

Article

Substrate–Genotype Interaction Influences Growth and Phytochemical Composition of Wild and Commercial Purslane (*Portulaca oleracea* L.) Microgreens

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Abstract

Purslane is highly suitable for intensive microgreen cultivation due to its rapid growth, high germination rate, and exceptional nutritional profile, including omega-3 fatty acids, essential vitamins, and minerals. While previous studies have mostly emphasized its basic composition, our research investigated additional functional traits, such as pigment accumulation and antioxidant activity. We also explored the cultivation potential of a wild purslane genotype (G2), naturally growing in the Botanical Garden of the Slovak University of Agriculture in Nitra, as a sustainable alternative to commercially available seeds (G1). This study examined how genotype and substrate interactions influence growth performance, pigment concentration, and antioxidant capacity in *Portulaca oleracea* microgreens. Both genotypes were grown on two different substrates: agar mixed with perlite and mineral wool. Although conserved DNA-derived polymorphism marker analysis revealed a high degree of genetic similarity between G1 and G2, significant phenotypic differences were observed. G1 exhibited greater fresh biomass and shoot length, making it more visually appealing for commercial microgreen production. In contrast, G2 showed higher dry matter content and enhanced accumulation of chlorophylls and carotenoids. Antioxidant activity, measured by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power) assays, peaked in G1 cultivated on agar–perlite mix. These findings emphasize the importance of selecting the right genotype–substrate combination to optimize both quality and productivity in microgreen systems.

Keywords: purslane; genotype; rock wool; agar; perlite



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1. Introduction

Portulaca oleracea L. (purslane), known by various names worldwide, such as pigweed (English), berdolaga (Spanish), porcelaine (French), and rudravanti (Hindi), is a cosmopolitan succulent plant [1,2].

Nutritionally, purslane is distinguished by its high content of biologically active compounds. It is one of the richest leafy vegetables in omega-3 fatty acids, particularly

α -linolenic acid [3]. It also contains significant levels of carotenoids (α -/ β -carotene, lutein, zeaxanthin), vitamins A, C, and B-complex, and essential minerals such as iron, zinc, potassium, calcium, and magnesium [4,5]. In addition, flavonoids (e.g., apigenin, quercetin) and phenolic acids (e.g., gallic, caffeic) contribute to its antioxidant profile, along with glutathione and novel alkaloids such as oleracein A and B [6].

Purslane is cultivated and consumed in many countries, both fresh and cooked. In parts of Africa, its seeds are ground into flour, while in countries like China, Turkey, and the Netherlands, it features in traditional recipes [7,8]. Fresh purslane is composed of 92.9% water and provides approximately 20 kcal per 100 g. On a dry matter basis, it contains around 36% dietary fiber, 13% protein, and 3.75% fat [9,10].

In recent years, purslane has attracted growing interest because of its distinctive characteristics and remarkable adaptability to harsh environments. This resilient and fast-spreading species can withstand drought, high salinity, and nutrient-poor soils, making it a promising candidate for sustainable farming practices and the development of functional foods.

Microgreens are tender, immature vegetables, grains, or herbs harvested 7 to 28 days after sowing. They are typically collected at the cotyledon stage or when the first one or two true leaves emerge [11]. Their increasing popularity is attributed to their intense flavor, rapid growth cycle, and health benefits. Due to their early harvest stage, microgreens often exhibit a higher concentration of bioactive compounds and micronutrients such as minerals, polyphenols, vitamins, and other antioxidants, making them valuable contributors to a healthy diet. Microgreens are considered a functional food and their inclusion in diets is rising globally due to increasing interest in healthy lifestyles and balanced nutrition [12,13].

The short growth cycle of these seedlings makes them particularly suited for urban cultivation. They require minimal inputs and can be produced sustainably even in limited spaces. Within the broader context of sustainable and urban agriculture, microgreen cultivation represents an efficient and innovative farming model with high added value, offering short growth cycles, low input requirements, and favorable economic returns. This makes it particularly suitable for small-scale growers, farmers in developing regions, and start-up entrepreneurs [14]. As global food security faces mounting challenges due to resource depletion and climate variability, microgreens also provide a practical strategy for dietary diversification and adaptation to shifting agricultural conditions [15].

A diverse array of organic and inorganic substrates, such as peat, sand, perlite, coconut fiber, and cellulose mats, can be used for cultivation of these plants. Essential qualities of a suitable substrate include lightness, porosity, airiness, low bulk density, and a pH between 5.5 and 7. Good drainage is also crucial to prevent waterlogging and ensure healthy root development [16,17]. The yield and content of bioactive compounds in microgreens are influenced by the choice of growing medium; therefore, substrate selection must consider the specific needs of the plant species [18].

Mineral wool is made from basalt and phenolic resins, and has a porosity of 97% and water-holding capacity of 82%. It is manufactured at a melting temperature of 1600 °C and available in various forms such as cubes, slabs, and mats. These substrates are sterile and offer excellent physical properties [19]. In addition to its structure, mineral wool contains essential nutrients like phosphorus (P) and potassium (K), and has superior water retention capabilities compared to other planting media that support photosynthesis and plant hydration [20].

Perlite, another commonly used substrate, has a low bulk density (0.05–0.3 g cm^{−3}), is sterile, and exhibits high water absorption with a pH range of 6.5–8.0 [19]. It enhances soil aeration and prevents compaction, contributing to healthier root development. Studies have demonstrated that incorporating perlite into growing media helps mitigate the negative

effects of high electrical conductivity (EC) and salinity, thereby improving overall plant growth and height [21].

Building on the growing recognition of purslane's exceptional nutritional profile and environmental resilience, its potential as a functional crop can be further enhanced through innovative cultivation approaches such as microgreen production. Despite extensive documentation of its bioactive composition and adaptability, studies exploring purslane in its microgreen form remain limited. The investigation of optimal cultivation conditions, particularly the influence of different substrates, may unlock new opportunities for sustainable, space-efficient, and nutrient-rich food production systems.

The aim of this study was to evaluate the effect of two different types of substrates (rock wool and agar–perlite mixture) on phytochemical, nutritional and yield parameters of two purslane genotypes (G1—commercially available genotype and G2—genotype of naturally occurring purslane) cultivated in controlled conditions. Additionally, the genetic profiles of conserved DNA-derived polymorphism of these purslane genotypes were determined and compared.

2. Materials and Methods

2.1. Plant Material

Two genotypes of purslane were used in this experiment (Figure 1). Genotype G1 is commercially available *Portulaca oleracea* (DeBolster, Epe, The Netherlands). The second tested genotype, G2, is purslane naturally occurring in the Botanical Garden of Slovak University of Agriculture (SUA) and the seeds were harvested in September 2023. For the analysis of genetic profiles of these two purslane genotypes, plants were cultivated from seeds in the demonstration garden of Institute of Horticulture in 2024 and samples were sent to The Institute of Plant and Environmental Sciences for genetic analysis.

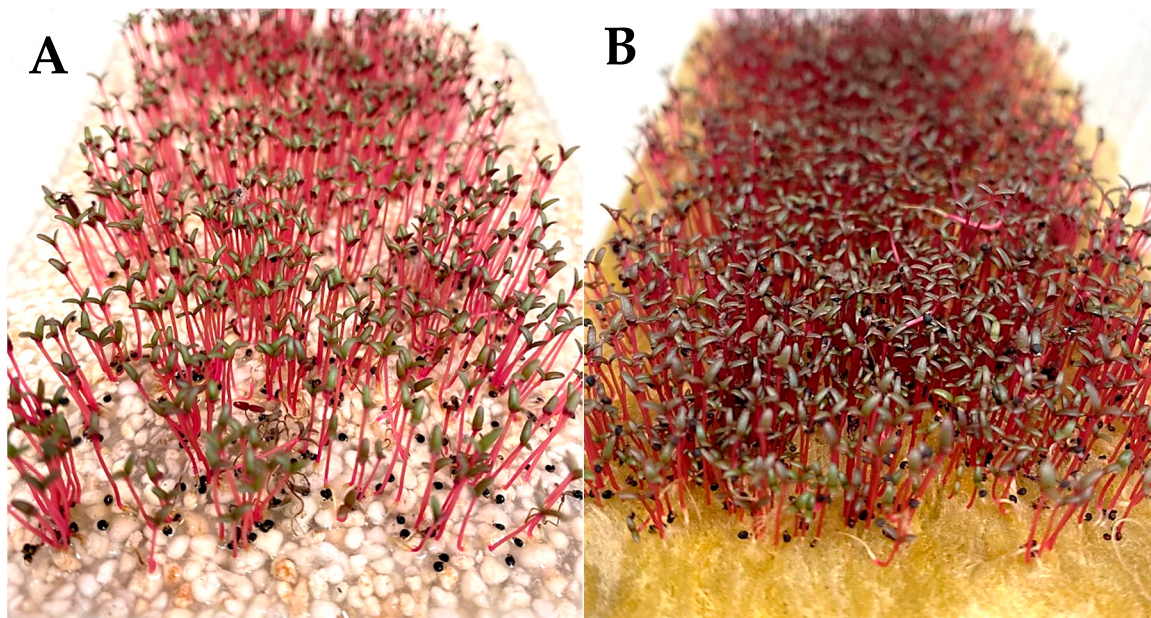


Figure 1. Purslane microgreens on substrates: (A)—genotype G1 on agar–perlite substrate, (B)—genotype G2 on rock wool substrate.

2.1.1. Germination Test

To ensure the quality of seeds from both purslane genotypes, a germination test was conducted in September 2024. Three test plastic Petri dishes (10 cm diameter) were prepared for each genotype, each containing filter paper (77 g.m^{−2}, pH 7, thickness 0.16/0.18 mm)

moistened with distilled water (1.5 mL) and 100 seeds arranged in a 10 × 10 grid. The Petri dishes were placed in a growing chamber with RH 70%, temperatures of 20/25 °C, and photoperiod of 16/8 h, suitable for purslane seed germination according to [22,23]. The number of germinated seeds was recorded every 12 h, and subsequently, a germination curve was plotted along with the calculation of mean germination rates for both purslane genotypes.

2.1.2. Plant Cultivation

Cultivation was conducted in the laboratory of AgroBioTech Research Centre, Slovak University of Agriculture in February 2025. From each genotype, 12 seed samples of 0.5 g were weighed. Subsequently, the seeds were soaked in 50 mL of deionized water for half an hour. Pre-soaking of the seeds was performed to improve germination. Seeds were sown in densities of 1 g per 210 cm² (47.62 g.m⁻²). For the substrate, two materials were used. The first was rock wool growing medium (GROWLAND, Púchov, Slovakia), 12 g per tray (tray area was 105 cm² with depth of 3 cm) with 150 mL of deionized water added to saturate the substrate, and the second one was a mixture of 230 mL of agar (1 g per 100 mL of water) (AGAR AGAR 700, Dipos, Banská Bystrica, Slovakia) and 23 g of perlite per tray (0–4 mm) (Perlit s.r.o., Šenov u Nového Jičína, Czech Republic). The soaked seeds were strained and placed on top of prepared substrate. All 24 prepared trays (6 repetitions of each variant) were placed into the growing chamber with phytotron system KK 750 FIT TOP+ (POL-EKO-APARATURA, Wodzisław Śląski, Poland), with forced air convection and chamber capacity of 749 L and controller microprocessor PID. The artificial lighting was provided through a 16/8 h photoperiod by fluorescent lamps, MASTER TL5 HO 39W/840 SLV/20 (Philips N.V., Eindhoven, Netherlands), Color Code 840 (4000 K), Luminous Flux 3500 lm. These lights are integrated in the growth chamber from the factory, with a maximum illumination power of 15,000 lux. Day/night temperatures and relative humidity were set at 25/20 °C and 70/70%.

2.1.3. Plant Harvest and Postharvest Processing

Microgreen plants were harvested when they reached the stage of cotyledon leaves (BBCH10) [24], which was 12 days after sowing. Trays were removed from the growing chamber and the plants were trimmed above the substrate with sharp scissors. For further analyses both fresh and dry biomass was used.

2.2. Genetic Analysis

Total genomic DNA was extracted by a commercial EliGene® Plant DNA Isolation Kit (Elisabeth Pharmacon, Brno, Czech Republic) following the instructions of the manufacturer. The concentration and quality of the isolated DNA were measured by NanoPhotometer™ P360 (Implen, Munich, Germany). All PCR reactions were performed by SureCycler 8800 thermocycler (Agilent, Santa Clara, CA, USA). For the CDDP (conserved DNA-derived polymorphism) methodology, 3 primer pairs were utilized, WRKY F + WRKY R1, WRKY F + WRKY R2, and WRKY F + WRKY R3, shown in Table 1 [25]. The temperature profile of the reactions was as follows: 95 °C—5 min, 35 cycles of 95 °C—45 s, 54 °C—45 s, 72 °C—1 min, and final polymerization 72 °C—5 min. The composition of the reaction mixture for was as follows: in a 10 µL volume: EliZyme Robust PCR Master Mix (2X), 400 nM of primers and 30 ng of DNA. The PCR reaction products were separated on 2% agarose gels stained with the intercalating dye GelRed® Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA). The fragments were separated at 115 V for 120 min. They were subsequently visualized using a BDA digital system 30 transilluminator (Analytik Jena, Jena, Germany). The obtained electropherogram images were processed using the GelAnalyzer (23.1) software

(www.gelalyzer.com, [accessed on 30th May 2025]). The amplicons were evaluated using a size standard (Hyperladder 50 bp Meridian Bioscience®, Cincinnati, OH, USA).

Table 1. Primer sequences.

Gene	Primer	Primer Sequence (5' to 3')	Length	Reference
WRKY	F	TGGCGSAAGTACGGCCAG	18	[25]
	R1	GTGGTTGTGCTTGCC	15	
	R2	GCCCTCGTASGTSGT	15	
	R3	GCASGTGTGCTCGCC	15	

2.3. Determination of Yield Parameters

The yield was measured immediately after the cutting of the monitored species. Firstly, the fresh weight (FW) was measured. For each variant, the biomass weight (in grams) was determined relative to 0.5 g of sown seeds or 105 cm². Six measurements were taken from each species and on each substrate. After drying at room temperature, when the plants were sufficiently dried (reached constant weight), the dry weight (DW) was measured, and the dry matter content was calculated. From each variant, 100 measurements were performed for shoot length and root length of the purslane microgreen plants.

2.4. Phytochemical and Nutritional Analysis

2.4.1. Chlorophyll *a* and *b* Content

An average sample of fresh plant material was prepared, and 1 g was weighed (in three repetitions). This sample was homogenized in acetone (Heidolph Silent Crusher M, Heidolph Instruments, Schwabach, Germany). After homogenization, the acetone extract was filtered using filter paper 84 g/m² (Munktell, Bärenstein, Germany). The prepared extract was quantitatively transferred into the volumetric flask (50 mL) and filled with acetone to the final volume. The intensity of extract color was measured at 649 nm for chlorophyll *a* and at 665 nm for chlorophyll *b* (spectrophotometer, PHARO 200, Spectoquant, Darmstadt, Germany). The zero position was controlled by pure acetone at a 750 nm wavelength. The possible dispersion value was deducted from individual absorbance values. The calculation of chlorophyll content was performed according to [26].

$$\text{Chlorophyll } a \text{ (mg kg}^{-1} \text{ FW)} = \frac{(11.64 \cdot A_{665} - 2.39 \cdot A_{649}) \cdot df \cdot 1000}{20 \cdot w}$$

$$\text{Chlorophyll } b \text{ (mg kg}^{-1} \text{ FW)} = \frac{(20.11 \cdot A_{649} - 5.18 \cdot A_{665}) \cdot df \cdot 1000}{20 \cdot w}$$

where:

A_{665} = absorbance at 665 nm;

A_{649} = absorbance at 649 nm;

df = dilution factor (if needed);

w = sample weight.

2.4.2. Total Carotenoid Content

An average sample of fresh plant material was prepared, and 1 g was weighed (in three repetitions). This weighed sample was further homogenized (Heidolph Silent Crusher M, Heidolph Instruments, Schwabach, Germany) in approximately 50 mL of acetone, and the prepared mixture was then filtered. The filtered extract was shaken twice in a separatory funnel with 10 mL of petroleum ether, during which the carotenes and other pigments transferred into the petroleum ether layer. The petroleum ether layers were transferred to a second separatory funnel and mixed with 50 mL of distilled water, into which acetone

residues were extracted. After the phases separated, the aqueous phase was discarded, and the petroleum ether layer was dried over anhydrous sodium sulphate (Na_2SO_4). The clarified petroleum ether extract was quantitatively transferred to a 50 mL volumetric flask. Absorbance was measured at a wavelength of 450 nm (spectrophotometer, PHARO 200, Spectoquant, Darmstadt, Germany), using petroleum ether as the control sample [26]. The calculation was performed according to [27].

$$TC \left(\text{mg kg}^{-1} \right) = \frac{(A_{450} \cdot V1 \cdot M \cdot 1000 \cdot 1000) \cdot V2}{V3 \cdot d \cdot \epsilon \cdot w}$$

where:

A_{450} = absorbance at 450 nm;

$V1$ = extraction volume (cm^3);

$V2$ = dilution volume (if applicable) (cm^3);

$V3$ = pipetted volume at dilution (cm^3);

d = cuvette path length (1 cm);

M = average molecular weight of carotenoids (548 g/mol);

ϵ = specific average absorbance of carotenoids (135,310 L/mol/cm);

w = sample weight (g).

2.4.3. Anthocyanin Content

Dried microgreens samples were homogenized (Heidolph Silent Crusher M, Heidolph Instruments, Schwabach, Germany) and methanolic extract was prepared (0.2 g dry plant matter and 10 mL of 80% methanol), shaking on an orbital shaker (Biosan, Riga, Latvia, PSU-10i) for two hours and filtered (filter paper 84 g/m², Munktell, Germany). Before the analysis, extracts were filtered through a Q-Max injection filter (0.22 mm, 25 mm; Frisette ApS, Knebel, Denmark). Prepared methanolic extracts were analyzed using HPLC-DAD (Agilent 1260 infinity high-performance liquid chromatography (Agilent Technologies, Waldbronn, Germany) with a diode array detector. For the analysis, standards of HPLC quality were used. Data were evaluated by AgilentOpenLab ChemStation (2.8) software for LC 3D Systems.

2.4.4. Antioxidant Activity

Antioxidant activity of purslane microgreen plants was evaluated in laboratories of the Agrobiotech research center in Nitra from the same methanolic extract prepared for HPLC analysis using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power) assays. The DPPH assay measures the scavenging ability of antioxidants against the stable purple DPPH radical, indicated by a decrease in absorbance (yellow color) after mixing with the sample. The ABTS method is based on the reduction in the blue-green ABTS⁺ radical cation by antioxidants, with absorbance measured at 750 nm after 30 min of incubation. The FRAP assay quantifies the reducing power of antioxidants through the conversion of the Fe^{3+} -TPTZ complex to its ferrous form, resulting in a blue color with maximum absorbance at 593 nm. All assays were performed in 96-well plates, using Trolox (Sigma Aldrich, Schnelldorf, Germany) as a standard and antioxidant activity was expressed as μmol Trolox Equivalent per 1 g of dry weight ($\mu\text{mol TE g}^{-1} \text{ DW}$).

2.4.5. Statistical Analysis

Statistical analysis was performed using Statgraphics Centurion XVII software (Stat-Point Inc., The Plains, VA, USA). The obtained results were evaluated by analysis of variance (ANOVA), and average values were compared using the Least Significant Differ-

ence (LSD) test at significance levels of $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***). In addition, correlation analysis was conducted using Microsoft Excel (Microsoft 365 Apps for enterprise), where the strength of the relationships between variables was expressed by the coefficient of determination (R^2), and the corresponding Pearson correlation coefficient (r) was calculated as the square root of R^2 .

3. Results and Discussion

3.1. Germination Test

Purslane seeds germinate very easily and rapidly under optimal conditions. According to [7], up to 90% of seeds can germinate within 24 h. In our case, we observed germination rates exceeding 90% for the cultivated purslane genotype G1 already after 12 h of testing. In contrast, the wild purslane seeds (G2) did not exceed the 90% germination threshold until 48 h. As shown in Figure 2, although wild purslane seeds germinated more slowly than the commercial seeds, the final average germination rate of G2 reached 99% after 108 h, compared to 97.3% for G1.

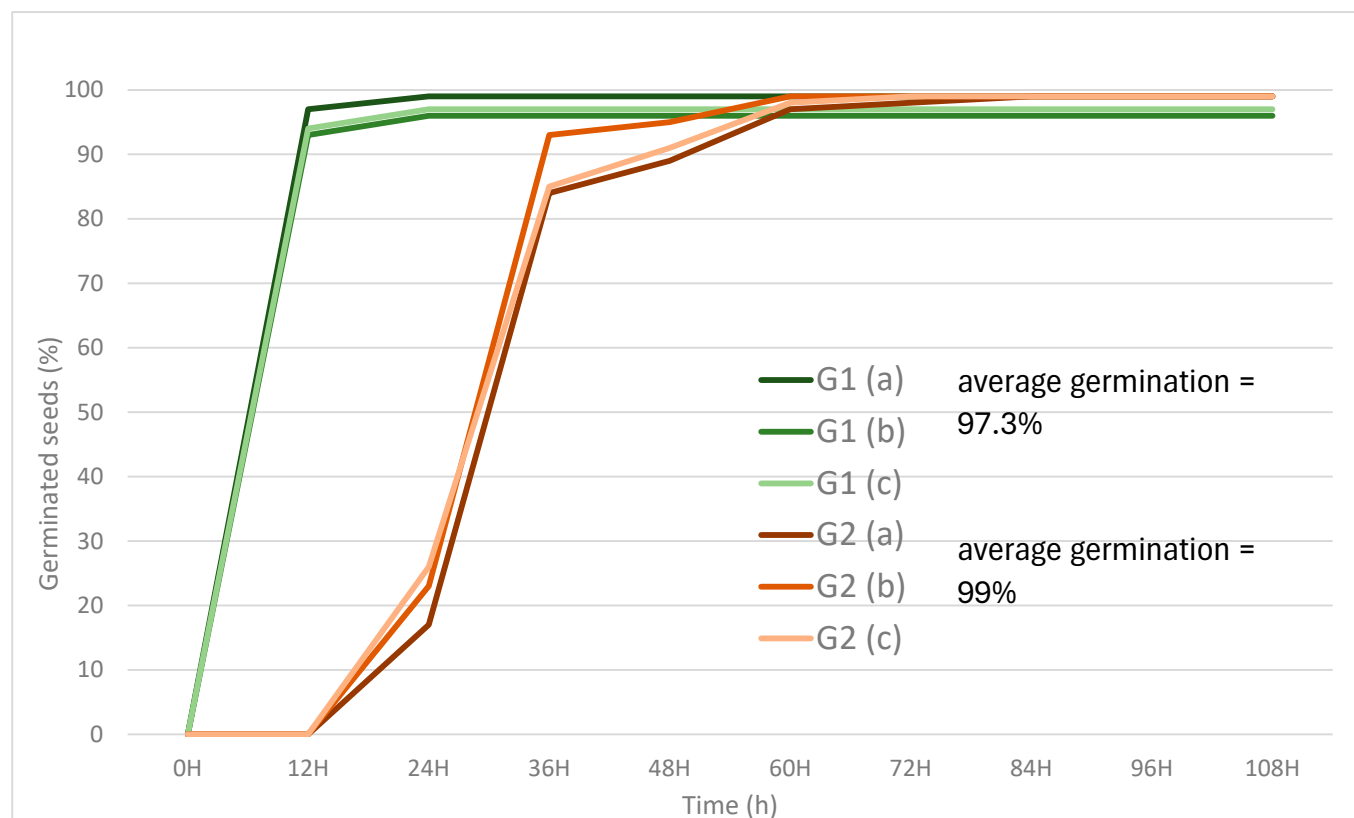


Figure 2. Results of germination test; different letters (a, b, c) depict repetitions. G1—commercial genotype, G2—wild genotype.

3.2. Genetic Analysis

Refs. [25,28,29] all used primer combinations of the CDDP technique, which showed the same profile for both of the analyzed genotypes, with the only difference in primer combination WRKY F + WRKY R3, where the amplified loci were more abundant in the genotype G2, but of the same length (Figure 3). A total of five distinctive amplicons were amplified in the primer combination WRKY F + WRKY R1, six amplicons for the primer combination WRKY F + WRKY R2, and nine amplicons for primer combination WRKY F + WRKY R3.

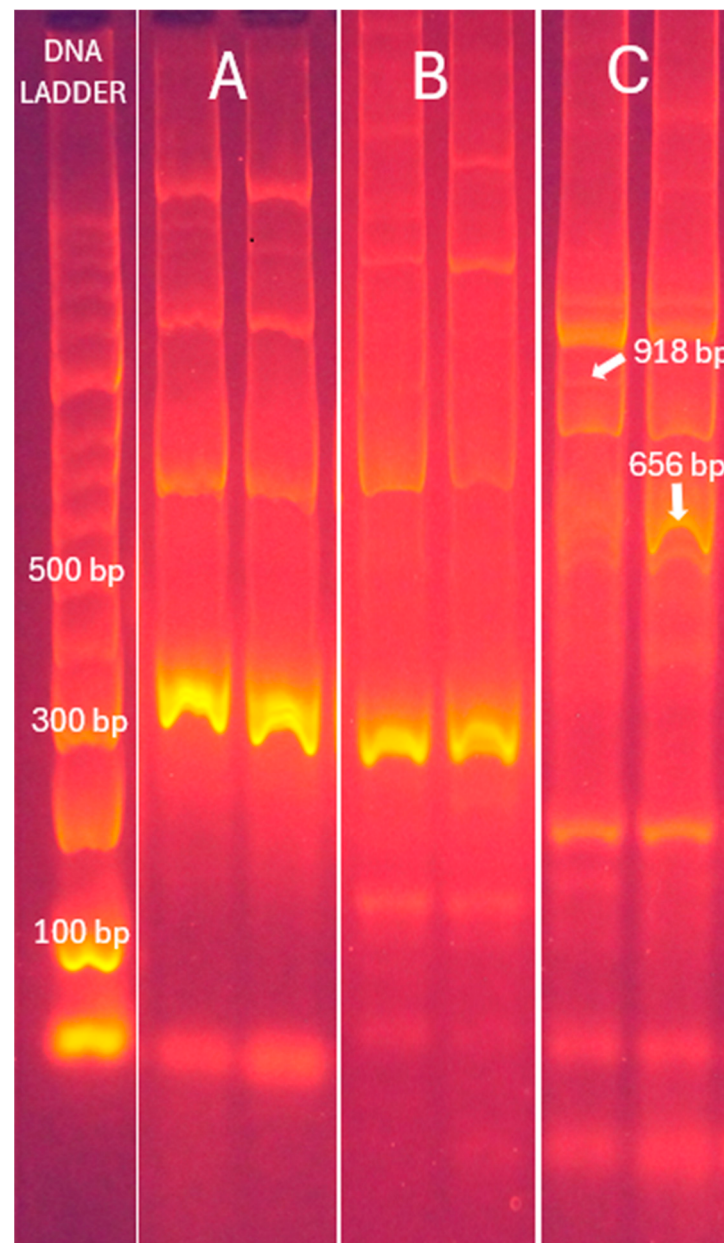


Figure 3. CCDP fingerprint of analyzed genotypes of *Portulaca oleracea* amplified by primer combinations (A) WRKY F + WRKY R1, G1 (left), G2 (right) (B) WRKY F + WRKY R2, G1 (left), G2 (right), and (C) WRKY F + WRKY R3, G1 (left), G2 (right).

Genetic diversity in plant species has been extensively examined through the analysis of DNA markers across species. Various molecular markers, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), sequence-characterized amplified regions (SCARs), and inter-simple sequence repeats (ISSRs), have been employed to evaluate the extent and patterns of genetic variation within and among plant populations [28]. CCDP markers were successfully used in characterization of genetic diversity among *Pistacia* species [29], where the findings showed high levels of polymorphism, enabling the differentiation between closely related species and genotypes.

Intraspecific stability of analyzed *Portulaca oleracea* genotypes was analyzed by the CCDP method, which was proven to be effective in distinguishing plant genotypes due to their reproducibility, genome-wide coverage, and independence from prior genomic sequence information. In a study by [25], CCDP markers were recognized as a reliable tool

in assessing genetic diversity in both cultivated and wild plant taxa. Here, using the CDDP, both of the analyzed genotypes were confirmed to share the same genetic background.

3.3. Yield Parameters

Results of our study (Table 2) show that yield and growth parameters were primarily determined by the main effects of substrate and genotype, with little evidence of mutual interaction. Regarding the yield and dry matter content, the substrate $\times$ genotype interaction was not significant. This indicates that both genotypes responded similarly to the substrate. G1 generally exhibited higher FW, while G2 showed a higher proportion of dry matter. These differences remained consistent regardless of the substrate. The substrate $\times$ genotype interaction effect on the measurements of the plants was also not significant. Genotype G1 was generally taller, and rockwool supported greater elongation, but this effect was consistent across both genotypes. G1 appeared to benefit more from agar–perlite in root growth, whereas G2 was overall shorter, and substrate-related differences were less pronounced for this genotype.

Table 2. Yield parameters of purslane microgreens and statistical analysis of effects of substrate and genotype and their interaction.

Genotype	Substrate	FW (g per 0.5 g Seeds)	Dry Matter Content (%)	Shoot Length (cm)	Root Length (cm)	Total Plant Length (cm)
G1	agar + perlite	1.93 $\pm$ 0.22 b	10.12 $\pm$ 1.10 ab	1.78 $\pm$ 0.11 c	3.02 $\pm$ 1.55 c	4.8 $\pm$ 1.53 c
G2	agar + perlite	1.23 $\pm$ 0.18 a	13.63 $\pm$ 0.41 c	0.92 $\pm$ 0.17 a	1.87 $\pm$ 0.65 b	2.79 $\pm$ 0.64 ab
G1	rock wool	2.49 $\pm$ 0.29 c	8.21 $\pm$ 0.86 a	1.91 $\pm$ 0.41 c	1.2 $\pm$ 0.52 ab	3.11 $\pm$ 0.40 b
G2	rock wool	2.02 $\pm$ 0.31 b	10.68 $\pm$ 0.52 b	1.17 $\pm$ 0.13 b	1.03 $\pm$ 0.30 a	2.2 $\pm$ 0.34 a
<i>p</i> -value (substrate)		0.0000 ***	0.0066 **	0.0165 *	0.0000 ***	0.0003 ***
<i>p</i> -value (genotype)		0.0000 ***	0.0027 **	0.0000 ***	0.0284 *	0.0000 ***
<i>p</i> -value (interaction substrate $\times$ genotype)		0.3227	0.3955	0.4353	0.0906	0.0536

G1—commercial genotype, G2—wild genotype; results are presented as mean values $\pm$ standard deviation, and the letters a, b, c indicate a significant difference within the parameter at $p \leq 0.05$, based on the LSD test, ANOVA. Statistically significant differences at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) (Statgraphics XVII).

The yield of purslane microgreen ranged between 1.23 g and 2.49 g per 0.5 g of seeds. The highest yield (FW) was measured in variant with G1 genotype purslane cultivated on rock wool. The highest dry matter content was present in the sample of the G2 purslane genotype cultivated on agar–perlite substrate (13.63%) compared to the lowest dry matter content present in G1 plants cultivated on rock wool (8.21%). Genotype G1 plants were generally longer, with longer shoots, roots, and total length. The shoot length of purslane microgreens ranged from 0.92 cm to 1.91 cm, with the shortest shoots observed in genotype G2 grown on agar–perlite and the longest in genotype G1 cultivated on rock wool. Root length varied more substantially, with the longest roots (3.02 cm) found in G1 microgreens grown on agar–perlite, while the shortest roots (1.03 cm) were recorded in G2 plants grown on rock wool. Consequently, the total plant length ranged from 2.20 cm (G2 on rock wool) to 4.80 cm (G1 on agar–perlite).

According to [30], the yield of purslane microgreens cultivated on peat-based substrate was 1.19 kg of fresh weight per square meter and the dry matter content was 6.23%. The hypocotyl was around 4.35 cm long. Compared to our experiment, where the plants were harvested at the cotyledon leaf stage 12 days after sowing, in the experiment from 2021, the microgreens were harvested 23 days after sowing at the emergence of the first true leaf. A similar length of the growing period was executed in the experiment of [31]. The plants

were harvested 14 days after sowing of 0.75 g of seeds on grow mats. The yield of purslane microgreens was 1.93 g per container, and the dry matter content was 11.3%; both metrics match our results.

In the experiment of [32], purslane microgreens were cultivated on a jute map in a growth chamber with alternating day/night temperatures of 25 °C/20 °C and a constant relative humidity (80%), and harvested 14 days after sowing. The yield of fresh weight was 0.638 kg FW m⁻² and the dry matter content was 5.29%. The experiment conducted by [33] focused on cultivation of purslane microgreens on cellulose growth viscose pads. Results show the yield values of 1.51 kg m⁻². Purslane microgreens cultivated on peat substrate had a yield of approximately 1 kg of microgreens per m² after 29 days of cultivation [34]. These plants had dry matter content of 87.4 g kg⁻¹ fresh weight, comparable to our experiment's results.

Figure 4 represents the relationship between root length and shoot length. In conventional cultivation of mature plants grown in soil, it is commonly observed that a larger root system is associated with more extensive aboveground growth. To evaluate whether this relationship also applies to microgreens, we conducted a correlation analysis between shoot length and root length. However, given the extremely small size and early developmental stage of microgreens, the correlation was negligible, with a correlation coefficient r of only 0.064. This suggests that, unlike mature plants, the balance between root and shoot growth in microgreens may be influenced more by genetic or environmental variability than by direct proportionality.

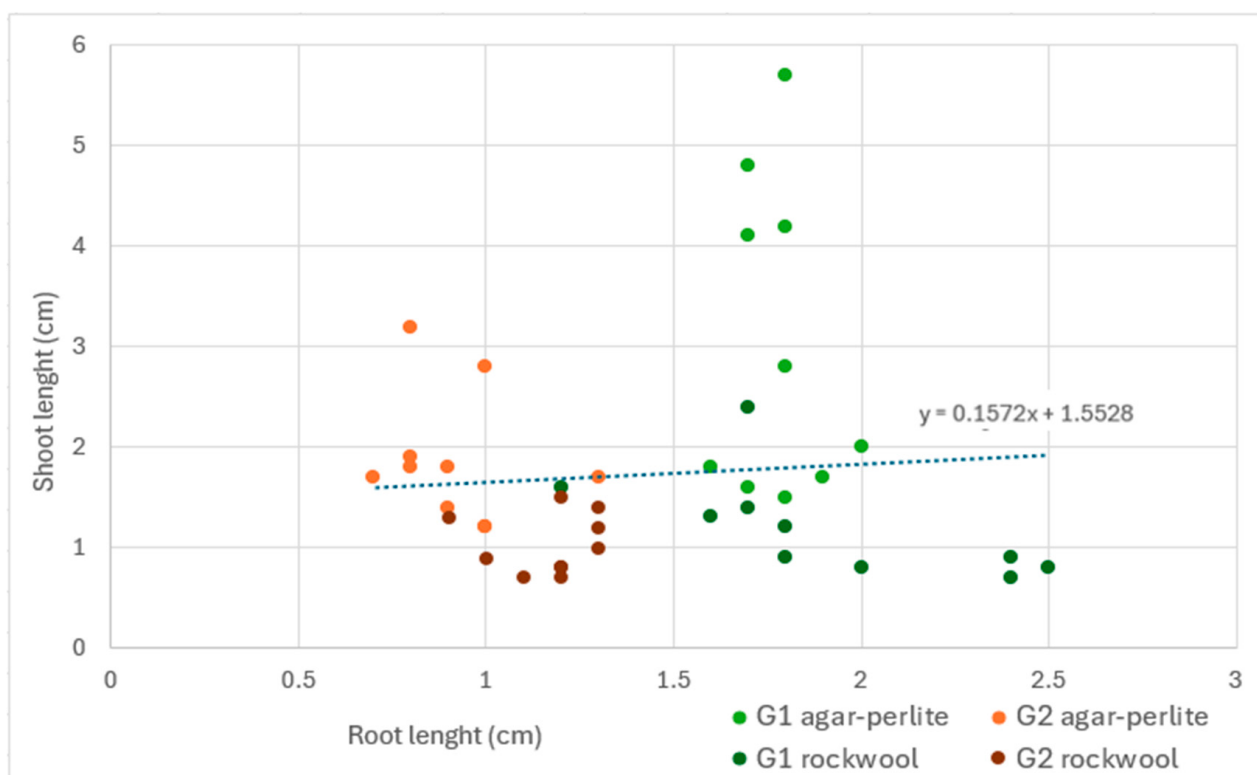


Figure 4. Relationship between shoot and root length of purslane microgreens. G1—commercial genotype, G2—wild genotype.

3.4. Phytochemical and Nutritional Parameters

3.4.1. Chlorophyll *a* and *b* Content

Chlorophyll is a green pigment present in plants, algae, and some bacteria, and is essential for photosynthesis, where it facilitates the absorption of light energy and its

transformation into chemical energy. Additionally, chlorophyll content serves as a valuable indicator for monitoring various stages of plant growth, physiological performance, and overall development [35,36]

Based on the statistical analysis, a significant substrate $\times$ genotype interaction was detected, indicating that the effect of substrate on chlorophyll content differed between genotypes. G2 accumulated more chlorophyll *a* and *b* on agar+perlite than on rock wool, whereas G1 plants contained more chlorophyll *a* when cultivated on rock wool.

Results of our study (Table 3) show that the highest content of both chlorophyll *a* and chlorophyll *b* was in purslane microgreens of genotype G2 cultivated on agar–perlite substrate (610.05 mg chl *a* kg^{−1} FW and 229.07 mg chl *b* kg^{−1} FW). The lowest chlorophyll content was evaluated in G2 plants cultivated on rock wool (452.84 mg chl *a* kg^{−1} FW and 154.79 mg chl *b* kg^{−1} FW). The data suggest that, for the G2 genotype, the agar–perlite substrate is more suitable for chlorophyll synthesis compared to rock wool. A potential cause of this is differences in water retention, aeration, and nutrient availability between the two substrates. The agar–perlite mixture may have provided more stable moisture levels and better root support, enhancing nutrient uptake efficiency.

Table 3. Content of plant pigments in purslane microgreens and statistical analysis of effects of substrate and genotype and their interaction.

Genotype	Substrate	Chlorophyll <i>a</i> (mg kg ^{−1} FW)	Chlorophyll <i>b</i> (mg kg ^{−1} FW)	Total Carotenoids (mg kg ^{−1} FW)	Cyanidin-3-G (μg g ^{−1} DW)
G1	agar + perlite	458.20 ± 24.30 a	176.55 ± 7.72 b	196.23 ± 0.56 b	26.13 ± 0.28 c
G2	agar + perlite	610.05 ± 42.41 b	229.07 ± 2.40 c	269.79 ± 0.57 d	15.93 ± 0.18 b
G1	rock wool	503.42 ± 3.65 a	175.42 ± 3.70 b	187.58 ± 0.34 a	16.19 ± 0.63 b
G2	rock wool	452.84 ± 21.91 a	154.79 ± 5.27 a	235.08 ± 0.38 c	13.22 ± 0.48 a
<i>p</i> -value (substrate)		0.2991	0.0734	0.0138 *	0.0006 ***
<i>p</i> -value (genotype)		0.3429	0.3831	0.0001 ***	0.0005 ***
<i>p</i> -value (interaction substrate $\times$ genotype)		0.0060 **	0.0006 ***	0.0000 ***	0.0000 ***

G1—commercial genotype, G2—wild genotype; results are presented as mean values $\pm$ standard deviation, and the letters a, b, c, d indicate a significant difference within the parameter at $p \leq 0.05$, based on the LSD test, ANOVA. Statistically significant differences at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) (Statgraphics XVII).

In the experiment of [33], the total chlorophyll content ranged from 800 to 1400 mg kg^{−1} FW. Total chlorophyll content in purslane microgreens was evaluated by [30] with results of 635.9 ± 17.5 mg kg^{−1} FW. According to [32], the total content of chlorophylls in purslane microgreens is 0.629 mg g^{−1} FW. Both of these experiments are comparable to our results.

Chlorophyll *a/b* Ratio

All measured *a/b* ratios ranged from 2.60 to 2.92 (Figure 5), which, according to [37], fall within the typical range for C3 plants. However, the species analyzed in this study was *Portulaca oleracea*, a well-known C4 plant, for which a typical *a/b* ratio ranges from 3.4 to 4.5. The relatively lower values observed may be explained by the fact that the microgreens were harvested at a very early developmental stage, when C4 characteristics may not yet be fully expressed. Higher *a/b* ratios are generally associated with adaptation to more intense light conditions, due to a greater proportion of chlorophyll *a*. The highest *a/b* ratios were observed in both genotypes grown on rockwool substrate.

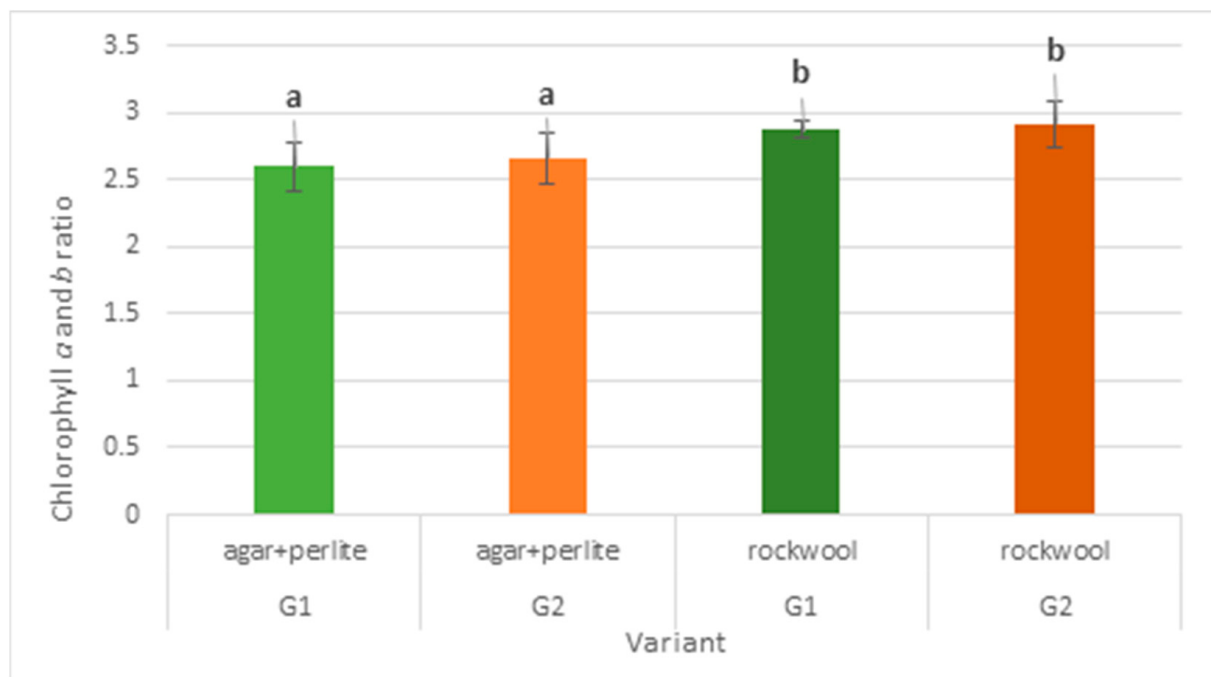


Figure 5. Chlorophyll *a/b* ratio in tested variants. G1—commercial genotype, G2—wild genotype; the letters a and b indicate a significant difference at $p \leq 0.05$, based on the LSD test, ANOVA (Statgraphics XVII).

3.4.2. Total Carotenoid Content

Carotenoids are among the most extensively studied natural pigments. These fat-soluble compounds are widely distributed in fruits and vegetables, where they contribute to orange, yellow, and red coloration. Approximately 600 carotenoids have been identified, which can be structurally classified into two main groups: carotenes, composed solely of carbon and hydrogen atoms, and xanthophylls, which also contain oxygen atoms. The concentration of carotenoids in plant-derived foods is influenced by multiple factors, including cultivar, maturity stage, climatic and environmental conditions, harvest time, and postharvest handling such as processing and storage [38]. In our experiment, the total carotenoid content was significantly affected by the substrate $\times$ genotype interaction, indicating that substrate effects differed between genotypes. G2 accumulated more carotenoids on agar+perlite than on rock wool, whereas G1 showed a slight decrease on rock wool compared with agar+perlite. Using the spectrophotometric analysis, we evaluated that total carotenoid content of purslane plants ranged from $187.58 \text{ mg kg}^{-1} \text{ FW}$ (G1 genotype cultivated on rock wool) to $269.79 \text{ mg kg}^{-1} \text{ FW}$ (G2 genotype cultivated on agar-perlite mixture). These results suggest that G2 is more responsive to substrate composition in terms of carotenoid biosynthesis, potentially reflecting differences in light capture, nutrient availability, or stress signaling. The genotype-specific response highlights that selecting an appropriate substrate can optimize carotenoid accumulation. Table 3 shows all values and statistical analysis of substrate and genotype influence on the content of total carotenoids in purslane microgreens.

In the experiment conducted by [30], the lutein and β -carotene contents were evaluated separately, and the results show that purslane microgreens contain $25.99 \text{ }\mu\text{g}$ of lutein per g of dry weight and $197.4 \text{ }\mu\text{g}$ of β -carotene per g of DW. According to [39], purslane microgreens contain 107 mg of lutein and 254.3 mg of β -carotene per kilogram of dry weight. Ref. [32] state that purslane microgreens in control variant contain 0.101 mg of total carotenoids per 1 g of fresh weight, which is lower compared to our experiment.

3.4.3. Anthocyanin Content

Anthocyanins are water-soluble polyphenolic pigments belonging to the flavonoid class of secondary plant metabolites. They are widely distributed in most vascular plants, where they are responsible for the vibrant pink, red, purple, and blue coloration observed in fruits, vegetables, and flowers. Beyond their visual appeal, anthocyanins play essential roles in plant physiology, including functions related to reproduction, ecophysiological adaptation, and defense mechanisms against biotic and abiotic stresses [40]. In our experiment we evaluated that the content of cyanidin-3-glucoside was significantly influenced ($p < 0.001$) by substrate, genotype, and also the interaction of substrate $\times$ genotype. We also evaluated that purslane microgreens contain cyanidin-3-glucoside as a main anthocyanin. The value (Table 3) of cyanidin-3-G in purslane microgreens was highest in G1 genotype plants cultivated on agar-perlite substrate ($26.13 \mu\text{g g}^{-1}$ DW) and lowest in G2 plants cultivated on rock wool ($13.22 \mu\text{g g}^{-1}$ DW). The pattern suggests that G1 is particularly sensitive to substrate composition for anthocyanin biosynthesis, possibly due to differences in nutrient availability, water retention, or microenvironmental stress signals provided by agar-perlite mixture. The stronger anthocyanin response in G1 may indicate a genotype-specific capacity for secondary metabolite accumulation under favorable substrate conditions, highlighting the importance of substrate selection in optimizing anthocyanin content in different genotypes.

In the experiment of [41], they evaluated the anthocyanin content in 15 accessions of mature purslane plants. The highest cyanidin-3-G content was $0.80 \mu\text{g g}^{-1}$ DW for purslane PA 3. According to [42], the content of anthocyanins in mature purslane leaves is $1.08 \mu\text{g}$ of cyanidin 3-glucoside equivalent per g of dry weight, and in stems the value of cyanidin 3-glucoside equivalent is $4.61 \mu\text{g g}^{-1}$ dry weight. These values were considerably lower than those observed in our experiment, which aligns with the widely recognized trend that microgreens typically contain higher concentrations of bioactive compounds, such as phenolics, anthocyanins, vitamins, and minerals, compared to mature plants [43].

3.4.4. Antioxidant Activity

Each of the methods used (DPPH, ABTS, and FRAP) interact differently with antioxidant compounds based on their chemical properties. The ABTS assay is capable of measuring both hydrophilic and lipophilic antioxidants and its flexible pH range. The DPPH assay, in contrast, is more suitable for assessing lipophilic antioxidants. Its limited water solubility makes it less effective for hydrophilic compounds, and its performance is sensitive to environmental factors such as light, pH, and oxygen. The FRAP assay is primarily responsive to hydrophilic antioxidants due to the aqueous-phase affinity of the Fe^{3+} -TPTZ complex used in the reaction. Its reduced sensitivity to lipophilic compounds presents a limitation in capturing the full antioxidant potential of lipid-soluble substances [44]. Given that *Portulaca oleracea* contains a complex mixture of both hydrophilic (e.g., phenolic acids, flavonoids) and lipophilic (e.g., carotenoids, tocopherols) antioxidants, applying all three assays provides a more comprehensive assessment of its antioxidant capacity.

For DPPH and ABTS, the substrate $\times$ genotype interaction was significant, with differences between substrates observed in only one genotype (G1), while genotype G2 remained stable. This indicates that antioxidant activity is determined by the combination of genetic traits and nutrient availability from the substrate.

The three antioxidant assays showed clear differences between genotypes and substrates. The highest antioxidant activity was recorded in the commercial genotype G1 grown on agar-perlite, reaching $62.26 \mu\text{mol TE g}^{-1}$ DW (DPPH), $97.64 \mu\text{mol TE g}^{-1}$ DW (ABTS), and $44.37 \mu\text{mol TE g}^{-1}$ DW (FRAP). This combination demonstrated the strongest antioxidant potential among all tested variants.

In contrast, G1 grown on rock wool showed the lowest antioxidant activity in all three parameters, highlighting the significant influence of substrate on antioxidant synthesis. The wild genotype G2 showed more consistent results, with slightly higher ABTS values on rock wool ($93.11 \mu\text{mol TE g}^{-1} \text{ DW}$) than on agar–perlite. All values are depicted in Table 4, including the statistical analysis of substrate and genotype effects on the antioxidant activity.

Table 4. Results of antioxidant activity of purslane microgreens and statistical analysis of effects of substrate and genotype and their interaction.

Genotype	Substrate	DPPH ($\mu\text{mol TE g}^{-1} \text{ DW}$)	ABTS ($\mu\text{mol TE g}^{-1} \text{ DW}$)	FRAP ($\mu\text{mol TE g}^{-1} \text{ DW}$)
G1	agar + perlite	$62.26 \pm 3.00 \text{ b}$	$97.64 \pm 1.19 \text{ c}$	$44.37 \pm 3.78 \text{ c}$
G2	agar + perlite	$55.93 \pm 4.49 \text{ a}$	$89.36 \pm 6.69 \text{ b}$	$41.89 \pm 2.30 \text{ bc}$
G1	rock wool	$54.25 \pm 2.19 \text{ a}$	$83.56 \pm 0.99 \text{ a}$	$35.84 \pm 1.44 \text{ a}$
G2	rock wool	$56.86 \pm 4.11 \text{ a}$	$93.11 \pm 2.47 \text{ b}$	$39.0 \pm 1.82 \text{ b}$
<i>p</i> -value (substrate)		0.0527	0.0455 *	0.0001 ***
<i>p</i> -value (genotype)		0.2926	0.7967	0.7749
<i>p</i> -value (interaction substrate × genotype)		0.0060 **	0.0000 ***	0.0121 *

G1—commercial genotype, G2—wild genotype; results are presented as mean values $\pm$ standard deviation, and the letters a, b, c, indicate a significant difference within the parameter at $p \leq 0.05$, based on the LSD test, ANOVA. Statistically significant differences at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***) (Statgraphics XVII).

These findings suggest that G1 combined with agar–perlite is a promising choice for growing functional microgreens with high antioxidant value, while G2 offers stable antioxidant properties across different substrates.

The FRAP method was used in experiment [32] and the values for purslane microgreens were $28.0 \mu\text{mol FE(II) g}^{-1}$ fresh weight. The antioxidant capacity was expressed in mg of reduced DPPH per kilogram of fresh weight in [33]. For purslane microgreens cultivated on viscose pads, the result was $175 \text{ mg red. DPPH kg}^{-1} \text{ FW}$). In the experiment of [39], they evaluated lipophilic antioxidant activity by the ABTS method, and the values ranged from 80.47 to $94.49 \text{ mmol TE } 100 \text{ g}^{-1} \text{ DW}$ based on the color of lighting used during purslane cultivation. All three analytical methods were used in the experiment of [30], and the results are comparable to those of our study, with AOA values of $45.4 \text{ mmol TE kg}^{-1} \text{ DW}$ (DPPH), $82.83 \text{ mmol TE kg}^{-1} \text{ DW}$ (ABTS), and $45.40 \text{ mmol TE kg}^{-1} \text{ DW}$ (FRAP).

Correlation Between Antioxidant Activity Methods

Since assays used in this experiment are based on different chemical principles—radical scavenging in DPPH and ABTS, and electron transfer capacity in FRAP—correlation analysis was conducted to assess how closely the results from these methods align (Figures 6–8).

The strongest correlation coefficient (r) was observed between DPPH and ABTS, with $r = 0.779$, indicating a strong positive correlation. This suggests that these two assays measured antioxidant capacity in a very similar manner under the conditions tested. In contrast, the correlation between ABTS and FRAP yielded $r = 0.626$, and DPPH and FRAP showed $r = 0.579$, both of which represent moderate positive correlations. These results imply a meaningful, but not particularly strong, linear relationship between the methods. While the assays are related and tend to follow the same trend, approximately 60% of the variation remains unexplained. This indicates that other factors—such as the specific chemical nature of antioxidants present in the samples—may influence the results

differently across assays. Overall, the correlation analysis confirms that while the three methods were consistent to a certain extent, they should not be considered interchangeable, and their combined use offers a more comprehensive evaluation of antioxidant activity.

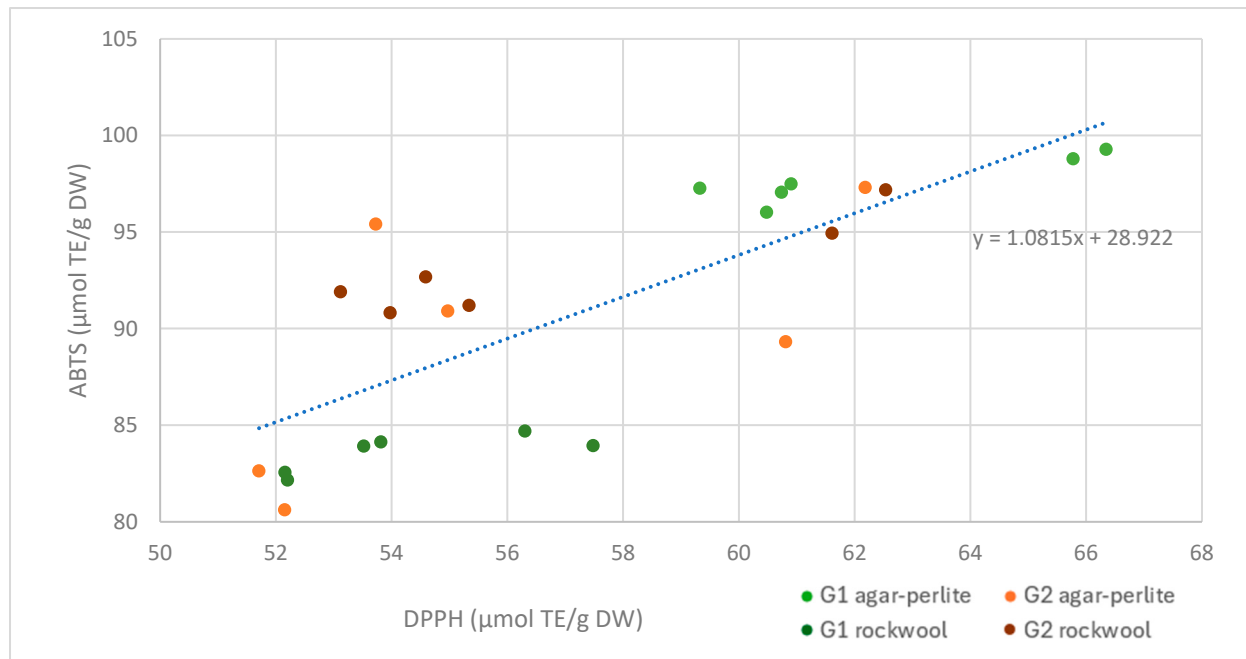


Figure 6. Relationship between DPPH and ABTS methods. G1—commercial genotype, G2—wild genotype.

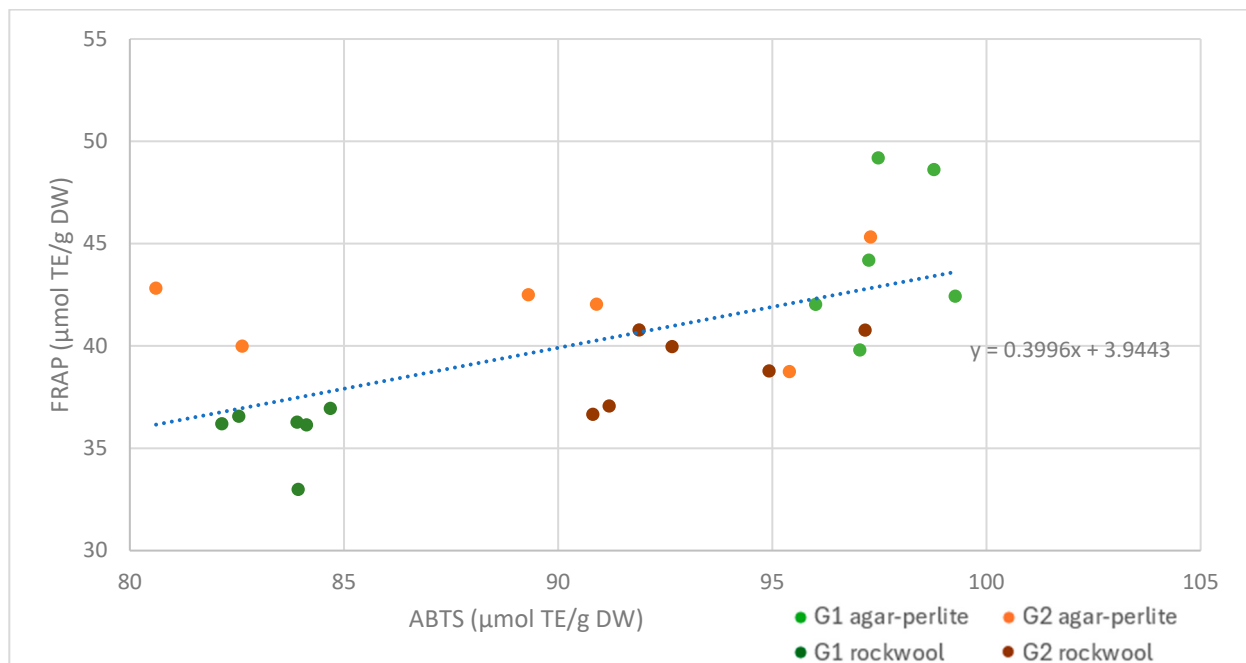


Figure 7. Relationship between ABTS and FRAP methods. G1—commercial genotype, G2—wild genotype.

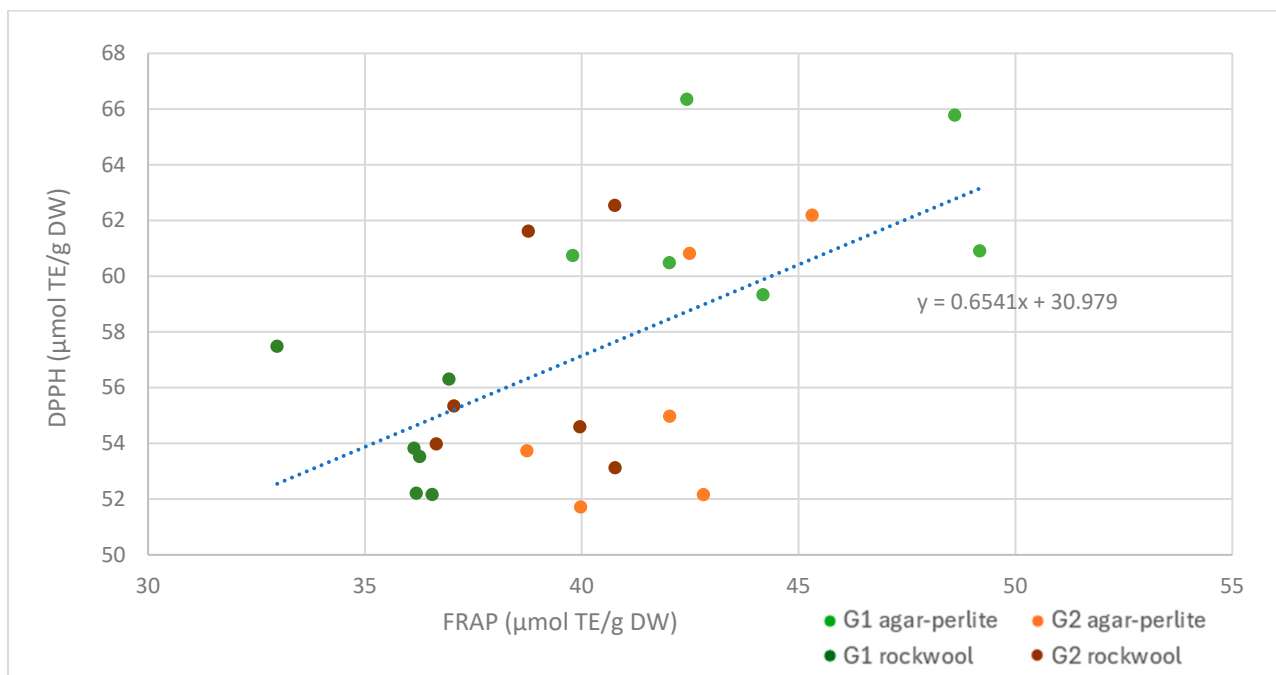


Figure 8. Relationship between DPPH and FRAP methods. G1—commercial genotype, G2—wild genotype.

4. Conclusions

The findings of this study clearly demonstrate that both genotype selection and cultivation substrate play a crucial role in determining the yield and functional composition of purslane (*Portulaca oleracea*) microgreens.

The two tested genotypes of purslane differed in several key traits. The commercial genotype G1 achieved the highest fresh weight (up to 2.49 g per 0.5 g of seeds) and shoot length, making it ideal for producing visually appealing microgreens. The combination of G1 and agar with perlite also showed the strongest antioxidant activity across all three assays (DPPH, ABTS, FRAP).

In contrast, the wild genotype G2 was notable for its higher dry matter content (up to 13.63%) and significantly greater accumulation of photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids), especially when cultivated on agar with perlite. This highlights its potential for use in functional foods or for extracting bioactive compounds.

Agar with perlite supported stronger root and pigment development, while rock wool favored fresh shoot biomass. These results suggest that a targeted selection of genotypes and substrate can effectively optimize specific microgreen traits.

Growth parameters were primarily determined by the main effects of substrate and genotype, with minimal interaction. In contrast, pigment accumulation and antioxidant activity were highly genotype-specific and strongly influenced by substrate × genotype interactions. These results indicate that optimizing yield can rely on either factor independently, whereas enhancing bioactive compound content requires careful selection of specific genotype–substrate combinations.

Genetic analysis using CDDP markers confirmed high genetic similarity between the two genotypes, despite notable phenotypic differences. The low polymorphism suggests a narrow genetic background or possible shared origin. This underlines the importance of evaluating underutilized wild sources for their cultivation potential.

Overall, these findings provide valuable guidance for improving the sustainable cultivation of purslane microgreens while enhancing their nutritional and functional value.

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