agronomy

MDPI

Article

Evaluation of Italian and Spanish Accessions of *Brassica rapa* L.: Effect of Flowering Earliness on Fresh Yield and Biological Value

María Elena Cartea [1,*], Maria Concetta Di Bella [2], Pablo Velasco [1], Pilar Soengas [1], Stefania Toscano [2] and Ferdinando Branca [2,*]

1 Group of Genetics, Breeding and Biochemistry of Brassicas, Misión Biológica de Galicia (MBG-CSIC), E-36080 Pontevedra, Spain; pvelasco@mbg.csic.es (P.V.); psoengas@mbg.csic.es (P.S.)

2 Department of Agriculture, Food and Environment (Di3A), Università degli Studi di Catania, Via Valdisavoia 5, 95123 Catania, Italy; maria.dibella@unict.it (M.C.D.B.); stefania.toscano@unict.it (S.T.)

* Correspondence: ecartea@mbg.csic.es (M.E.C.); fbranca@unict.it (F.B.)

Abstract: A comparative study for evaluating Italian and Spanish accessions of *Brassica rapa* var. *rapa* L., including turnip greens and turnip tops, was carried out at different locations with a view to determine the effect of earliness on crop production, antioxidant activity, glucosinolates amount, and profile (GLSs) and total phenolics content. The accessions evaluated were represented by two turnip top local varieties (one Italian variety and a Galician one), four new synthetic varieties established by Misión Biológica de Galicia (MBG-CSIC), and three commercial varieties widely used by growers in Galicia and in Italy. The results showed a great variability regarding flowering time, fresh and dry weight of the leaves and flower buds, and the branch number per plant. The highest turnip greens production was found in two synthetic varieties ("SIN07" and "SIN01") for both countries. Local varieties "BRS550" and "CM39" were also suitable for turnip greens production in Spain and Italy, respectively. For turnip tops, the highest production was found for "SIN07" in Spain, for "CM39" in Italy and for "BRS550" in both countries. We found a high diversity in the total and individual glucosinolate, phenolic content, and antioxidant activity among genotypes, geographical origins, and the different parts of the plant (leaf and flower). Varieties "SIN01" and "SIN07" showed the highest values in total GLSs, total aliphatic and gluconapin contents in turnip greens followed by the two commercial varieties. For turnip tops, the highest values in gluconapin, aliphatic, and total GLSs contents were found in "SIN01" and "BRS550". Even though different varieties stand out over the rest depending on the location, "SIN01", "SIN07", "CM39", and "BRS550" could be recommended for turnip greens production because of its high antioxidant activity. The study showed that the latest varieties are more productive and show higher bioactive compounds than the earlier ones and that it is possible to improve genotypes for different growing cycles. Therefore, these varieties could be proposed for further breeding programs for *B. rapa* production.

Keywords: turnip greens; turnip tops; synthetic varieties; fresh production; glucosinolates; phenolic compounds; antioxidant activity

Citation: Cartea, M.E.; Di Bella, M.C.; Velasco, P.; Soengas, P.; Toscano, S.; Branca, F. Evaluation of Italian and Spanish Accessions of *Brassica rapa* L.: Effect of Flowering Earliness on Fresh Yield and Biological Value. *Agronomy* **2021**, *11*, 29. https://dx.doi.org/10.3390/agronomy11010029

Received: 5 December 2020
Accepted: 23 December 2020
Published: 25 December 2020

1. Introduction

Species belonging to the Brassicaceae family support one of the world's most economically important crop groups. They range from noxious weeds to leaf and root vegetables and to oilseed and condiment crops. *Brassica rapa* is an important oil and vegetable crop in many parts of the world, with seeds being used for oil, and leaves, flowers, stems, and roots being used as vegetables [1]. The cultivation of this species for many centuries has caused a large variation in the plant organs that are consumed, which represent the results of human selection of different morphotypes, depending on local preferences [1,2]. Based on their morphological characteristics, there are three well defined groups of *B. rapa*:

(1) the oleiferous type of which canola is a specific form, having low erucic acid levels in its oil and a low glucosinolate (GLSs) content in its meal protein; (2) the rapiferous type, comprising the rapifera group (turnip, rapini), and the ruvo group (turnip broccoli, Italian turnip, "cima di rapa") and (3) the leafy type, including the rapa group (turnip greens), the chinensis group (pak-choi), the pekinensis group (Chinese cabbage), and the perviridis group (tendergreen) [3].

Vegetable *B. rapa* crops, including rapifera and leafy types, are widely grown in Asia and Europe. In Europe, they are notably popular in Portugal, Italy and Spain [4–6], where they play an important role in traditional farming and in the diet. In these countries, *B. rapa* includes two main crops, turnip greens and turnip tops, as vegetable products. Turnip greens are the young leaves harvested trough the vegetative period, while turnip tops are the young inflorescences with the flower buds and their surrounding leaves, which are consumed before opening and while they are still green In Italy, turnip tops (or "cima di rapa") is a typical vegetable grown mainly in the Southernmost Italian regions, such as in Apulia, where it is sold at local markets nearly all year round. They are commonly consumed as boiled vegetables, being used in the preparation of soups and stews and they have a slightly spicy flavor like mustard greens [6]. Turnip greens and turnip tops have a good commercial potential in both countries, and the number of industries producing *B. rapa* canned products has been increasing in the last years.

A collection of vegetable *B. rapa* from Spain is currently kept at Misión Biológica de Galicia (CSIC, Spain). Agronomical and nutritional evaluations of this collection were previously performed concerning genetic diversity [6] and the GLSs amount and profile in leaves [7,8] under specific environmental conditions in NW Spain. These works highlighted the enormous diversity existing in Galician *B. rapa* local varieties and their use to produce improved new varieties that are adapted to modern consumer habits and to a wide range of environmental conditions.

Despite the wide consumption of *B. rapa* in Italy and Spain, little information about the genetic resources preserved and used in these countries is available. With a view to expand the use of underexploited local varieties/landraces and new varieties, a more exhaustive evaluation of their agronomic performance and biological value on human health in a wide range of environmental conditions, like the Mediterranean and Atlantic areas, becomes necessary.

The high genetic diversity described within *B. rapa* species [2,9] would be useful to select varieties with a high production and an improved nutritional value. One of the biggest concerns on *B. rapa* production is the harvest period. Turnip greens are harvested throughout the vegetative period (autumn), while, in the case of turnip tops, it is in late winter, as they require a prolonged cold exposure before flowering. Turnip tops have a very short harvest period between the appearance of the first flower buds and before bolting. Therefore, the development of turnip greens and turnip tops varieties with different earliness cycles will be important, not only to have a continuous production through autumn–winter for fresh consumption but also to have varieties adapted to requirements of the agro-food industry. These vegetables are consumed as fresh vegetables and as processed foods (frozen and canned). Developing new *B. rapa* varieties with new attributes would be interesting for both fresh and industrial markets. The introduction of new *B. rapa* varieties in fresh and agro-food markets can render a profit, not only for producers but also for consumers, due to the possibility of including new vegetables of high biological value in their diet.

Most efforts made in *B. rapa* breeding have been focused on the oleiferous types, where canola varieties were successfully developed in the 1980s. However, breeding for vegetables types like turnip greens or turnip tops was scarcely developed, and so far, no programs have been carried out for obtaining new varieties with different earliness cycles. Except for oil seed types, most major types of *B. rapa* are self-incompatible, and pure line selection is complicated, labor-intensive, and it often leads to severe inbreeding depression. Therefore, most cultivars are produced by mass or family selection. Consequently, there is a strong interest in developing new cultivars by using simple recurrent selection procedures to select local varieties with a benefit for consumers and producers. Varieties with a higher production and a wide range of adaptability to edaphic and climatic conditions are essential for breeding programs. The evaluation of different genotypes at different locations across the years will provide us with valuable information about their agronomic performance and will allow us to identify superior cultivars on several environments.

Brassica crops are considered to be important vegetables due to several evidences of their health-promoting effects, such as a reduction in the risk of chronic diseases, particularly cardiovascular diseases and several types of cancer [10–15]. The beneficial effects that are associated to *B. rapa* consumption have been linked to the presence of phenolics and GLSs in these plants and to many constituents having a strong antioxidant activity [7,16–18]. GLSs and their degradation products have been extensively researched for their role in cancer prevention and plant defense [12,19,20]. On the other hand, antioxidant compounds are being extensively researched due to their potent antioxidant activity, their ability to wipe up harmful free radicals, and the health benefits associated to them. The antioxidant properties of some *Brassica* vegetables such as cabbage, broccoli, cauliflower, kale, and turnip have been studied extensively [9,16,21–23]. However, relatively little is known about the antioxidant properties of *B. rapa* var. *rapa* leaves and floral buds, which are common foods in some European Mediterranean countries.

In a previous study carried out by our group, the effect of earliness and plant habit in the total and individual GLSs content was studied from a set of local varieties of *B. rapa* [8]. Authors suggest that the variation on GLSs concentrations was affected by earliness. Early varieties had the lowest gluconapin content, although the result was dependent on the environment. Therefore, further exhaustive studies will be necessary with other genotypes and environments in order to verify these first results.

To the best of our knowledge, few information is available on (i) the effect of earliness of *B. rapa* genotypes on fresh production in turnip greens and turnip tops or (ii) the effect of earliness on bioactive compounds and antioxidant capacity. Thus, the objectives of the present study were to evaluate the agronomic attributes of new *B. rapa* genotypes with different earliness when compared to *B. rapa* commercial varieties under different environments and (ii) to evaluate the effect of the genotypes and the origin on the GLSs profile and content, total phenolic content, and the antioxidant activity.

2. Materials and Methods

2.1. Plant Material

Seven varieties of *B. rapa* var. *rapa* from Galicia (Spain) and two varieties from Apulia (Italy) were chosen for this study (Table 1). Out of these ones, four synthetic varieties (denominated "SIN01", "SIN05", "SIN06", and "SIN07") were developed at Misión Biológica de Galicia (MBG-CSIC, Galicia, NW Spain). Synthetic variety "SIN01" was obtained after four cycles of mass selection according to its agronomic performance for turnip greens fresh production in a typical production area for *B. rapa* crops (Oroso, A Coruña, Spain). Synthetic varieties "SIN05", "SIN06", and "SIN07" were obtained after three cycles of mass selection and were designed to obtain three synthetic turnip tops varieties with different earliness (early, medium, and late, respectively). Each synthetic variety was obtained in 2011 (cycle 0) after cross-pollination of a set of Galician local varieties kept at the MBG *Brassica* germplasm bank and selected depending on the abovementioned traits for each synthetic variety. In 2011, approximately 300 plants from cycle 0 (C0) for each synthetic variety were transplanted in the field. For synthetic "SIN01", the best 60 plants for turnip greens production were selected (≈20% selection intensity). For synthetic varieties "SIN05", "SIN06", and "SIN07", the 60 earliest, medium, and latest plants, respectively, were selected (≈20% selection intensity). The non-selected plants were removed before flowering, and cross-pollination among the selected plants in each plot was obtained by using bumblebees (*Bombus terrestris*). In 2012, seeds were taken from the selected plants belonging to each synthetic variety to create cycle 1. From 2013 to 2015, this process was repeated for three successive generation cycles for "SIN05", "SIN06", and "SIN07" and for four cycles (from 2013 to 2016) for "SIN01". Two commercial varieties ("Grelos de Santiago" and "Nabo Globo de Lugo") of turnip greens that are widely used in Spain and a local variety ("BRS0550") used by canning companies for turnip greens production were included as checks for comparison with the new synthetic varieties. The two accessions used in Italy are Nabo sessantino ("CM39"), an extra-early variety, and Broccoletto di Rapa Sessantino Riccio San Marzano ("CM24"), an Italian commercial variety (Pagano sementi) that shows a dark green lamina, while the petiole is whitish.

Table 1. Description of *Brassica rapa* varieties studied and their classification according to their flowering earliness.

Variety Name	Description	Origin	Source [1]	Flowering Earliness
SIN05 C3	Synthetic	Spain	MBG	Early
SIN06 C3	Synthetic	Spain	MBG	Medium
SIN07 C3	Synthetic	Spain	MBG	Late
SIN01 C4	Synthetic	Spain	MBG	Medium
BRS0550	Landrace	Spain	MBG	Extra-late
Nabo globo de Lugo	Commercial	Spain	Rocalba	Medium
Grelos de Santiago	Commercial	Spain	Rocalba	Late
Broccoletto di Rapa sessantino Riccio San Marzano ("CM24")	Landrace	Italy	UNICT 817	Extra-early
Nabo sessantino ("CM39")	Commercial	Italy	UNICT 3272	Extra-early

[1] MBG: Germplasm Bank at the Misión Biológica de Galicia, Pontevedra, Spain; UNICT: active genebank collection at the University of Catania, Italy.

2.2. Experimental Design

These varieties were evaluated for 2 years (2016–2017 and 2017–2018) in Spain, at two representative locations, Oroso (A Coruña) (43°1′ N, 8°26′ W, 280 m.a.s.l.), and Salcedo (Pontevedra) (42°24′ N, 8°38′ W, 20 m.a.s.l), and in Catania (Sicily, Italy) (37°31′ N, 15°4′ E 105 m.a.s.l.). In Spain, transplanting dates were 23 September 2016 in Oroso, and 10 October and 27 October 2017 in Oroso and Pontevedra, respectively. In Catania, transplanting dates were 14 February 2016 and 9 November 2017. Varieties were planted in multipot-trays, and seedlings were transplanted into the field at the five-six leaf stage. Varieties were transplanted in a randomized complete block design with three replications. The experimental plots consisted of two rows with 15 plants per row. Rows were spaced 0.8 m apart and plants within rows were spaced 0.5 m apart. Irrigation was done after transplanting into the field and when it was required, depending on the rainfall, by drip irrigation. Cultural operations, fertilization, and weed control were made according to local practices. For pest control, Force® (Syngenta, Basel, Switzerland) was added at the time of transplantation to combat soil insects, Pyganic 1,4 (Biograd, Grassobbio (BG)) for aphids' control, and BTK ® 32 WG (Xeda, Forlì, Italy) based on *Bacillus thuringiensis* sub. *kurstaki* for controlling *Pieris brassicae*.

2.3. Biomorphometric Traits

Morphological and agronomical traits were recorded for turnip greens and turnip tops related to earliness and fresh production, along with the maturity cycle of varieties. Traits measured (Table 2) were adapted from the International Board for Plant Genetic Resources *Brassica* L. and *Raphanus* L. descriptors list [24].

2.4. GLSs Identification and Quantification

Two sample types were collected and analyzed: leaves (turnip greens), three months after sowing, and flower buds (turnip tops), taken sequentially depending on the maturity of each genotype, just after flower bud formation and before flower opening. Five samples of healthy leaves and young shoots from five plants per plot were used. Samples were frozen in situ on dry ice, immediately transferred to the laboratory, and frozen at −80 °C. All samples were lyophilized (BETA 2–8 LD plus, Christ, GmbH, Osterode am Harz, Germany) for 72 h. The dried material was powdered by using an IKA-A10 (IKA-Werke GmbH & Co. KG, Staufen, Germany) mill, and the fine powder obtained was used for GLS analysis. Sample extraction and desulfation were performed according to Kliebenstein et al. [25] with minor modifications. Ten microliters of the desulfo-GLSs extract were used to identify and quantify GLSs. Chromatographic analyses were carried out on an Ultra-High-Performance Liquid-Chromatograph, UHPLC Nexera LC-30AD (Shimadzu, Kyoto, Japan) equipped with a Nexera SIL-30AC injector (Shimadzu, Kyoto, Japan) and one SPD-M20A UV/VIS photodiode array detector (Shimadzu, Kyoto, Japan). The UHPLC column was a XSelect HSS T3 XP Column C18 protected with a C18 guard cartridge (Waters Corporation, Milford, MA, USA). The oven temperature was set at 30 °C. Compounds were separated by using the following method in aqueous acetonitrile, with a flow of 0.5 mL min^{-1}: 1.5 min at 100% H_2O, an 11 min gradient from 0% to 25% (v/v) acetonitrile, 1.5 min at 25% (v/v) acetonitrile, a minute gradient from 25% to 0% (v/v) acetonitrile, and a final 3 min at 100% H_2O. Data were recorded on a computer with the LabSolutions software (Shimadzu, Kyoto, Japan). All GLSs were quantified at 229 nm by using glucotropaolin (GTP, monohydrate from Phytoplan, Diehm & Neuberger GmbH, Heidelberg, Germany) as an internal standard and quantified by comparison to purified standards. GLSs are reported as µmol g^{-1} dry weight (dw).

Table 2. Agronomic traits used in the evaluation of varieties of *Brassica rapa* from northwestern Spain and South Italy.

Agronomic Traits	Description
Time to flowering (d)	Days from transplanting until 50% of plants have the first flower
Turnip greens fresh production (g)	Average fresh weight of 30 leaves per plot
Turnip greens moisture (%)	Leaf water content
Branch number (n)	Average number of secondary stems per plant at first flower opening of five plants per plot
Leaf per plant (n)	Average number of leaves per plant at first flower opening of five plants per plot
Turnip tops fresh production (g)	Average fresh weight of the turnip top of five plants per plot
Turnip tops moisture (%)	Turnip top water content

2.5. Evaluation of Antioxidant Activity: ABTS Assay

Freeze-dried and ground samples (10 mg) were extracted with 1 mL of 80% aqueous methanol in dark maceration for 24 h. After centrifugation (3700 rpm, 5 min), methanolic extracts were employed in order to determine the antioxidant activity by [2,2′-azino-bis (3-ethylbenzothiazoline-6-sul-fonic acid)] (Sigma–Aldrich Chemie GmbH (Steinheim, Germany) cation assay (ABTS). Three technical replications were analyzed for each sample. A standard prepared with different concentrations of Trolox (0, 0.008, 0.016, 0.024, 0.032, 0.040 mM) (Sigma–Aldrich Chemie GmbH (Steinheim, Germany) was also measured. The antioxidant activity was normalized to Trolox equivalents per gram (g) of dry weight (dw). The method of decolorization of free radicals ABTS employed was a modified version of that used by Samarth et al. [26] and initially reported by Re et al. [27]. ABTS was generated by oxidation of ABTS 7 mM with potassium persulphate 2.45 mM in water, at room temperature for 16 h. For each analysis, the ABTS solution was freshly diluted with water in order to obtain an initial absorbance around 0.8 at 734 nm. An aliquot of 10 µL methanolic extract for the sample was added to 250 µL of ABTS solution. Absorbances were measured at 734 nm after 30 min of incubation in the dark at room temperature.

2.6. Estimation of Phenolic Content

Phenolic content was estimated according to the phenolic colorimetric method described by Dewanto et al. [28]. The same methanolic extracts employed for antioxidant activity assays were employed to determine phenolic content. Extracts were oxidized with

50 mL of 0.5 M Folin reagent (Sigma–Aldrich Chemie GmbH (Steinheim, Germany). After 5 min, 200 mL of a 20% Na_2CO_3 solution were added to neutralize the reaction. Then, the absorbance was read at 760 nm after 2 h of incubation in the dark at room temperature. Standards prepared with different concentrations of gallic acid (Sigma–Aldrich Chemie GmbH (Steinheim, Germany) (0, 0.008, 0.016, 0.024, 0.032, and 0.04 mM) were also measured. Results were expressed as micromoles of gallic acid equivalents per gram of dry weight.

2.7. Statistical Analysis

Each location × year interaction was considered as an environment. We conducted a mixed model ANOVA according to a randomized complete block design where the main effects of environments, varieties, and plant organs and their interactions were considered as fixed factors. Blocks and their interactions with varieties, environments, and plant organs were considered as random factors. As we found a significant variety × environment interaction, we also performed these analyses individually for each environment, considering the variety as a fixed effect and block, and the interaction between block and variety as random factors. Means comparisons were done with Fisher's protected least significant difference (LSD) at a 0.05 level of probability [29]. Analyses were made by using the GLM procedure of SAS 2007 statistical software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Agronomical Traits

The combined analysis of variance showed significant differences among environments and varieties for most traits ($p < 0.01$). The variety × environment interaction was highly significant for most of them. Because of the significance of the interaction, individual analyses by environment were carried out for each trait. When comparing the results from the individual analyses for the most relevant agronomic traits, different varieties stand out over the rest, depending on the country but not on the location. Then, both magnitude and rank changes contributed to these interactions and analyses of variance were combined across countries. Therefore, comparisons were made between countries.

The means of combined data across environments for each country are shown in Table 3. These traits will allow us to define the most appropriate varieties for turnip greens and turnip tops fresh production. Turnip greens fresh matter ranged from 7.23 g to 28.38 g, the best varieties being "BRS0550", "SIN01", and "SIN07"in both countries, along with "CM39" in Catania (Table 3).

All these varieties were significantly better than the three commercial varieties in most environments.

Varieties flowered earlier in Italy than in Spain (148 days and 91 days, respectively) and turnip tops production was higher in Spain than in Italy (51.3 g and 28.2 g, respectively). No significant differences between countries ($p > 0.05$) were found for turnip greens fresh production and moisture. In conclusion, plants performed similarly in both countries for turnip greens production, but varieties had a better agronomic performance for turnip tops production in Spain.

The potential of these genotypes for turnip tops fresh production was evaluated at two locations in Spain and at one location in Italy, all of them during the 2017–2018 period because of these varieties' problems to produce this plant organ at the other environments. Branch number and earliness are useful traits to select varieties that are suitable for turnip top production. Varieties "SIN07" and "BRS0550" along with commercial variety "Grelos de Santiago" in Spain and two local varieties ("BRS0550" and "CM39") in Catania showed the highest turnip tops fresh production (more than 60 g per branch), differing significantly from all other varieties (Table 3). Variety "CM39" was under environmental conditions and growing seasons in Spain too early. Data for turnip tops fresh yield were recorded only at one environment in Spain, as plants performed badly and reached bolting when they were still small

Table 3. Means of several agronomical traits of nine *Brassica rapa* genotypes evaluated at three environments in Northwestern Spain and two environments in Italy.

Country	Varieties	Time to Flowering (d)	Turnip Greens Fresh Production(g)	Turnip Greens Moisture (%)	Branch Number[1] (n)	Leaf per Plant (n)	Turnip Tops Fresh Production [1] (g)	Turnip Top Moisture [1] (%)
Spain	BRS550	205.67 ± 2.36	27.73 ± 1.25	89.95 ± 0.62	15.72 ± 1.26	65.21 ± 10.2	65.27 ± 6.95	91.43 ± 2.56
	CM24	70.33 ± 1.33	8.30 ± 1.57	91.27 ± 0.30	7.21 ± 0.84	18.10 ± 2.9	24.66 ± 1.75	91.71 ± 1.65
	CM39	84.32 ± 3.11	7.23 ± 0.87	82.43 ± 3.41	6.53 ± 1.54	24.13 ± 7.5	20.52 ± 2.10	85.81 ± 0.78
	SIN01C4	145.11 ± 4.16	21.15 ± 1.68	90.34 ± 0.90	12.77 ± 0.79	63.84 ± 10.2	54.16 ± 6.65	85.05 ± 1.05
	SIN05 C3	117.00 ± 3.34	11.21 ± 1.29	89.65 ± 3.19	8.54 ± 1.84	42.51 ± 5.3	29.69 ± 4.73	79.38 ± 0.88
	SIN06 C3	140.00 ± 4.69	17.92 ± 1.63	90.08 ± 1.18	11.39 ± 1.41	55.14 ± 8.9	47.98 ± 5.69	83.85 ± 1.22
	SIN07 C3	152.11 ± 2.19	21.02 ± 1.93	90.55 ± 0.78	14.74 ± 0.59	72.23 ± 11.5	65.67 ± 12.71	84.06 ± 2.02
	Grelos de Santiago	161.33 ± 3.26	18.28 ± 1.38	89.84 ± 1.11	13.26 ± 0.89	63.61 ± 6.3	65.36 ± 13.45	85.92 ± 0.92
	Nabo Globo Lugo	156.44 ± 2.79	18.07 ± 1.36	89.77 ± 0.99	13.32 ± 1.21	69.32 ± 8.9	61.42 ± 8.94	84.72 ± 1.25
	LSD (5%)	3.84	3.52	1.08	2.95	26.31	11.73	3.68
Italy	BRS550	-[2]	16.50 ± 4.23	87.41 ± 1.05	6.33 ± 0.88	26.78 ± 5.9	74.00 ± 26.00	87.25 ± 2.19
	CM24	58.50 ± 6.50	9.81 ± 2.06	84.94 ± 3.00	6.13 ± 0.81	23.10 ± 5.6	20.00 ± 0.99	82.14 ± 1.12
	CM39	64.60 ± 0.40	26.52 ± 4.02	85.61 ± 0.53	7.83 ± 0.46	16.78 ± 2.8	60.40 ± 7.60	83.15 ± 0.43
	SIN01C4	82.67 ± 5.20	23.03 ± 2.42	87.09 ± 0.89	4.89 ± 0.61	46.89 ± 18.9	20.42 ± 4.08	86.29 ± 0.99
	SIN05 C3	78.83 ± 4.03	15.22 ± 2.32	87.96 ± 1.11	6.78 ± 0.83	26.89 ± 7.5	18.43 ± 2.00	83.16 ± 0.91
	SIN06 C3	84.25 ± 6.36	14.80 ± 2.33	88.43 ± 1.50	4.56 ± 0.38	45.8 ± 10.2	17.67 ± 3.84	86.52 ± 1.20
	SIN07 C3	92.50 ± 3.48	28.38 ± 4.10	86.62 ± 0.53	7.22 ± 0.89	41.71 ± 8.9	20.88 ± 8.08	83.62 ± 0.73
	Grelos de Santiago	111.17 ± 0.65	15.81 ± 1.95	85.55 ± 0.82	6.44 ± 0.71	53.22 ± 11.9	22.72 ± 5.90	81.55 ± 0.92
	Nabo Globo Lugo	130.00 ± 1.15	20.90 ± 3.66	86.61 ± 0.95	8.44 ± 1.71	55.50 ± 25.6	31.60 ± 10.28	84.61 ± 0.85
	LSD (5%)	4.52	8.35	3.62	4.18	23.59	30.61	4.25

[1] Data for turnip tops were recorded in the growing cycle 2017–2018 for two locations in Spain and one location in Italy; [2] Flowering data were not recorded for this variety since it did not get to bloom in this country.

From the point of view of the farmer-producer, flowering time is an important agronomic trait for *B. rapa* crops. Variety "BRS0550" showed the longest time to flowering (206 days in Spain and it was too late for Catania, where this variety did not bloom). On the contrary, the two Italian varieties, "CM24" and "CM39", were the earliest ones in all environments (70 and 84 days to flowering in Spain, and 59 and 65 days to flowering in Catania), differing significantly from all other varieties.

Besides flowering, branch number is another relevant trait to select varieties that are suitable for turnip tops production, since this trait may greatly affect the yield. Two varieties, "BRS550" and "SIN07", along with the two commercial varieties, "Grelos de Santiago" and "Nabo Globo de Lugo", presented the highest branch number (more than 13) in Spain. Similar results were found for varieties evaluated in Italy, where "CM39", "SIN07" and commercial variety "Nabo Globo de Lugo" showed more than 7 branches per plant, differing significantly from all other varieties (Table 3). All these varieties, except for "CM39", performed well for branch number and they were among the latest varieties, thus indicating that earliness was inversely related with fresh production.

Means for traits related to turnip greens and turnip tops production at each environment are shown in Figure 1. Significant differences among environments ($p < 0.001$) were found for turnip greens fresh production, being Pontevedra and Catania in 2017 the locations where varieties had the highest values (Figure 1a). Regarding turnip tops production, data were taken at three locations in 2017. Once again, Pontevedra was the location where varieties performed better for branch number and turnip tops fresh production (Figure 1b).

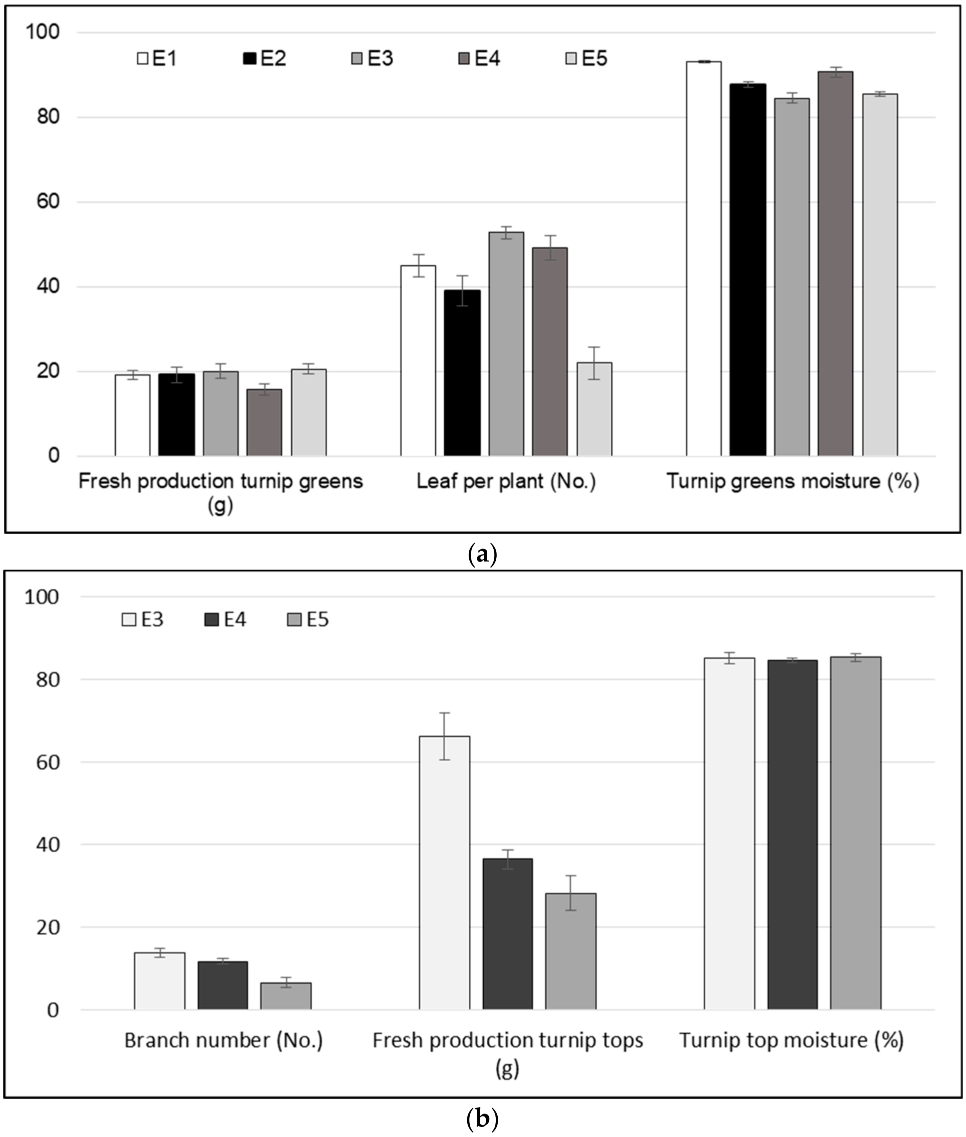

Figure 1. (**a**) Means for traits related to turnip greens fresh production: fresh production turnip greens (g), leaf per plant (No.), and turnip greens moisture (%) at the five environments where varieties were grown (E1 = Pontevedra 2016; E2 = Catania 2016; E3 = Pontevedra 2017; E4 = Oroso 2017; E5 = Catania 2017). (**b**) Means for traits related to turnip tops fresh production: branch number (No.), fresh production turnip tops (g) and turnip top moisture (%) at the three environments where varieties were grown (E3 = Pontevedra 2017; E4 = Oroso 2017; E5 = Catania 2017).

3.2. Variation of GSLs among Varieties, Locations, and Plant Organs

The chemical profile of *B. rapa* varieties studied in this work was composed of nine GLSs belonging to the three chemical classes: four aliphatic (progoitrin, glucoraphanin, gluconapin, and glucobrassicanapin), four indolic (glucobrassicin, 4-hydroxyglucobrassicin, methoxyglucobrassicin and neoglucobrassicin) and one aromatic (gluconasturtiin). Other aliphatic GLS such as glucoiberverin and gluconapoleiferin were found in minor quantities (Tables 4 and 5). GLS analyses for turnip greens were performed at five environments from Spain and Italy, whereas GLSs for turnip tops were evaluated at two Spanish locations during the 2017–2018 growing season, where plants were able to produce floral buds.

Table 4. Mean (µmol gram^{-1} dw) glucosinolate content in turnip greens from the *B. rapa* varieties evaluated at three environments in Northwestern Spain and two environments in Italy.

Variety	PRO	GRA	GNA	GBN	GBS	OHGBS	MGBS	NGBS	ALIPH	INDOL	AROM	Total GLSs
SIN01C4	0.80 ± 0.13	0.03 ± 0.01	40.14 ± 2.63	1.95 ± 0.22	1.68 ± 0.18	5.60 ± 0.63	1.34 ± 0.13	0.41 ± 0.06	42.97 ± 2.69	9.02 ± 0.84	0.78 ± 0.15	52.77 ± 3.29
SIN05C3	1.74 ± 0.22	0.07 ± 0.04	21.63 ± 2.43	1.23 ± 0.21	1.17 ± 0.14	3.23 ± 0.51	0.83 ± 0.11	0.20 ± 0.04	24.67 ± 2.64	5.42 ± 0.73	0.35 ± 0.10	30.45 ± 3.32
SIN06C3	1.20 ± 0.16	0.15 ± 0.06	30.91 ± 2.81	2.10 ± 0.36	1.43 ± 0.16	6.32 ± 0.94	1.16 ± 0.11	0.34 ± 0.05	34.50 ± 2.87	9.25 ± 1.15	0.61 ± 0.11	44.36 ± 3.56
SIN07C3	1.49 ± 0.20	0.02 ± 0.02	36.14 ± 2.08	3.61 ± 0.45	1.60 ± 0.14	6.81 ± 0.85	1.33 ± 0.13	0.33 ± 0.03	41.44 ± 2.16	10.07 ± 1.04	0.98 ± 0.14	52.50 ± 2.83
BRS550	2.59 ± 0.19	0.23 ± 0.57	32.49 ± 2.50	2.57 ± 0.30	1.60 ± 0.10	9.18 ± 0.90	1.92 ± 0.12	0.19 ± 0.02	37.98 ± 2.63	12.85 ± 1.05	0.69 ± 0.14	51.52 ± 3.24
CM24	0.09 ± 0.05	0	5.35 ± 0.87	6.33 ± 1.24	0.79 ± 0.19	1.01 ± 0.17	0.29 ± 0.02	0.21 ± 0.04	12.09 ± 2.17	2.30 ± 0.37	1.36 ± 0.29	15.74 ± 2.76
CM39	1.58 ± 0.32	0	6.58 ± 0.51	8.73 ± 0.75	1.16 ± 0.18	2.00 ± 0.49	0.71 ± 0.09	0.39 ± 0.06	18.71 ± 1.38	4.25 ± 0.66	0.97 ± 0.15	23.94 ± 1.88
Grelos de Santiago	0.21 ± 0.10	0.32 ± 0.08	33.62 ± 2.22	1.56 ± 0.24	1.39 ± 0.12	5.37 ± 0.55	1.16 ± 0.13	0.40 ± 0.06	35.85 ± 2.31	8.32 ± 0.74	1.35 ± 0.25	45.52 ± 2.93
Nabo G. Lugo	0.44 ± 0.19	0.04 ± 0.02	33.97 ± 2.70	1.21 ± 0.17	1.51 ± 0.16	4.48 ± 0.35	1.20 ± 0.13	0.32 ± 0.04	35.84 ± 2.72	7.51 ± 0.57	0.85 ± 0.14	44.20 ± 3.24
LSD (5%)	0.46	0.12	3.89	1.01	0.30	1.45	0.20	0.12	4.04	1.74	0.29	5.13

PRO, progoitrin; GRA, glucoraphanin; GNA, gluconapin; GBN, glucobrassicanapin; GBS, glucobrassicin; OHGBS, 4-hydroxyglucobrassicin; MGBS, methoxyglucobrassicin; NGBS, neoglucobrassicin; ALIPH, total aliphatics; INDOL, total indolics; AROM, total aromatic glucosinolates; total GLSs, total glucosinolates; LSD, least significant difference.

Table 5. Mean (µmol gram^{-1} dw) glucosinolate content in turnip tops from the *B. rapa* varieties evaluated at two environments in Northwestern Spain at the growing season 2017–2018.

Variety	PRO	GRA	GNA	GBN	GBS	OHGBS	MGBS	NGBS	ALIPH	INDOL	AROM	Total GLSs
SIN01C4	1.00 ± 0.55	0.01 ± 0.01	68.93 ± 4.27	2.04 ± 0.41	2.00 ± 0.16	0.12 ± 0.07	0.99 ± 0.08	0.34 ± 0.10	71.98 ± 3.98	3.46 ± 0.27	2.01 ± 0.14	77.44 ± 4.09
SIN05C3	3.98 ± 0.42	0.45 ± 0.11	36.13 ± 3.32	0.85 ± 0.12	2.02 ± 0.24	3.30 ± 0.83	0.49 ± 0.12	0.13 ± 0.03	41.43 ± 3.55	5.95 ± 1.15	2.20 ± 0.19	49.57 ± 4.51
SIN06C3	2.10 ± 0.74	0.25 ± 1.71	46.91 ± 5.37	1.96 ± 0.46	2.16 ± 0.31	0.20 ± 0.07	0.66 ± 0.08	0.20 ± 0.03	51.23 ± 5.51	3.20 ± 0.35	1.51 ± 0.19	55.94 ± 5.73
SIN07C3	1.77 ± 0.46	0.04 ± 0.02	59.91 ± 4.45	2.84 ± 0.63	1.89 ± 0.20	0.68 ± 0.15	0.59 ± 0.11	0.20 ± 0.05	64.62 ± 4.24	3.36 ± 0.30	2.15 ± 0.28	70.14 ± 4.34
CM24	0	0	8.93 ± 0.93	9.49 ± 0.75	0.94 ± 0.14	2.80 ± 0.30	0.09 ± 0.05	0.29 ± 0.07	19.51 ± 1.71	4.13 ± 0.48	3.13 ± 0.34	26.77 ± 2.35
BRS0550	5.64 ± 0.73	0	62.51 ± 3.34	1.71 ± 0.18	0.79 ± 0.04	1.48 ± 0.05	0.42 ± 0.05	0.19 ± 0.03	69.94 ± 3.29	2.88 ± 0.11	0.94 ± 0.03	73.76 ± 3.41
Grelos de Santiago	0.04 ± 0.02	0.44 ± 0.10	53.00 ± 2.35	1.33 ± 0.28	1.34 ± 0.08	0.30 ± 0.08	0.62 ± 0.10	0.18 ± 0.03	54.83 ± 2.38	2.44 ± 0.16	2.04 ± 0.25	59.29 ± 2.36
Nabo Globo Lugo	0	0.10 ± 0.04	57.47 ± 3.54	0.96 ± 0.25	1.30 ± 0.13	0.57 ± 0.10	0.50 ± 0.11	0.24 ± 0.07	58.68 ± 3.49	2.61 ± 0.20	1.42 ± 0.23	62.71 ± 3.49
LSD (5%)	1.32	0.26	11.06	1.21	0.56	0.43	0.26	0.18	10.7	0.87	0.57	11.09

PRO, progoitrin; GRA, glucoraphanin; GNA, gluconapin; GBN, glucobrassicanapin; GBS, glucobrassicin; OHGBS, 4-hydroxyglucobrassicin; MGBS, methoxyglucobrassicin; NGBS, neoglucobrassicin; ALIPH, total aliphatics; INDOL, total indolics; AROM, total aromatic glucosinolates; Total GLSs, total glucosinolates; LSD, least significant difference.

GLS quantification showed that aliphatic GSLs were predominant, representing 79 and 91% of the total GLS content in turnip greens and turnip tops, respectively. As it was expected for *B. rapa* crops, gluconapin was by far the most abundant GSL in Spanish varieties. However, Italian varieties had a different profile, where glucobrassicanapin was the main GSL in both plant organs. Gluconapin levels represented 67% and 83% of the total GLSs content in turnip greens and turnip tops, respectively. The mean value of gluconapin was 26.8 μmol g^{-1} dw in turnip greens and ranged from 5.4 μmol g^{-1} dw in "CM24" to 40.1 μmol g^{-1} dw in "SIN01". In turnip tops, the mean value of gluconapin was 49.3 μmol g^{-1} dw and ranged from 8.9 μmol g^{-1} dw in "CM24" to 68.9 μmol g^{-1} dw in "SIN01". In turnip greens, the second GLS in abundance was hydroxyglucobrassicin, representing 12% of the total GLS content. The mean value of hydroxyglucobrassicin in this plant organ was 4.9 μmol g^{-1} dw. In turnip tops, the second GLS in abundance of the total GLS content was glucobrassicanapin, representing 4.5% of the total GLS content. The mean value for glucobrassicanapin was 2.8 μmol g^{-1} dw.

The indolic group of GLSs represented between 19% and 6% of the total GLS content in turnip greens and turnip tops, respectively. Means for total indolic GLSs were 7.7 μmol g^{-1} dw in turnip greens and 3.50 μmol g^{-1} dw in turnip tops. Gluconasturtiin was the only aromatic GLS found in a concentration of 0.9 μmol g^{-1} dw and 1.90 μmol g^{-1} dw in turnip greens and turnip tops, respectively Aromatic GLSs were minor and ranged between 2% and 3% of the total GLS content in turnip greens and turnip tops, respectively.

Leaves of all varieties were harvested before bud opening at the same date, regardless of their earliness to reduce the effects due to different environmental factors, which notably affects the final GLS content. At this period, early varieties are close to the flowering stage; the medium ones are at the pre-flowering stage with floral bud formation starting, and late ones are at the vegetative stage. Because turnip tops are the other edible part that is very appreciated for food purposes, GLS content was also evaluated in trials carried out 2017 at two locations in Spain. Flower buds were collected according to the earliness of each variety.

In the individual analysis of variance performed for each plant organ, significant differences ($p \leq 0.001$) were found among varieties and environments for most individual and total GLS content in both plant organs. For turnip greens, varieties were significantly different for the total GLS content, as well as for most of the individual GLS content, except for glucobrassicin, glucoraphanin, neoglucobrassicin, and glucoiberverin (all of them minor GLSs in this crop). Differences among environments were significant ($p < 0.01$) for gluconapoleiferin (a minor GLS in this species).

Similar results were found in turnip tops. Varieties were significantly different for most of the individual and total GLS content, except for neoglucobrassicin, and differences among environments were significant for the two major GLSs (gluconapin and glucobrassicanapin) and three minor ones (glucoraphanin, 4-hydroxyglucobrassicin and gluconasturtiin).

Total and most individual GLS content showed a significant variety × environment interaction in both organs. Therefore, individual analyses of variance for these compounds were made at each environment. Comparing the results from the individual analyses, varieties showed a similar performance across environments, i.e., varieties with extreme values were the same at different locations for most traits. Then, magnitude changes rather than rank changes contributed to these interactions. For this reason, data are presented as means of the two locations.

Our results showed high variability for many of the components and for total glucosinolates among different genotypes in both plant organs. Varieties "SIN01" and "SIN07" showed the highest values on total GLS, total aliphatic and gluconapin contents in turnip greens, followed by the two commercial varieties (Table 4). For turnip tops, the highest values on gluconapin, aliphatic and total GLS contents were found for "SIN01" and local variety "BRS550" (Table 5). No data for turnip tops were taken for this last variety "BRS550" at Oroso because this variety did not flower at this location. Therefore, results for this variety should be taken cautiously because of the effect of the environment in GLS values. The two commercial varieties also had high GLS levels, but they were significantly lower than in synthetic varieties "SIN01" and "SIN07".

For indolic GLSs, varieties with the highest contents were different depending on the plant organ. In turnip greens, "BRS550" and "SIN07" had the highest indolic GLS content, whereas in turnip tops, "SIN05" and "CM24" showed the highest indolic GLS content. For aromatic GLSs, Italian commercial variety "CM24" had the highest values for gluconasturtiin content both in turnip greens and in turnip tops.

Furthermore, significant differences ($p < 0.01$) were found between plant organs (turnip greens and turnip tops) for most GLSs analyzed. Similar to what happened with varieties, the location × plant organ interaction was significant for most GLSs (data not shown). In spite of these interactions, varieties and plant organs showed similar behaviors across locations for gluconasturtiin and total GLS content, and therefore, a combined analysis of variance across locations was made. As it has been already reported, the concentration of GLSs in *B. rapa* varies across stage development.

Comparisons between the two plant organs were carried out at two Spanish locations where plants could produce turnip greens and turnip tops. Data show that total GLS and total aliphatic and gluconapin content were higher in turnip tops than in turnip greens (Figure 2). In turnip greens, the total GLS content ranged from 20 µmol g^{-1} in CM24 to 64.2 µmol g^{-1} dw in "SIN01", with a mean value of 51.9 µmol g^{-1} dw. In turnip tops, the total GSL content ranged from 26.8 in "CM24" to 77.4 µmol g^{-1} dw in "SIN01" with a mean value of 58.9 µmol g^{-1} dw (Figure 2).

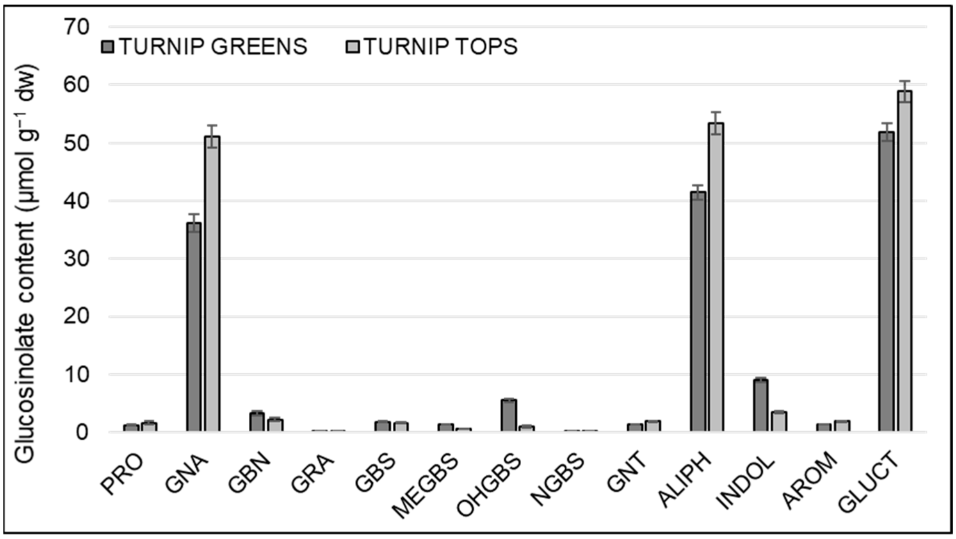

Figure 2. Total and individual glucosinolate content (µmol g^{-1} dw) measured in leaves (turnip greens) and flower buds (turnip tops) of nine *B. rapa* varieties grown at two locations from Spain in 2017–2018 growing season.

3.3. Variation of Phenolic Content and Antioxidant Activity among Varieties, Locations, and Plant Organs

For turnip greens, the combined analysis of variance across environments showed significant differences ($p < 0.01$) among varieties and environments for DPPH assays and for the total phenolic content. The environment × variety interaction was also highly significant. Therefore, individual analyses of variance for these compounds were made at each environment. When environments were analyzed by year and by location, varieties showed similar behaviors for all compounds across years for each location. Therefore, data are presented as means of the two years for each location (Figure 3).

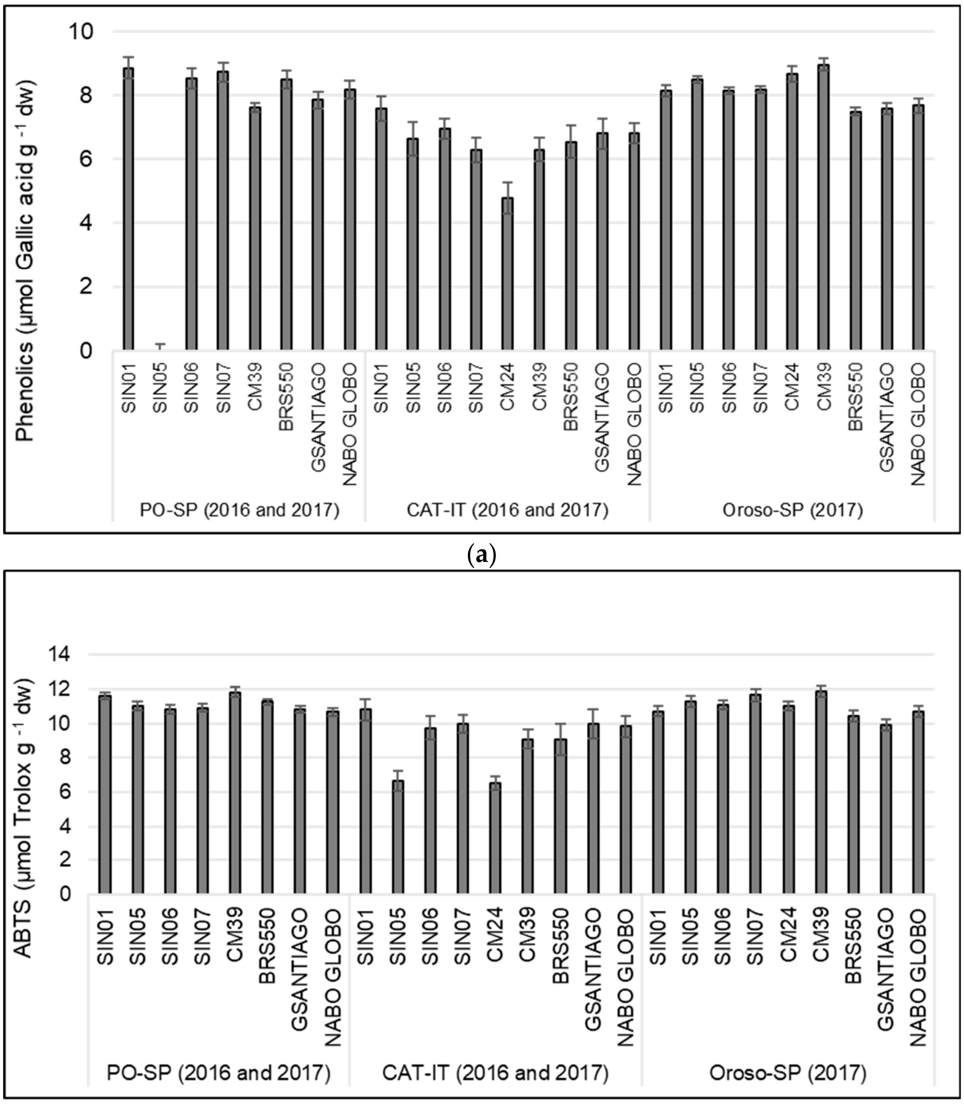

Figure 3. Phenolic content (**a**) and antioxidant activity measured with ABTS assays (**b**) in leaves (turnip greens) of nine *B. rapa* varieties grown at five environments in North Weast Spain and in South Italy for two years. Significant differences among environments ($p < 0.001$).

In this work, different varieties stand out over the rest, depending on the location. In Pontevedra variety "SIN01" had significantly higher leaf phenolic content than the other varieties (8.85 µmol Gallic acid g^{-1} dw), followed by "SIN07" (8.72 µmol Gallic acid g^{-1} dw), which significantly differ from the rest of varieties (Figure 3a). Synthetic "SIN01", along with the two commercial varieties, stand out over the rest in Catania, while "CM24" showed the lowest values for total phenolic content in leaves. The two Catania varieties, "CM24" and "CM39", showed the highest values for total phenolic content in Oroso, whereas Galician local variety "BRS550" and "Grelos de Santiago" had the lowest values (Figure 3a).

Variety "SIN01" had significantly higher antioxidant activity in leaves than the other varieties for ABTS in Pontevedra (11.6 µmol Trolox g^{-1} dw) and Catania (10.8 µmol Trolox g^{-1} dw), and it did not differ significantly from the varieties with the highest ABTS content in Oroso (Figure 3b). For this assay, "SIN01" was followed by "CM39" and "BRS550" in Pontevedra and by the two commercial varieties in Catania, which significantly differ from the rest of crops for both ABTS assays.

When comparing the antioxidant activity and the total phenolic content of the consumed organs (leaves and flowering buds) (Figure 4), no significant differences ($p > 0.05$) were found between plant organs for the compounds analyzed. In the combined analyses of variance across locations, the location × plant organ interaction was significant for the total phenolic content and ABTS assays. Varieties showed a different performance at each location, and therefore, data are shown for each location.

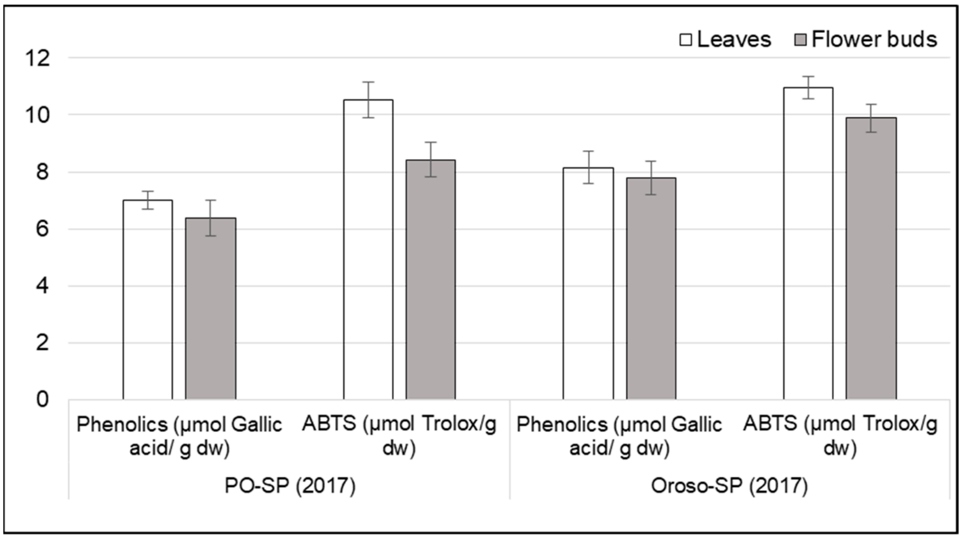

Figure 4. Phenolic content (µmol Gallic acid/g dw) and antioxidant activity measured with ABTS assays (µmol Trolox/g dw) of nine *B. rapa* varieties measured in leaves (turnip greens) and flower buds (turnip tops) in two locations from Spain in 2017–2018 growing season. Significant differences among environments ($p < 0.001$).

The earliest varieties "SIN05" and "CM24" had the lowest levels of antioxidant activity in leaves, whereas the latest varieties such us "SIN07" and "BRS550" showed the highest total phenolic content, thus suggesting a relationship between earliness and the antioxidant activity once again. However, this effect was not noticed in turnip tops, where variety "SIN05" had the highest total phenolic content and ABTS values in Pontevedra, while BRS550 and "SIN07" had the lowest values.

Total phenolic content showed a mean value of 6.99 and 6.37 µmol Gallic acid g^{-1} dw in Pontevedra in turnip greens and turnip tops, respectively. These values were similar in Oroso, where the total phenolic contents for turnip greens and tops were 8.15 and 7.79 µmol Gallic acid g^{-1} dw, respectively (Figure 4). For ABTS assays, contents ranged from 10.5 in turnip greens to 8.42 µmol Trolox g^{-1} dw in turnip tops grown in Pontevedra, whereas we found values from 11 µmol Trolox g^{-1} dw in turnip greens to 9.9 µmol Trolox g^{-1} dw in turnip tops in Oroso (Figure 4).

4. Discussion

Brassica rapa spp. *rapa* is an important vegetable species. Several plant parts, including turnip tubers, leaves and turnip tops, are important for human consumption. In the present study, agronomic value and bioactive compounds were determined for nine genotypes with different genetic background. Genotypes included landraces, synthetic and commercial varieties with different geographical origins and earliness. The overall goal of this study was to compare the agronomic value of synthetic varieties and local varieties versus commercial varieties and to select high yielding *B. rapa* varieties suitable for growing in different environments.

Due to the importance of a continuous turnip tops and turnip greens production throughout the autumn and winter for the fresh market and the processing industry, we developed three synthetic varieties (early, medium, and late). Late varieties delay turnip greens production, which can be very interesting for producers and agro-industrial purposes, while early varieties allow offering turnip tops on the market before their usual dates. Besides, the earliest varieties have greater flexibility to grow in different seasons due to lower cold and photoperiod requirements, and they facilitate the product arrival in the markets very soon, which is desirable for the consumer. The convenience of one or the other will depend on the market and producers' decisions.

The best plants for turnip greens production should have a high leaf number, large leaves and high fresh weight content. The best varieties for turnip greens fresh yield were local variety extra-late BRS0550 and two synthetic varieties developed at MBG ("SIN01" and "SIN07"). Besides, agronomic performance of these genotypes was stable in both countries. The Italian variety "CM39" also showed good turnip greens production under the environmental conditions of Sicily, because it is well adapted to local conditions. Furthermore, all these varieties were significantly better than both commercial varieties "Grelos de Santiago" and "Nabo Globo de Lugo" in most environments tested in Spain and Italy.

Because turnip tops are the other plant organ that is highly appreciated for human consumption, its fresh production was also evaluated in trials performed in 2017. The highest turnip tops fresh production was noticed for extra-late variety BRS0550 in both countries. Synthetic "SIN07" and commercial variety "Grelos of Santiago" were suitable varieties for turnip tops in Pontevedra, whereas local variety "CM39", as it was noticed for turnip greens production, stood out over the other varieties in Catania once again.

Local varieties ("BRS550" and "CM39") could be valuable resources for turnip greens production since they are adapted to the climatic conditions of the area. In our work, "BRS550" was a very extra-late variety under the environmental conditions and sowing cycles used in each country, and it did not flower in some environments after more than six months had passed. In a previous evaluation carried out by Francisco et al. [7], authors also found that "BRS0550" was appropriate for turnip greens production, although it was very late for turnip tops production. An extra-late variety could be desirable for canning companies, as leaf production occurs for a long time. However, late varieties mean that a variety growing out of the usual dates is not able to produce edible turnip tops.

Regarding "SIN01", this variety is derived from four cycles of mass selection by turnip greens fresh production in NW Spain, and therefore, it is expected to have a good agronomic performance. On the other hand, "SIN07" was developed after three cycles of mass selection by lateness in selecting and crossing the latest plants at each cycle, but no selection for fresh yield was performed.

Flowering time is a complex trait controlled by multiple loci in *Brassica* species [30]. We found an effect of the earliness on traits related to yield (leaf number, branch number, and fresh weight of leaves and flower buds). The earliest varieties like "SIN05" or "CM24" had the lowest turnip greens production, whereas the latest varieties like "SIN07" and "BRS550" were the best in most environments. However, "SIN01" had a medium cycle. Similar results had previously been reported by various authors evaluating germplasm of *B. rapa* from Galicia, where they found a relationship between plant habit and earliness with fresh production [8].

Considering the yield contributing traits in connection with the earliness, it might be concluded that varieties "BRS0550", "SIN07", and "SIN01" are suitable for both turnip greens and turnip tops production. Therefore, they could be proposed for further breeding programs for *B. rapa* production. On the other hand, early local variety "CM39" was also suitable for turnip tops and turnip greens production in Catania but not for Atlantic areas like those in NW Spain where trials were performed. These varieties could be used for further breeding programs in each country.

Trials were performed at two geographical sites: Galicia, in NW Spain, and South Italy (Sicily). These sites represent Atlantic coast and Mediterranean conditions. On the Atlantic coast, the weather is often cloudy with frequent rainfall. On the Mediterranean coast, the weather is mild with rain in spring and autumn. These differences in temperatures and rainfall clearly affect the performance of varieties for flowering and turnip tops fresh production. Varieties flowered earlier in Italy than in Spain, but no significant differences were found between countries for turnip greens fresh production. In conclusion, plants performed similarly in both countries for turnip greens production, but varieties had a better agronomic performance for turnip tops production in Spain.

Bioactive Compounds

Firstly, our goal was to be able to select turnip greens and turnip tops genotypes of high agronomic potential and superiority to current cultivars and, secondly, to maintain their GLS and phenolic contents and antioxidant activity in order to preserve their beneficial health properties. Therefore, identification of varieties with high levels of these compounds provides a value-added opportunity for marketing these crops.

GLS profile analyses in leaves and flower buds showed that aliphatic ones were the most abundant, being gluconapin the major of them in both plant organs. Yang and Quiros [31] studied the GLS variation in more than 80 crops of *B. rapa,* and they found that the major compound was gluconapin. The identities of the main GLS compounds in *B. rapa* reported here were different in leaves and flower buds. The GLS profile in turnip tops was the expected for this species, with GNA being the major one, followed by another aliphatic one, that is, glucobrassicanapin. However, gluconapin was the most abundant GLS in leaves, followed by the indolic hydroxyglucobrassicin, which differs with the GLS profile detected in previous studies [6,7,32]. These authors reported two aliphatic GLSs, gluconapin and glucobrassicanapin, as the most abundant both in leaves and in flower buds.

The most promising varieties for future breeding purposes would be those with the highest total GLS content and profile, with beneficial effects related to human health. In our work, we found that the new *B. rapa* varieties developed in our group, like "SIN01" and "SIN07", as well as local Galician variety "BRS550", could be used as a source of GLSs in our diet. However, "BRS550" is too late for the environmental conditions of NW Spain and S Italy. Considering the two organs analyzed, synthetic variety "SIN01" has stood out as the best variety to have *B. rapa* crops enriched in GLS.

A significant environmental influence on locations for total and individual GLS content was found, even though varieties with the highest and lowest contents were stable in most environments. This result agrees with other works where GLS content in *B. rapa* varieties was dependent upon the crops and genotypes [7,31,33,34]. In this work, GLS levels varied depending on the edible part evaluated, and they were higher in flower buds than in leaves, as it was already reported by other authors [7,35,36]. The high GLS and gluconapin levels found in turnip tops content could be related with the pungent and bitter flavor attributed to this vegetable [37,38]. Considering the beneficial effects of GLSs, turnip greens and turnip tops, which contain a relatively high content of GNA and aliphatic GLSs, are of high dietary value.

Because phenolic compounds are also important as health-protective agents in human nutrition, the development of varieties with an improved nutritional value would be useful [39]. In this sense, the *B. rapa* varieties evaluated in this work had different total phenolic content in turnip greens, hence showing that the environmental effect is important for these compounds, as well as it was for GSL content. The influence of the genotype and the environment on the total and individual phenolic contents in *B. rapa* crops was previously reported by Francisco et al. [7]. Authors concluded that both hydroxycinnamic acids and flavonoids are highly influenced by the environment and the genotype × environment interactions.

The antioxidant activity of *B. rapa* crops, including measurements of antioxidant potential on different plant organs, has not been conveniently studied yet, and these crops may be promising subjects in the field of antioxidant activity [17]. Variations in the content of phenolic compounds in *Brassica* spp. are affected by differences in genetics, environmental conditions, and plant organs [7,40,41]. In this work, the antioxidant activity was more dependent on the environmental effects rather than the plant part. Besides, different varieties stand out over the rest depending on the location. Unlike what was observed for GLSs, the concentration of total phenolic content and the antioxidant activity did not vary for plant organs. However, other authors found that the antioxidant activity depends on the plant development stage, and it reaches its maximum when plants are young [42,43]. Varieties with the highest levels of total phenolic compounds in leaves were the two synthetic varieties, "SIN01" and "SIN07", and the two Italian varieties, "CM39" and "CM34". Thus, these varieties could be recommended for turnip greens production because of its high antioxidant activity.

5. Conclusions

In conclusion, analyses from nine *B. rapa* genotypes showed considerable variation in agronomical traits, total GLS, phenolic content and antioxidant activity among genotypes (landraces, synthetic, and commercial varieties), the different parts of the plant (leaf and flower buds), and the geographical origin, which confirmed the relationship between the content of these traits and the environmental and genetic factors. The study showed that the latest genotypes are more productive than the earlier ones and that it is possible to improve genotypes for different growing cycles. Thus, varieties selected in our work have not only good commercial perspectives for fresh production, but they also may provide considerable amounts of bioactive compounds and constitute an important natural source of dietary antioxidants.

We demonstrate that the mass selection carried out in *B. rapa* was successful. The development of synthetic varieties such as the four selections evaluated in this work offers us new *B. rapa* crops with good agronomic performance, preserving their positive health effects based on their antioxidant potential and their GLS and phenolic compounds content. As such, they will be useful material for the agro-food sector or could be used as parents in breeding programs to develop new varieties for future releases.

Author Contributions: M.E.C. conceived the study, carried out the field experiments in Spain, performed statistical analysis of the data and wrote the manuscript; F.B. and M.C.D.B. carried out the field experiments in Italy; P.V. carried out the glucosinolate analysis and discussed the results; P.S. carried out the antioxidant analysis and discussed the results; F.B. and S.T. reviewed and edited; F.B. conceived the study and discussed the results. All authors read, edited, and approved the final version of the manuscript.

Funding: This research was supported by projects AGL2015-66256-C2-R and RTI2018-096591-B-I00 (MCIU/AEI/FEDER, UE), through the Spanish Ministry of Science the European Regional Development Fund (ERDF), in which both institutions, MBG-CSIC and Di3A-UNICT, were involved.

Acknowledgments: Authors thank the invaluable help of Abilleira R. and Fernández J.C. for all the laboratory and field work.

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

References

1. Dixon, G.R. Origins and diversity of *Brassica* and its relatives. In *Vegetable Brassicas and Related Crucifers*; Dixon, G.R., Ed.; CABI: Wallingford, UK, 2006; pp. 1–33. [CrossRef]
2. Zhao, J.; Wang, X.; Deng, B.; Lou, P.; Wu, J.; Sun, R.; Xu, Z.; Vromans, J.; Koornneef, M. Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. *Theor. Appl. Genet.* **2005**, *110*, 1301–1314. [CrossRef] [PubMed]
3. Prakash, S.; Hinata, K. Taxonomy, cytogenetics and origin of crop Brassicas, a review. *Opera. Bot.* **1980**, *55*, 3–57.
4. Monteiro, A.; Dias, J. Portuguese Brassica landraces: A perspective. *Acta Hortic.* **1996**, *407*, 87–94. [CrossRef]
5. Bianco, V.V.; Damato, G.; Pomarici, R. Sowing and transplant dates in four cima di rapa (*Brassica rapa* L.) cultivars.—I. Sowing dates. *Acta Hort.* **1996**, *407*, 293–295. [CrossRef]
6. Padilla, G.; Cartea, M.E.; Rodríguez, V.M.; Ordás, A. Genetic diversity in a germplasm collection of *Brassica rapa* subsp *rapa* L. from northwestern Spain. *Euphytica* **2005**, *145*, 171–180. [CrossRef]
7. Francisco, M.; Cartea, M.E.; Soengas, P.; Velasco, P. Effect of genotype and environmental conditions on health-promoting compounds in *Brassica rapa*. *J. Agric. Food Chem.* **2011**, *59*, 2421–2431. [CrossRef]
8. Cartea, M.E.; De Haro, A.; Obregón, S.; Soengas, P.; Velasco, P. Glucosinolate variation in leaves of *Brassica rapa* crops. *Plant Foods Hum. Nutr.* **2012**, *67*, 283–288. [CrossRef]

9. Soengas, P.; Cartea, M.E.; Francisco, M.; Velasco, P. Genetic structure and diversity of a collection of *Brassica rapa* subsp. *rapa* L. revealed by simple sequence repeat markers. *J. Agric. Sci.* **2011**, *149*, 617–624. [CrossRef]
10. Cartea, M.E.; Velasco, P. Glucosinolates in *Brassica* foods: Bioavailability in food and significance for human health. *Phytochem. Rev.* **2008**, *7*, 213–229. [CrossRef]
11. Hong, E.Y.; Kim, G.H. Anticancer and antimicrobial activities of β-phenylethyl isothiocyanate in *Brassica rapa* L. *Food Sci. Technol. Res.* **2008**, *14*, 377–382. [CrossRef]
12. Traka, M.; Mithen, R. Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.* **2009**, *8*, 269–282. [CrossRef]
13. Paul, S.; Geng, C.A.; Yang, T.H.; Yang, Y.P.; Chen, J.J. Phytochemical and health-beneficial progress of turnip (*Brassica rapa*). *J. Food Sci.* **2019**, *84*, 19–30. [CrossRef] [PubMed]
14. Terzo, M.N.; Pezzino, F.; Amodeo, L.; Catalano, D.; Viola, M.; Tribulato, A.; Travali, S.; Branca, F. Evaluation of a Sicilian black broccoli extract on in vitro cell models. *Acta Hortic.* **2018**, *1202*, 135–142. [CrossRef]
15. Terzo, M.N.; Russo, A.; Ficili, B.; Pezzino, F.M.; Tribulato, A.; Russello, D.; Branca, F.; Travali, S. Neglected Sicilian landraces of black broccoli (*Brassica oleracea* var. *italica* Plenck) and health benefits: An in vivo study. *Acta Hortic.* **2020**, *1267*, 91–96. [CrossRef]
16. Fernandes, F.; Valentao, P.; Sousa, C.; Pereira, J.A.; Seabra, R.M.; Andrade, P.B. Chemical and antioxidative assessment of dietary turnip (*Brassica rapa* var. *rapa* L.). *Food Chem.* **2007**, *105*, 1003–1010. [CrossRef]
17. Shawon, R.A.; Kang, B.S.; Lee, S.G.; Kim, S.K.; Lee, H.J.; Katrich, E.; Gorinstein, S.; Ku, Y.G. Influence of drought stress on bioactive compounds, antioxidant enzymes and glucosinolate contents of Chinese cabbage (*Brassica rapa*). *Food Chem.* **2020**, *308*, 125657. [CrossRef] [PubMed]
18. Cámara-Martos, F.; Obregón-Cano, S.; Mesa-Plata, O.; Cartea-González, M.E.; de Haro–Bailón, A. Quantification and in vitro bioaccessibility of glucosinolates and trace elements in Brassicaceae leafy vegetables. *Food Chem.* **2021**, *339*, 127860. [CrossRef]
19. Singh, A. Glucosinolates and plant defense. In *Glucosinolates*; Mérillon, J.M., Ramawat, K.G., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 1–26. [CrossRef]
20. Núñez-Iglesias, M.J.; Novio, S.; García, C.; Soengas, P.; Cartea, M.E.; Velasco, P.; Freire-Garabal, M. Glucosinolate-degradation products as coadjuvant therapy on prostate cancer in vitro. *Int. J. Mol. Sci.* **2019**, *20*, 4977. [CrossRef]
21. Llorach, R.; Espian, J.C.; S-Barberan, F.A.T.; Ferreres, F. Valorization of cauliflower (*Brassica oleracea* L. var. botrytis) by-products as a source of antioxidant phenolics. *J. Agric. Food Chem.* **2003**, *51*, 2181–2187. [CrossRef]
22. Branca, F.; Chiarenza, G.L.; Cavallaro, C.; Gu, H.; Zhao, Z.; Tribulato, A. Diversity of Sicilian broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis*) landraces and their distinctive bio-morphological, antioxidant, and genetic traits. *Genet. Resour. Crop. Ev.* **2018**, *6*, 485–502. [CrossRef]
23. Di Bella, M.C.; Niklas, A.; Toscano, S.; Picchi, V.; Romano, D.; Lo Scalzo, R.; Branca, F. Morphometric characteristics, polyphenols and ascorbic acid variation in *Brassica oleracea* L. Novel Foods: Sprouts, Microgreens and Baby Leaves. *Agronomy* **2020**, *10*, 782. [CrossRef]
24. IBPGR. *Descriptors for Brassica and Raphanus*; International Board for Plant Genetic Resources: Rome, Italy, 1990.
25. Kliebenstein, D.J.; Lambrix, V.; Reichelt, M.; Gershenzon, J.; Mitchell-Olds, T. Gene duplication and the diversification of secondary metabolism: Sidechain modification of glucosinolates in *Arabidopsis thaliana*. *Plant Cell* **2001**, *13*, 681–693. [CrossRef]
26. Samarth, R.; Panwar, M.; Kumar, M.; Soni, A. Evaluation of antioxidant and radical-scavenging activities of certain radioprotective plant extracts. *Food Chem.* **2008**, *106*, 868–873. [CrossRef]
27. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
28. Dewanto, V.; Wu, X.; Adom, K.; Liu, R. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014. [CrossRef]
29. Steel, R.D.G.; Torrie, J.H.; Dickey, D.A. *Principles and Procedures in Statistics: A Biometrical Approach*; McGraw Hill: New York, NY, USA, 1997.
30. Lou, P.; Zhao, J.; Kim, J.S.; Shen, S.; Del Carpio, D.P.; Song, X.; Jin, M.; Vreugdenhil, D.; Wang, X.; Koornneef, M.; et al. Quantitative trait loci for flowering time and morphological traits in multiple populations of *Brassica rapa*. *J. Experim. Bot.* **2007**, *58*, 4005–4016. [CrossRef]
31. Yang, B.; Quiros, C. Survey of glucosinolate variation in leaves of *Brassica rapa* crops. *Gen. Res. Crop Evol.* **2010**, *57*, 1079–1089. [CrossRef]
32. Kim, S.J.; Kawaguchi, S.; Watanabe, Y. Glucosinolates in vegetative tissues and seeds of twelve cultivars of vegetable turnip rape (*Brassica rapa* L.). *Soil Sci. Plant Nutr.* **2003**, *49*, 337–346. [CrossRef]
33. Charron, C.S.; Saxton, A.M.; Sams, C.E. Relationship of climate ad genotype to seasonal variation in the glucosinolate-mirosinase system I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J. Sci. Food Agric.* **2005**, *85*, 671–681. [CrossRef]
34. Ciska, E.; Martyniak-Przybyszewska, B.; Kozlowska, H. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *J. Agric. Food Chem.* **2000**, *48*, 2862–2867. [CrossRef]
35. Velasco, P.; Cartea, M.E.; González, C.; Vilar, M.; Ordás, A. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem.* **2007**, *55*, 955–962. [CrossRef] [PubMed]
36. Bonnema, G.; Lee, J.G.; Shuhang, W.; Lagarrigue, D.; Bucher, J.; Wehrens, R.; de Vos, R.; Beekwilder, J. Glucosinolate variability between turnip organs during development. *PLoS ONE* **2019**, *14*, e0217862. [CrossRef] [PubMed]

37. Rosa, E.A.S.; Heaney, R.K.; Fenwick, G.R.; Portas, C.A.M. Glucosinolates in crop plants. *Hortic. Rev.* **1997**, *19*, 99–215.
38. Francisco, M.; Velasco, P.; Romero, A.; Vazquez, L.; Cartea, M.E. Sensory quality of turnip greens and turnip tops grown in northwestern Spain. *Eur. Food Res. Technol.* **2009**, *230*, 281–290. [CrossRef]
39. Cartea, M.E.; Francisco, M.; Soengas, P.; Velasco, P. Phenolic compounds in *Brassica* vegetables. *Molecules* **2011**, *16*, 251–280. [CrossRef] [PubMed]
40. Bhandari, S.R.; Jo, J.S.; Lee, J.G. Comparison of glucosinolate profiles in different tissues of nine *Brassica* crops. *Molecules* **2015**, *20*, 15827–15841. [CrossRef]
41. Kim, J.K.; Chu, S.M.; Kim, S.J.; Lee, D.J.; Lee, S.Y.; Lim, S.H.; Ha, S.H.; Kweon, S.J.; Cho, H.S. Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. *pekinensis*. *Food Chem.* **2010**, *119*, 423–428. [CrossRef]
42. Samec, D.; Piljac-Zagarac, J.; Bogovic, M.; Habjanic, K.; Gruz, J. Antioxidant potency of white (*Brassica oleracea* L. var. *capitata*) and Chinese (*Brassica rapa* L. var. *pekinensis* (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection. *Sci. Hortic.* **2011**, *128*, 78–83. [CrossRef]
43. Soengas, P.; Cartea, M.E.; Francisco, M.; Sotelo, T.; Velasco, P. New insights into antioxidant activity of Brassica crops. *Food Chem.* **2012**, *134*, 725–733. [CrossRef]