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Effects of Greenhouse vs. Growth Chamber and Different Blue-Light Percentages on the Growth Performance and Quality of Broccoli Microgreens

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Abstract: Microgreens are a product category with a biochemical content that is currently earning them the status of a functional food. The genotype of the microgreens, and environmental factors, such as the photosynthetic photon flux density (PPFD) and light spectra, can influence the yield and biochemical profile. A landrace of broccoli called 'Mugnoli' was compared with a commercial variety ('Broccolo Natalino') in two microgreen growing systems (greenhouse vs. growth chamber) and under three growth chamber light spectra (blue, control, control + blue). The results showed that both Mugnoli and Broccolo Natalino can be used to produce microgreens, achieving similar yields, but that Mugnoli showed notably higher polyphenols and antioxidant contents. Due the higher PFFD of the greenhouse environment, microgreens yields were 18% higher than the yields from cultivation in the growth chamber. Regarding the results under different growth chamber spectra, monochromatic blue caused reductions in the microgreens yield and polyphenols content of 13.5% and 14.2%, respectively. In conclusion, Mugnoli can be considered a valuable genetic source for the production of microgreens given its fast crop cycle, good fresh weight production, and, compared to Broccolo Natalino, its superior biochemical content and lower susceptibility to PPFD variations.

Keywords: biodiversity; Brassicaceae; functional food; landraces; light-emitting diodes; polyphenols

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1. Introduction

Consumer interest in good health has been the driving force in the creation of foods that are healthier (low fat, low calories) and have health-promoting functions (e.g., high antioxidant activity, pro-/prebiotics) [1]. In this context, new foods, such as microgreens, have emerged as food sources that may promote health [2].

Microgreens comprise a category of products with characteristics that are quite distinct from those identified in sprouts or baby leaf [3], and which should not be confused with mini-vegetables, which can be obtained using particular cultivation techniques (e.g., high sowing density, early harvest) [4]. The term 'microgreens' has no legal definition but is used by marketers to describe a particular product category. Compared to sprouts, microgreens are grown in greenhouses [5] or indoor environments [6], with or without growing media [7], and with natural or artificial light [8]. Moreover, microgreens have a longer cycle than sprouts, and the edible portion consists only and entirely of the aerial part deprived of the roots. Instead, for baby leaf, the edible portion consists only of the true leaves (no more than eight), which must be cut before marketing [3]. Microgreens may be marketed before harvest, maintaining the growing medium and leaving the final consumer (or chef) with the choice of cutting the product immediately before consumption.

Thanks to their distinctive qualities, microgreens comprise a rich food source, and have earned the title of 'functional food' or 'superfood' [9,10], particularly among demanding categories of consumers, such as vegetarians and vegans, who can diversify and enrich their diet using a large variety of available microgreens [11]. Additionally, as microgreens are usually consumed raw, they can also satisfy the specific needs of so-called 'raw foods' and be prepared in this role as 'tailored foods' [12,13].

Many species and local varieties of several botanical families, such as Brassicaceae, Asteraceae, Fabaceae, etc., can be used for microgreens production [14,15]. Italy is widely regarded as the center of genetic diversity for several cultivated *Brassica* races, such as *Brassica oleracea* L. var. *italica* Plenck (broccoli; [16]). It is no surprise that Brassicaceae are among the most used vegetables in the world for the production of microgreens [17] given the widespread global distribution of this family, which is rich in compounds that are functional for the prevention of diseases and the preservation of human health. Palmitessa et al. [18], for example, found that a genotype of *Brassica oleracea* L. var. *italica* Plenck called 'Broccolo Natalino' had a very high content of mineral elements, carotenoids, tocopherols, fiber, and proteins. Another class of molecules with a bioactive function that are widespread in vegetable tissues are polyphenols [19]. Polyphenols show antioxidant activity and play important roles in the defense of cells against free radical damage, and thereby against diseases, such as cancer and cardiovascular disorders [20]. *Brassica* microgreens are normally considered a good source of food polyphenols [17], but their synthesis in the course of seedling growth is strongly influenced by the photosynthetic photon flux density (PPFD; [21]) and light spectra [8]. Nowadays, light-emitting diodes (LEDs) provide the most efficient artificial lighting technology [22], allowing optimization of the PPFD and spectral qualities for several plants and different physiological processes [23]. Recently, various experiments were conducted on the effects of various PPFD [24–26] and artificial light spectra [27] on microgreens' growth and nutritional value. In particular, after examining the effects of blue spectra on microgreens' growth and yield, it was found that their action was genotypic dependent [28], but this result was contradictory between experiments [29]. More generally, it was found that monochromatic blue light increased stomatal conductance [30], induced a more compact plant size [31], increased the net photosynthesis rate [32], reduced the plants' height, and increased the leaf area [33].

Starting from these remarks, the aim of this research was to investigate how two Apulian *Brassica* genotypes respond to microgreens production in two agricultural systems: greenhouse vs. indoor farming. Furthermore, in the latter, three artificial light treatments, with a different amount of blue radiation, were supplied to investigate the sensibility of the two genotypes to this spectrum. The genotypes selected for this research activity were a *B. oleracea* L. var. *italica* Plenck called 'Broccolo Natalino', as the reference genotype, and a landrace of *B. oleracea* L. var. *italica* Plenck called 'Mugnoli' that might be considered as an early genotype in the evolution of broccoli [16]. This crop is frequently found in the neighborhood of the towns of Lecce and Brindisi. Its cultivation in the area seems to be more traditional than the cultivation of broccoli [16] and it could be a valuable genetic source for the production of microgreens due to its high nutritional value [34].

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The research activities were carried out at the facilities of the Department of Agricultural and Environmental Science of the University of Bari 'Aldo Moro' (Italy). Microgreens were cultivated in a growth chamber (Fitotron SGC 120 Plant Growth Chamber, Weiss Umwelttechnik GmbH, Reiskirchen, Germany) with the dimensions 1.31 m × 0.675 m × 1.41 m (capacity 1200 L) and in an unheated plastic greenhouse situated at the University of Bari 'Aldo Moro' (41°06′40.0″ N 16°52′54.0″ E; southern Italy). The growth chamber is equipped with three levels for cultivation, with each level providing a different light treatment by means of LEDs (Phytofy RL, OSRAM, Munich, Germany) operating at different photosynthetic photon flux densities (PPFDs) and/or different light spectra.

Two genotypes of broccoli (*Brassica oleracea* L. var. *italica*) were used: the landrace called 'Mugnoli' and the cultivar 'Broccolo Natalino'. For both types, seed was supplied by Riccardo Larosa Sementi (seed company), Andria, Italy. The seed was sown at a density of 4 seeds·cm^{-2}, in square plastic vessels with a length per side of 16.5 cm and height of 3 cm. Perforations in the bottom of the vessels allowed a subirrigation distribution of nutrient solution (NS). The substrate used was peat (Brill 3 Special, Brill Substrate GmbH & Co., Georgsdorf, Germany). Twelve vessels, six for each genotype, were placed at each light treatment level. From sowing to germination, the seeds were left in the dark at a temperature of 20 °C and RH 95%. During this period, 125 mL of distilled water were nebulized daily on each vessel. When 90% of the seeds were germinated, the LEDs were switched on, with a 14-h photoperiod. Day/night temperatures were set at 20°/16 °C and RH was maintained at 60%. After germination, by subirrigation, 100 mL of NS were supplied daily for each vessel. The NS composition (mg·L^{-1}) was 119 nitrogen, 16 phosphorus, 24 magnesium, 116 calcium, 58.4 potassium, 54 sulphur, 1.12 iron, 0.27 manganese, 0.13 zinc, 0.27 boron, 0.03 copper, and 0.01 molybdenum, resulting in an EC of 1.8 dS·m^{-1} and pH 6.3. The nitrogen source was NO_3-N:NH_4-N at a ratio of 84:16. The microgreens were grown until 11 days after sowing (when the cotyledonary leaves of both genotypes were fully expanded, under all light treatments) and were manually harvested.

2.2. Light Treatments

A plant-level PPFD of 200 µmol·m^{-2}·s^{-1} and a DLI of 10.1 mol·m^{-2}·d^{-1} were set for each artificial light treatment. The three light spectra were as follows:

1. Control (CTRL): 70% red + 5% far-red + 25% blue (Figure 1a);
2. Blue (B): 100% blue (Figure 1b); and
3. CTRL + B: CTRL spectrum from germination to day eight after germination, then B spectrum for the last two days of microgreens growth.

For the comparison of the microgreens yield in an indoor environment vs. greenhouse conditions, from 23 April 2020 to 3 May 2020, i.e., simultaneously with the growth-chamber cycle, a microgreens production cycle was carried out in the greenhouse, using the same cultivation system as in the growth chamber (12 vessels, 6 for each genotype) but with the application of solar radiation only. The temperature and RH conditions used in the greenhouse are reported in Figure 2. The solar light photoperiod during the trial was 14 h, with an average DLI of 11.67 mol·m^{-2}·d^{-1}.

2.3. Preliminary Determination of Seed Density

Determination of the weight per 1000 seeds is fundamental in establishing the quantity of seed to be used. For this measurement, using an electronic balance (series PFB, Sinargica-soluzioni, Milano, Italy), 1000 seeds of each genotype were weighed 3 times, with the average value taken as the reference. Subsequently, the germinability of each genotype was determined. In total, 3 repetitions of 50 seeds per genotype were placed on filter paper in Petri dishes and sprayed with distilled water. The dishes were placed randomly in the growth chamber, operated at 20 °C and RH 70%. The seeds were sprayed daily with distilled water. After four days, the percentage of germination was determined. Based on the 1000 seeds weight, percent of germination, growing vessel area, and seed density, the following formula was used to calculate the weight of the seeds needed for each vessel:

$$[\text{thousand seeds weight} + (\text{thousand seeds weight} \times \text{percentage of the seeds not germinated})] \times 1.089 \quad (1)$$

Multiplying the chosen density of 4 seeds·cm^{-2} [35] by the growing vessel area of 272.25 cm^2 results in a count of 1089 as the number of seedlings planned for each vessel. After dividing 1089 by 1000 (thousand seeds weight), the resulting value is 1.089.

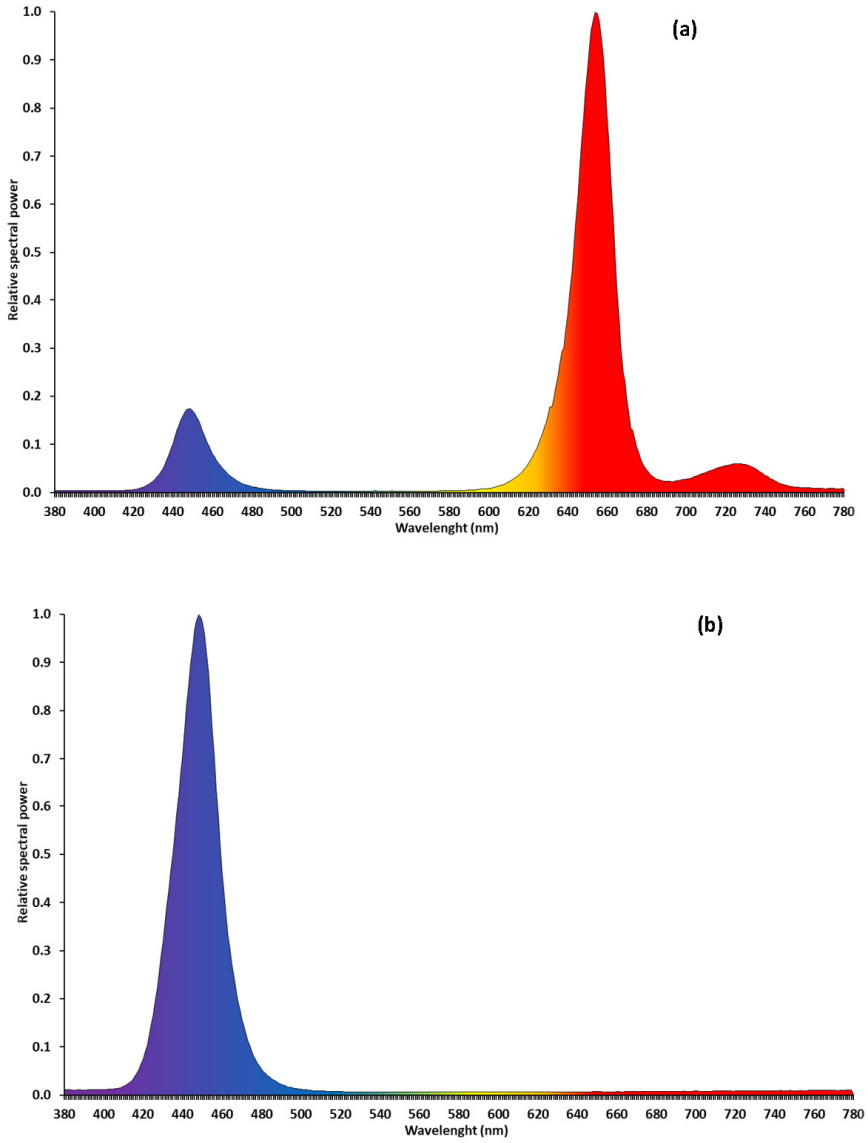

Figure 1. Relative spectral power of the CTRL light treatment (**a**) and B light treatment (**b**).

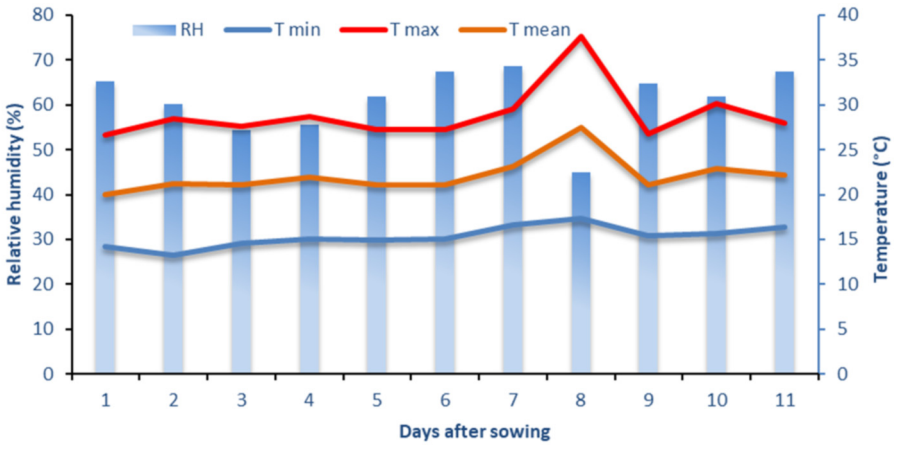

Figure 2. Greenhouse conditions during the microgreens crop cycle, 23 April 2020 to 3 May 2020: average temperature (T mean), maximum temperature (T max), minimum temperature (T min), and average relative humidity (RH).

2.4. Biometric Measurements

For each genotype and light treatment, data were recorded on the number of days from sowing until breaking seed integuments, radicle spillage, hypocotyl emission, cotyledons formation, first true leaf formation, and second true leaf formation (a true leaf was considered to have formed when it was at least 0.5 cm long). Other parameters were recorded immediately prior to harvesting: the presence of true leaves, leaf length (if a true leaf was present), shoot height, and substrate coverage. To determine the presence of true leaves, shoot height, and leaf length, 12 random microgreens were selected for each sub-parcel. The recording of the substrate coverage considered the microgreens distribution and overlap on the substrate, classified as follows: 1—low; 2—good; and 3—excessive. Each sub-parcel was observed at 30 cm, orthogonally from the growth plan, and when open space could be observed between the shoots, category 1 was assigned. If the growing media was not visible and overlap between the shoots was not observed, category 2 was assigned, or if overlap between the shoots was observed, category 3 was assigned. The harvested microgreens were weighed to determine the shoot fresh weight (FW) per unit area.

The dry matter (DM) was measured in triplicate by oven-drying at 65 °C until a constant sample weight was obtained. For chemical analysis, freeze-dried samples were used (ScanVac CoolSafe 55-9 Pro; LaboGene ApS, Lynge, Denmark).

2.5. Antioxidant Activity and Polyphenols Content

The antioxidant activity of the microgreens by electron transfer mechanisms was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical scavenging capacity test, according to Difonzo et al. [36]. The freeze-dried samples (0.1 g) were extracted with 5 mL of methanol:water (80:20) for 2 h in tubes covered with aluminum foil. The extracts were then centrifuged for 15 min at 15,000× *g* and 24 °C. The supernatant was recovered and filtered with polytetrafluoroethylene (PTFE) septa (0.45 µm). The extracts (50 µL) were added to 950 µL of 0.08 mM DPPH in methanol. The mixture was shaken and left at room temperature in the dark for 30 min. The decrease in the absorbance at 517 nm was measured using a Cary 60 Agilent spectrophotometer (Agilent Technologies, Milan, Italy). The results were expressed in µmol Trolox equivalents (TE)·g^{-1} dry weight (DW). Each sample was analyzed in triplicate.

Total phenolic compounds (TPCs) were determined on the same methanolic extract using the Folin–Ciocalteu assay [37]. In particular, 100 µL of extract were mixed with 100 µL of Folin–Ciocalteu reagent and, after 4 min, with 800 µL of a 5% (*w*/*v*) solution of sodium carbonate. The mixture was then heated in a water bath at 40 °C for 20 min and the total phenol content was determined at 750 nm by an Agilent Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The total phenolic content was expressed as gallic acid equivalents (µg·g^{-1}).

2.6. Experimental Design and Statistical Analysis

The experimental treatments in the growth chamber (three lighting treatments and two genotypes) were arranged in a split-plot design with three replications: the light in the plots (layers of cultivation) and the genotypes in the sub-plots (half layer of cultivation). To correctly compare the cultivation in the growth chamber with that in the greenhouse, only the data collected from the microgreens sown on 23 April 2020 were considered (with the vessels of the layers as replications). The data were analyzed using the general linear model procedure of SAS software (SAS Version 9.1, SAS Institute, Cary, NC, USA). All means were compared using the Student–Newman–Keuls (SNK) test at $p = 0.05$, and the standard deviation (SD) was also calculated. The significance of the main factors and their interaction are reported in tables. The average values of the main factors are reported in tables while the average values of the significant interactions genotype × light are shown using histograms.

3. Results and Discussion

3.1. Seed Quantities Needed to Produce Broccolo Natalino and Mugnoli Microgreens

The first step in identifying the seed quantities necessary for the production of microgreens is to calculate the average 1000 seeds weight [35]: 3.04 and 3.14 g for Broccolo Natalino and Mugnoli, respectively (data not shown). The germination percentages, at 91% for Broccolo Natalino (Figure 3a) and 100% for Mugnoli (Figure 3b), are within the optimal range (90–100%) suggested by Di Gioia et al. [35], thereby obviating the need for any seed pre-treatment operations [38]. Considering the small size of the seeds of these 2 genotypes, a density of 4 seeds·cm^{-2} [35] was chosen, and based on the values of the average weight per 1000 seeds and the percentage of germination, Equation (1) was applied to calculate the seed weight per vessel. The values were 3.31 g for Broccolo Natalino and 3.73 g for Mugnoli, per vessel.

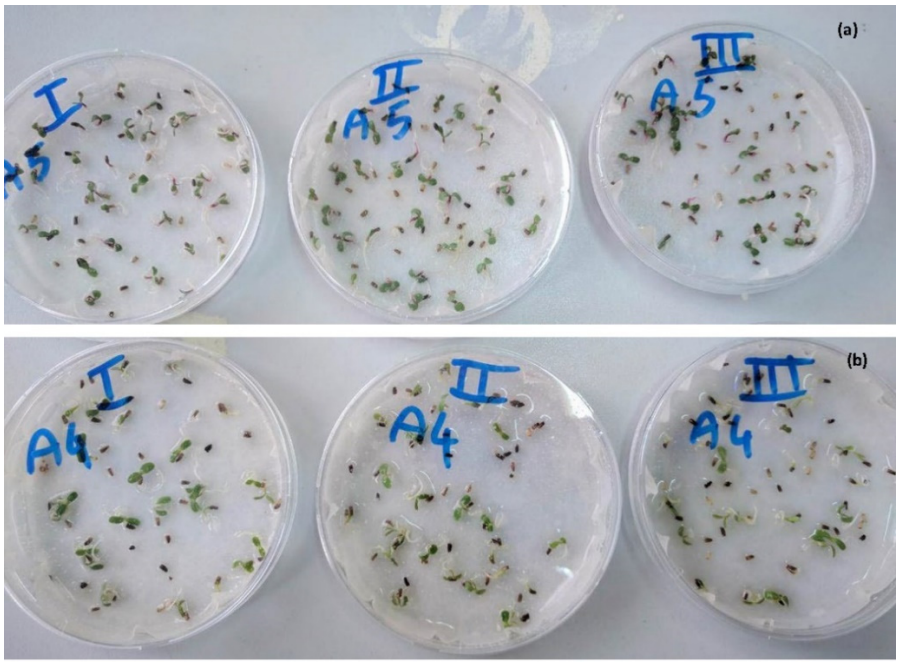

Figure 3. Test of the germinability in Petri dishes of Broccolo Natalino (**a**) and Mugnoli (**b**) seeds.

Commercial production of quality microgreens requires seed in large quantities and represents a major cost [5]. Some species will germinate easily and grow promptly while others are slow and require pre-sowing treatments for improved germination and standardization and shortening of the production cycle [38]. Therefore, a preliminary germination test per seed lot is advisable.

3.2. Seedling Development and Biometric Characteristics

In the growth chamber, Broccolo Natalino and Mugnoli seedlings did not emit true leaves until 11 days after sowing, meaning that the microgreens were harvested with cotyledonary leaves only (Table 1). Conversely, in the greenhouse, within 11 days after sowing (the same cycle length as the growth chamber), Mugnoli differentiated the second true leaf and Broccolo Natalino emitted the first true leaf (Table 2), i.e., the microgreens were observed to grow faster in the greenhouse than in the growth chamber (Table 2), probably due to the higher level of PPFD recorded in the greenhouse. Considering the length of the first true leaf emitted from the seedlings in the greenhouse, Mugnoli showed a length that was 57% greater than Broccolo Natalino (Table 2). Canopy development (substrate coverage; Tables 1 and 2) was uniform for the two genotypes under equal greenhouse light conditions. This parameter, however, is mainly influenced by the seed quality, sowing operation, and substrate characteristics (Tables 1 and 2). Effectively, the seed distribution was uniform,

the seed quality was high (germination > 90%), and the substate was uniformly watered. However, canopy development was not uniform under the different light treatments of the growth chamber (Table 1). Here, it was observed that the microgreens vessels obtained under B light had a scarce cotyledons coverage of the substrate surface compared with the other light treatments (Table 1). In fact, the monochromatic B light induced a more compact plant size [31] and reduced the plant height [33], reducing the substrate uniformity in comparison with the other light treatments. Instead, considering the average conditions of the substrate coverage and the uniformity between the growth chamber and greenhouse conditions, no significative differences were found (Table 2). In summary, the fastest crop cycle was observed in the greenhouse, for which Figures 4 and 5 report the microgreen growth steps over time. In the greenhouse, for both genotypes, seed integuments had broken by the day after sowing (Figures 4 and 5a). For Broccolo Natalino, the radicle was emitted on day 2 after sowing while hypocotyl emission was observed on day 3, and the complete cotyledons distension was observed on day 11 (Figures 4 and 5b,c). Instead, Mugnoli emitted the radicle earlier, and hypocotyl emission and cotyledons distension were observed one and two days earlier, respectively, than for Broccolo Natalino (Figure 4), i.e., the initial development was faster for Mugnoli than Broccolo Natalino (Figure 5d). This also implies that the two varieties cannot successfully be grown together during this initial phase, given the differing timing of development (Figure 2). The observed timing of development of Broccolo Natalino is, in fact, consistent with what was observed by Palmitessa et al. [18]. However, it is very important to clarify the three physiological phases of the seedling because the rupture of the integument is a signal that the environmental conditions and substrate humidity are adequate for germination. Radicle emission is, in turn, a signal that germination is continuing while after hypocotyl emission, it is important to change the environmental condition to avoid molds, caused by high RH, or plant tissue etiolation due to the absence of light. After hypocotyl emission, light must be supplied to start seedlings' photosynthetic activity. Broccolo Natalino was generally slower growing than Mugnoli, including in the phase following complete cotyledons distension (Figure 5e,f). In the greenhouse, in particular, the first true leaf was emitted on day 9 after sowing for Mugnoli and on day 11 for Broccolo Natalino (Figure 4). Compared to other *Brassicaceae* microgreens, Broccolo Natalino's seedling development is slower than broccoli raab [18] while Mugnoli has the same seedling development time as other *Brassica* genotypes, meaning that they could be sown and harvested together. For year-round market supply of microgreens, it is essential to know the timing of cultivation of the different species under fixed environmental conditions (light, temperature, RH, nutrient solution composition, etc.), thereby allowing the planning of sowing dates for subsequent daily harvesting and continuous production. Moreover, after the hypocotyl is cut, the shelf-life of microgreens is very short [39,40], again making it extremely important to understand the timing of microgreens development. In the current experiment, microgreens' growth in the greenhouse was faster than in the growth chamber, due to the higher temperature and PPFD recorded in the greenhouse environment, which promoted seedlings' photosynthetic activity and physiological processes (Figure 2), as also observed by Zhou et al. [41] in lettuce.

Table 1. Seedling stage, substrate coverage, and uniformity at the time of harvest of two brassica microgreens (Broccolo Natalino and Mugnoli) cultivated in a growth chamber under three different light spectra: Blue (B), Control (CTRL), and Control + Blue (CTRL + B). Means followed by different letters within a column indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

	Stage [(1)]	**Substrate Coverage** [(2)]	**Substrate Uniformity** [(3)]
Light treatments (L)			
Blue	1.00 ± 0.00	1.92 ± 0.23	1.92 ± 0.08 b
Control	1.00 ± 0.00	2.00 ± 0.14	2.00 ± 0.00 a
Control + Blue	1.00 ± 0.00	1.84 ± 0.28	2.11 ± 0.13 a

Table 1. *Cont.*

	Stage [1]	Substrate Coverage [2]	Substrate Uniformity [3]
Genotype (G)			
Broccolo Natalino	1.00 ± 0.00	1.86 ± 0.31	2.07 ± 0.14
Mugnoli	1.00 ± 0.00	1.94 ± 0.24	2.08 ± 0.11
Significance			
L	NS	NS	**
G	NS	NS	NS
L × G	NS	NS	NS

Development stage: [1] cotyledons; [2] small true leaves (≤5 mm); [3] true leaves (>5 mm). Seedling overlapping: [1] scarce; [2] optimal; [3] excessive. Seedling growth in the vessels: [1] malformed. only in the center; [2] optimal. uniform; [3] malformed. external sides. Significance: ** significant for $p \leq 0.01$; NS, not significant.

Table 2. Seedling stage, true leaf presence, first true leaf length, substrate coverage, and uniformity at the time of harvest of two brassica microgreens (Broccolo Natalino and Mugnoli) cultivated in a growth chamber and in a greenhouse with sunlight. Means followed by different letters within a column indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

	Stage [1]	True Leaf Presence	First True Leaf Length	Substrate Coverage [2]	Substrate Uniformity [3]
Growth system (S)		%	cm		
Growth chamber	1.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	1.93 ± 0.26	2.08 ± 0.38
Greenhouse	2.50 ± 0.52 a	85.00 ± 10.00 a	0.68 ± 0.21 a	2.00 ± 0.00	2.00 ± 0.00
Genotype (G)					
Broccolo Natalino	1.29 ± b	20.00 ± 2.50 b	0.14 ± 0.05 b	2.00 ± 0.00	2.19 ± 0.33
Mugnoli	1.50 ± a	25.00 ± 2.00 a	0.22 ± 0.08 a	1.92 ± 0.33	2.04 ± 0.43
Significance					
S	***	***	***	NS	NS
G	***	***	***	NS	NS
S × G	NS	NS	NS	NS	NS

Development stage: [1] cotyledons; [2] small true leaves (≤5 mm); [3] true leaves (>5 mm). [2] Seedling overlapping: [1] scarce; [2] optimal; [3] excessive. [3] Seedling growth in the vessels: [1] malformed. only in the center; [2] optimal. uniform; [3] malformed. external sides. Significance: *** significant for $p \leq 0.001$; NS, not significant.

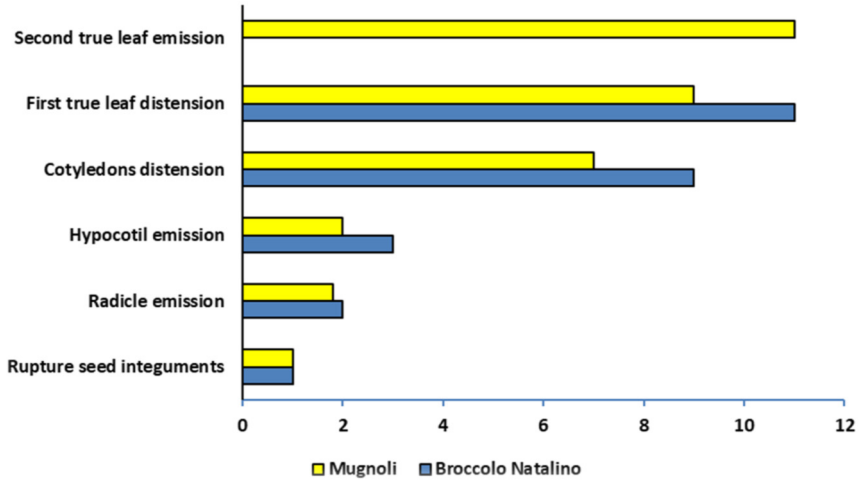

Figure 4. Timing of seedling development (in greenhouse environment) of two *Brassica* microgreens: Mugnoli and Broccolo Natalino.

Figure 5. Rupture seeds integuments (**a**), radicles emission (**b**), hypocotyl emission and cotyledons distension (**c**), different seedling growth between Broccolo Natalino and Mugnoli (**d**), complete cotyledons distension of Broccolo Natalino (**e**), and complete cotyledons distension of Mugnoli (**f**). The photos were taken in the greenhouse.

3.3. Microgreens Yield

Microgreens fresh weight (FW), dry weight (DW), and height did not significantly differ between genotypes, but the growing conditions and light treatments influenced these parameters (Tables 3 and 4). Related to the observations of the seedling development in Table 1, the yield of microgreens cultivated in the greenhouse was 22% higher than that realized under growth chamber conditions (Table 4). As already noted, the higher average temperature and DLI obtained in the greenhouse contributed to the accelerated seedling development and biomass production (Table 4). Considering the growth chamber environments, the yield of microgreens grown under B light was 13% and 5% lower than the CTRL and CTRL + B treatments, respectively (Table 3). The microgreens grown in the greenhouse had a DW content that was 34.7% higher than those of the growth chamber (Table 4). Considering only the growth chamber cultivation, DW under the B treatment was 6.4% higher than under CTRL (Table 3).

Table 3. Fresh weight, dry weight, and hypocotyl length of two *Brassica* microgreens (Broccolo Natalino and Mugnoli) cultivated in a growth chamber with three different light spectra: Blue (B), Control (CTRL), and Control + Blue (CTRL + B). Means followed by different letters within a column indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

	Fresh Weight	**Dry Weight**	**Hypocotil Lenght**
Light treatments (L)	$g \cdot m^{-2}$	$g \cdot 100\ g^{-1}$ FW	cm
Blue	1174 ± 80 c	5.11 ± 0.31 a	8.11 ± 0.95 b
Control	1358 ± 64 a	4.78 ± 0.28 b	9.31 ± 1.34 a
Control + Blue	1236 ± 76 b	4.91 ± 0.23 ab	8.54 ± 0.38 b
Genotype (G)			
Broccolo Natalino	1181 ± 112	5.92 ± 0.36	8.52 ± 3.62
Mugnoli	1255 ± 91	5.44 ± 0.37	8.84 ± 3.48
Significance [(1)]			
L	**	***	***
G	NS	NS	NS
L × G	NS	NS	NS

[(1)] Significance: *** and ** significant for $p \leq 0.001$ and $p \leq 0.01$, respectively; NS, not significant.

Table 4. Fresh weight, dry weight, and hypocotyl length of two *Brassica* microgreens (Broccolo Natalino and Mugnoli) cultivated in a growth chamber and greenhouse with sunlight. Means followed by different letters within a column indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

	Fresh Weight	**Dry Weight**	**Hypocotil Lenght**
Growth system (S)	$g \cdot m^{-2}$	$g \cdot 100\ g^{-1}$ FW	cm
Growth chamber	1426 ± 99 b	4.87 ± 0.35 b	9.65 ± 0.94 b
Greenhouse	1742 ± 87 a	7.46 ± 0.51 a	6.75 ± 0.35 a
Genotype (G)			
Broccolo Natalino	1481 ± 93	5.63 ± 1.04	8.31 ± 0.67
Mugnoli	1504 ± 92	5.70 ± 1.01	9.19 ± 0.64
Significance [(1)]			
S	**	***	***
G	NS	NS	NS
S × G	NS	NS	NS

[(1)] Significance: *** and ** significant for $p \leq 0.001$ and $p \leq 0.01$, respectively; NS, not significant.

Unlike for FW and DW, the height was almost 3 cm greater in microgreens cultivated in the growth chamber those those cultivated in the greenhouse (Table 4). For the latter, microgreens grown under CTRL were 11.8% taller than those grown under the B and CTRL + B light treatments (Table 3). The shorter growth of the microgreens in the greenhouse compared to the growth chamber was due to the higher greenhouse PPFD values, which significantly influence microgreens development. This result is consistent with Jones et al. [21], who reported that an increase in PPFD from 100 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ resulted in a decrease in the hypocotyl height of *Brassica* microgreens while the fresh and dry weights increased. Our study revealed that that blue (B) light also strongly influences microgreens production (Table 3). Generally, only low-intensity blue light is needed in the light spectrum for fully functional photosynthesis [42]. Blue light serves as a growth regulator through its involvement in several critical plant responses, such as phototropism, photomorphogenesis, stomatal opening, chloroplast development, and leaf expansion [32]. Among others, B light also increases the transpiration rate [43], causing greater water consumption and lower fresh weight (Table 3) than under other light treatments. Our findings are, in fact, in line with previous studies in which it was reported that an increase in blue light negatively affects plant elongation and leaf area, inhibiting cell division and expansion [44]. Finally, the higher DW found for microgreens grown under B light, relative to CTL and CTRL + B, is consistent with the results obtained in previous studies conducted

on microgreens, which observed that B light increases the carotenoids [45], dry weight, and mineral content [28].

3.4. Total Polyphenols Content and Antioxidant Activity

The polyphenols content of the microgreens, expressed as mg of gallic acid·g^{-1} of dry weight (DW), was influenced by the light treatments (Table 5), and in particular, it was 18.4% higher in microgreens grown under CTRL with respect to those grown under monochromatic blue (Table 5). Polyphenols are a class of bioactive molecules with a wide range of beneficial effects (e.g., antioxidant, anticarcinogenic, prebiotic, antidiabetic activity [46–48]). Broccolo Natalino grown in the greenhouse showed 12.4% more polyphenols than Mugnoli grown in the same environment (Figure 6) while in the growth chamber, Mugnoli showed a 26% higher concentration than 'Natalino' cultivated under equal conditions (Figure 6). Coherent with these observations of the polyphenols content, when grown under greenhouse conditions, Broccolo Natalino showed a 9.4% higher antioxidant activity than Mugnoli (Figure 7) while under growth chamber conditions, Mugnoli showed a 44.6% higher antioxidant activity than Natalino (Figure 7). In the growth chamber CTRL and CTRL + B environments, Mugnoli showed a higher antioxidant activity than Natalino (Figure 8), but under monochromatic B, Natalino showed a 29% higher antioxidant activity (Figure 8). In summary, the study observations reveal that the greenhouse environmental conditions positively influenced the polyphenols content and the antioxidant activity of *Brassica* microgreens. Coherent with the experimental observations of the FW and DW production, the higher PPFD of the greenhouse compared to the growth chamber conditions resulted in an increased polyphenols content and antioxidant activity in both Broccolo Natalino and Mugnoli (Table 6). These results are consistent with those obtained by Samouliene et al. [24] and Kowalczewski et al. [49], where high values of PPFD resulted in increases in the polyphenol and antioxidant compounds in two different genotypes. Furthermore, important differences in the polyphenols content and antioxidant activity between Broccolo Natalino and Mugnoli were detected (Figures 6 and 7), especially in the growth chamber conditions. Except under monochromatic B light, Mugnoli had a higher polyphenols content and antioxidant activity than Broccolo Natalino, confirming the results obtained by Argentieri et al. [34] on the nutraceutical profile of Mugnoli. Comparing the two genotypes, however, the total polyphenols content of Broccolo Natalino was more susceptible to PPFD variation (Figure 6), suggesting that independently of the environmental growth conditions, PFFD, and light spectra, the total polyphenols content of Mugnoli is more stable than that of Broccolo Natalino. Importantly, the use of Mugnoli would permit the production of microgreens with a stable total polyphenols content, independently of the growing conditions and seasons. Finally, during the experiment, it was observed that monochromatic B light reduced the productive performances and nutritional contents of Broccolo Natalino and Mugnoli. These results are species specific, as demonstrated by Puccinelli et al. [50], who found that monochromatic blue light increased the antioxidant content and polyphenols profile of the seeds of microgreens from flaxseed (*Linum usitatissimum* L.). In fact, it was demonstrated that a higher blue light intensity had a bigger effect on the accumulation of photosynthetic and carotenoid pigments. Meanwhile, the accumulation of metabolites, which are not directly connected with light reactions, such as tocopherols, is influenced more by a lower blue light dosage [45]. Furthermore, Alrifai et al. [51] showed that Brassicaceae microgreens can be grouped into several types considering their phenolic content and sensitivity to lighting: (i) genotypes that show high blue and amber dose dependence, producing high total phenolics; (ii) genotypes that show moderate to high sensitivity to overall lighting but no clear dose dependence; and (iii) genotypes with various responses to lighting.

Table 5. Polyphenols content and antioxidant activity, expressed as dry weight (DW), of two brassica microgreens (Broccolo Natalino and Mugnoli) cultivated in a growth chamber with three different light spectra: Blue (B), Control (CTRL), and Control + Blue (CTRL + B). Means followed by different letters within a column indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

	Total Polyphenols	**Antioxidant Activity**
Light treatments (L)	mg of gallic acid·g^{-1} DW	µmol Trolox equivalents (TE)·g^{-1} DW
Blue	10.3 ± 0.44 b	25.0 ± 2.20
Control	12.2 ± 0.95 a	63.6 ± 7.89
Control + Blue	11.6 ± 1.05 ab	40.9 ± 8.63
Genotype (G)		
Broccolo Natalino	10.0 ± 1.1 b	38.2 ± 7.51
Mugnoli	12.7 ± 0.9 a	48.1 ± 7.66
Significance [1]		
L	***	***
G	**	**
L × G [2]	NS	**

[1] Significance: *** and ** significant for $p \leq 0.001$ and $p \leq 0.01$, respectively; NS, not significant. [2]: Significative interactions are reported in the figures.

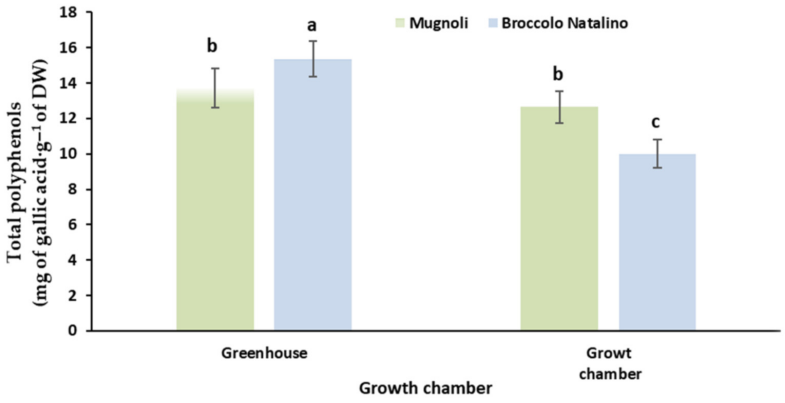

Figure 6. Polyphenols content, expressed as mg of gallic acid·g^{-1} of DW, of Mugnoli and Broccolo Natalino microgreens grown in a greenhouse and in a growth chamber. Vertical bars represent ± standard deviation of mean values. Letters on the columns indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

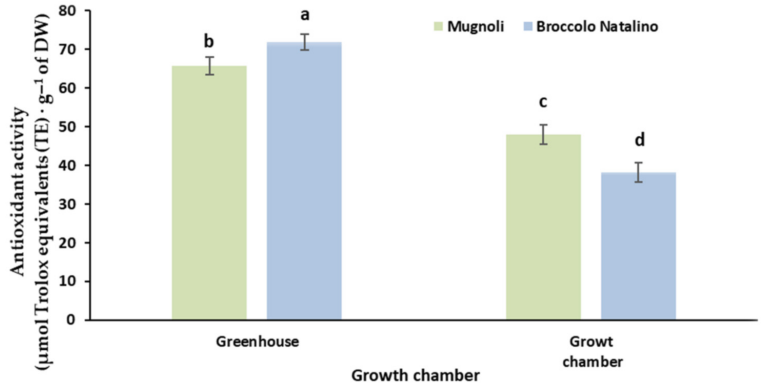

Figure 7. Antioxidant activity, expressed as µmol Trolox equivalents (TE) g^{-1} of DW, of Mugnoli and Broccolo Natalino microgreens grown in a greenhouse and in a growth chamber. Vertical bars represent ± standard deviation of mean values. Letters on the columns indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

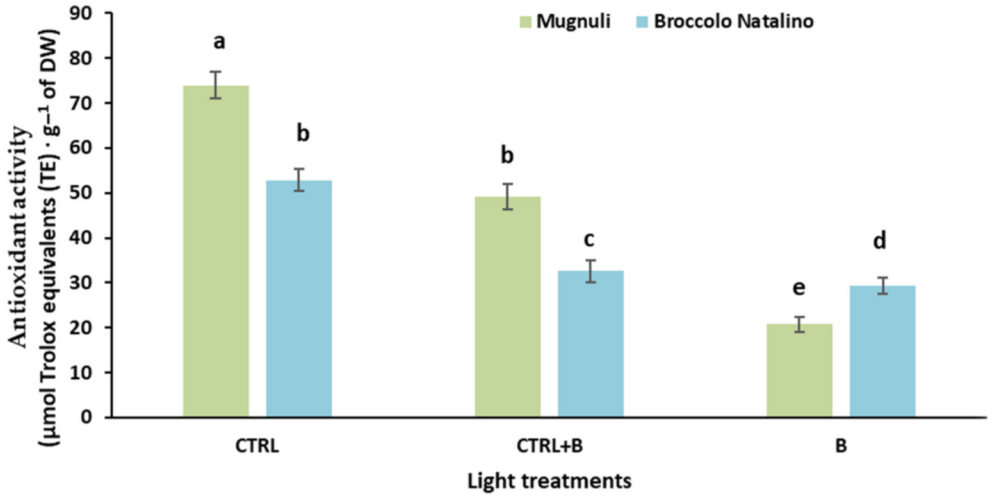

Figure 8. Antioxidant activity, expressed as µmol Trolox equivalents (TE)·g^{-1} of DW, of Mugnoli and Broccolo Natalino microgreens grown in a growth chamber under CTRL, CTRL + B, and B artificial light treatments. Vertical bars represent ± standard deviation of mean values. Letters on the columns indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

Table 6. Polyphenols content and antioxidant activity, expressed as dry weight (DW), of two brassica microgreens (Broccolo Natalino and Mugnoli) cultivated in a growth chamber and in a greenhouse with sunlight. Means followed by different letters within a column indicate significant differences according to Student–Newman–Keuls (SNK) test at $p = 0.05$.

	Polyphenols	**Antioxidant Activity**
Growth system (S)	mg of gallic acid·g^{-1} DW	µmol Trolox equivalents (TE)·g^{-1} DW
Growth chamber	12.1 ± 0.86	46.7 ± 0.33
Greenhouse	14.5 ± 0.45	68.8 ± 1.66
Genotype (G)		
Broccolo Natalino	11.3 ± 1.03	46.7 ± 6.5
Mugnoli	12.9 ± 0.95	52.5 ± 4.6
Significance [(1)]		
S	***	***
G	**	**
S × G [(2)]	**	***

[(1)] Significance: *** and ** significant for $p \leq 0.001$ and $p \leq 0.01$, respectively; NS, not significant. [(2)]: Significative interactions are reported in the figures.

4. Conclusions

In this study, two genotypes of *Brassica oleracea* L, var. *italica* Plenck were cultivated for microgreens production: 'Broccolo Natalino' and the landrace 'Mugnoli', with the latter being relatively unknown outside of a small geographic distribution in south-eastern Italy. Both genotypes showed high percentages of germination, obviating any need for the significant costs regarding pre-treatment procedures. The results also showed that Broccolo Natalino and Mugnoli can be successfully cultivated for microgreens production in both greenhouse and growth chamber environments, but some differences regarding seedling development, yield, and nutritional quality were observed. First, compared to the well-known Broccolo Natalino, it was observed that Mugnoli achieved the same or even better productive and qualitative performances, and could therefore be a valuable genetic source for the production of microgreens. In terms of the environmental conditions for cultivation, we observed that the higher temperatures and PPFD values of the greenhouse with respect to the growth chamber increased the microgreens biomass yield, reduced the microgreens crop cycle, and increased the polyphenols content and antioxidant activity

of the microgreens. The increasing yield and qualitative performance achieved under greenhouse conditions was greater for Broccolo Natalino than for Mugnoli. Finally, under growth chamber conditions, Mugnoli had higher nutritional value than 'Natalino', but for both genotypes, an increase in the percentage of blue radiation in the light spectrum caused a decrease in hypocotyl and cotyledons distension, resulting in reductions in the light absorption, fresh weight production, total polyphenols content, and antioxidant activity.

In conclusion, Mugnoli can be considered as a valuable genetic source for the production of microgreens, given its fast crop cycle, good fresh weight, and, compared to Broccolo Natalino, greater stability of its biochemical content under PPFD variations. Future research could evaluate other landraces regarding microgreens production, and assess whether the growth of microgreens under lighting with UV and/or different regions of the visible spectrum could serve in abiotic elicitation of phytochemicals synthesis.

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