acid. *Am. J. Clin. Nutr.* **1991**, *53*, 247S–250S. [CrossRef]
32. Shad, A.A.; Shah, H.U.; Bakht, J. Ethnobotanical assessment and nutritive potential of wild food plants. *J. Anim. Plant Sci.* **2013**, *23*, 92–97.