Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 1, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PHYSIOLOGICAL, BIOCHEMICAL AND AGROPRODUCTIVE CHARACTERISTICS OF HEMP MICROGREENS IN DIFFERENT GROWING ENVIRONMENTS

Lorena-Diana POPA[1], Gabriel-Ciprian TELIBAN[2], Ioana BUȚERCHI[2], Marian BURDUCEA[2, 3], Simona-Florina ISTICIOAIA[1], Florin NENCIU[4], Alexandra LEONTE[1], Alexandra-Andreea BUBURUZ[1], Andreea-Sabina PINTILIE[1], Ilie BODALE[2], Alexandru COJOCARU[2], Andrei LOBIUC[5], Vasile STOLERU[2]

[1]Agricultural Research and Development Station Secuieni-Neamt, 371 Principala Street, Secuieni, Neamt, Romania
[2]"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iasi, Romania
[3]"Al.I. Cuza" University of Iasi, 11 Carol I Blvd, Iasi, Romania
[4]National Institute of Research-Development for Machines and Installations Designed for Agriculture and Food Industry - INMA Bucharest, 6 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[5]"Ştefan cel Mare" University of Suceava, 13 University Street, Suceava, Romania

Corresponding author email: gabriel.teliban@iuls.ro

Abstract

Microgreens represent a healthy alternative in nutrition, due to their high nutritional value and unique sensory characteristics. The light, the temperature or the density influences the photosynthetic and metabolic activity of microgreens, having a beneficial effect on their nutritional quality. In 2023, an experiment was carried out with microgreens from a hemp variety at IULS Iasi. This explored two growing environments, controlled versus uncontrolled (growth chamber versus window) and seven different seeding densities ranging from 40 to 280 microgreens/100 cm^2. The results revealed that the variant with 280 microgreens/100 cm^2 in the growth chamber recorded the highest fresh matter of 12.864 g/100 cm^2, while the variant with 40 microgreens/100 cm^2 in the growth chamber presented the highest content of chlorophyll pigments (13.1 CCI). The highest value of vitamin C (58.0 mg/100 g product) was found in D160 variant and the highest content in total soluble solids (4.33°Bx) belongs to the D200 variant, both from the growth chamber. Results underline the importance of selecting appropriate growth conditions and seeding densities for optimizing the qualitative and quantitative properties of hemp microgreens.

Key words*: microgreens, hemp, growth chamber, density, qualitative properties.*

INTRODUCTION

Amidst a rising global concern for human well-being and environmental impact, consumers are increasingly seeking out nutritious and eco-friendly food options. This trend has spurred research into foods that are both nutrient-rich and easy to cultivate (Colantuoni et al., 2016; Caracciolo et al., 2019; Caracciolo et al., 2020). Thus, there has been a significant increase in interest among people for consuming microgreens, which are tender and edible young plants of vegetables, herbaceous plants, cereals, aromatic herbs or even wild species (Xiao et al., 2016; Sehrish et al., 2023). They are harvested 7-21 days or even 28 days after germination, depending on the species/variety and the cultivation conditions, after the formation of the first or even the second pair of true leaves (Rouphael et al., 2021; Sehrish et al., 2023).

Microgreens can be considered functional foods, which are consumed especially in a non-thermally processed form, thus reducing the side effects of processing and leading to a greater availability of the impressive phytochemical profile: phenolic compounds, carotenoids, vitamins, amino acids, macro- and microelements etc. (Caracciolo et al., 2020; Keutgen et al., 2021; Rouphael et al., 2021; Schayot, 2021; Gupta et al., 2023; Sehrish et al., 2023).

According to the scientific literature, microgreens present an ensemble of essential phytonutrients (ascorbic acid, β-carotene, α-tocopherol and others) richer than their mature counterparts (Ghoora et al., 2005; Maftei et al., 2018; El-Nakhel et al., 2020; Pannico et al., 2020; Paraschivu et al., 2021; Rouphael et al., 2021).

Microgreens appear more often in the daily human diet, among other things, due to the rather simple principles of cultivation, small areas and fewer resources, but also due to the short period of growth and development (Keutgen et al., 2021; Sehrish et al., 2023).

Thus, due to the many properties they possess, microgreens are also used for sensory purposes to improve the color, texture or flavor of different salads, soups, beverages, as condiments or decorative elements (Bahadoran et al., 2011; Delian et al., 2015; Wu and Xu, 2019; Zhang et al., 2021).

Considering the contribution of nutrients in the human body, it is of considerable interest to know the biochemical composition of microgreens (Rusu, 2021). The accumulation of vitamin C in young meristems is particularly pronounced, resulting in higher quantities of ascorbic acid in microgreens compared to mature plants, even in the bud and young leaf areas of mature plants (Di Bella et al., 2020; Bhaswant et al., 2023). The accumulation of vitamin C is directly influenced by the growth conditions and environment.

Particularly, hemp microgreens have become a subject of increased interest in the fields of nutrition and health due to their remarkable nutritional properties and potential to be a valuable source of bioactive substances. Hemp, a plant from the *Cannabaceae* family, is known for its diverse range of uses, from fibers and oils to food and medicines (Viskovic et al., 2023), appearing as a crop of the future (Popa et al., 2021).

Hemp microgreens are rich in bioactive compounds, including organic acids, amino acids, polyphenols, and cannabinoids, as highlighted in the study by Pannico et al., 2022.

This study aims to explore the potential of hemp microgreens as a nutritious dietary alternative, with a focus on their high nutritional value and productivity indicators. Microgreens, being at an early stage of plant growth, are particularly influenced by environmental factors such as light, temperature, and density, which can significantly impact their photosynthetic and metabolic activities, consequently affecting their nutritional quality (Lobiuc et al., 2017; Teliban et al., 2023).

Through an experiment conducted in 2023 at IULS, this research investigates two distinct growing environments (controlled and uncontrolled) and seven different seeding densities to assess their effects on the qualitative and quantitative properties of hemp microgreens. The findings underscore the importance of selecting appropriate growth conditions and seeding densities to optimize the nutritional quality and productivity indicators of hemp microgreens, offering valuable insights for their potential utilization as a nutritious dietary component.

MATERIALS AND METHODS

Biological Material and Growth Conditions

The biological material used consisted of seeds from the monoecious hemp variety Dacia-Secuieni, belonging to the variety owner – Agricultural Research and Development Station Secuieni (ARDS Secuieni). The experiment was conducted in November 2023 at the Faculty of Horticulture, "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS).

The experimental protocol was bifactorial, with the two factors represented by: (1) Growing environment with two gradations (a_1=controlled environment, involving the growth of microgreens in a climate-controlled chamber; a_2=uncontrolled environment, involving the growth of microgreens indoor, near a window) and (2) Seeding density, with seven gradations (b_1=40 microgreens/100 cm^2; b_2=80 microgreens/100 cm^2; b_3= 120 microgreens/100 cm^2; b_4= 160 microgreens/100 cm^2; b_5= 200 microgreens/100 cm^2; b_6= 240 microgreens/100 cm^2; b_7= 280 microgreens/100 cm^2).

The climate-controlled chamber provided the following growth conditions: an 8:16-hour photoperiod (Figure 1), a constant temperature of 20°C throughout the growth period, and a relative humidity of 70%.

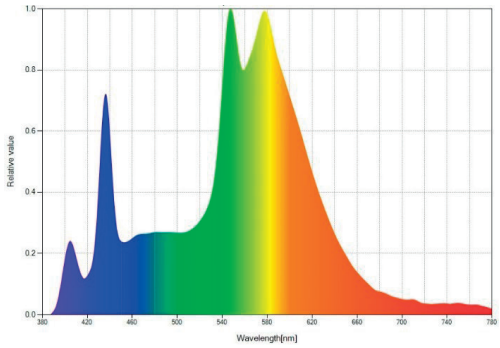

Figure 1. Light quality spectrum in the climate-controlled chamber

The microgreens grown in the uncontrolled environment, near the window, experienced temperatures ranging from a minimum of 15.3°C to a maximum of 28.2°C, with an average temperature during the growth period of 20.2°C and a relative humidity of 52.6% (Figure 2).

Figure 2. The evolution of temperature and humidity in the uncontrolled environment

The light recorded specific values for the month of November, which are presented in Figure 3.

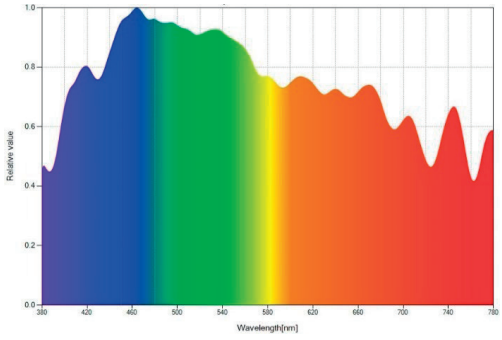

Figure 3. Light quality spectrum in the uncontrolled environment

The seeding was carried out in aluminum trays of 125 cm², using a mixture of soil and peat for both growing environments, with varying seeding densities according to the experimental protocol. Throughout the growth period, appropriate substrate humidity was maintained, and the microgreens were harvested for determinations and analyses after 18 days from sowing (Figure 4).

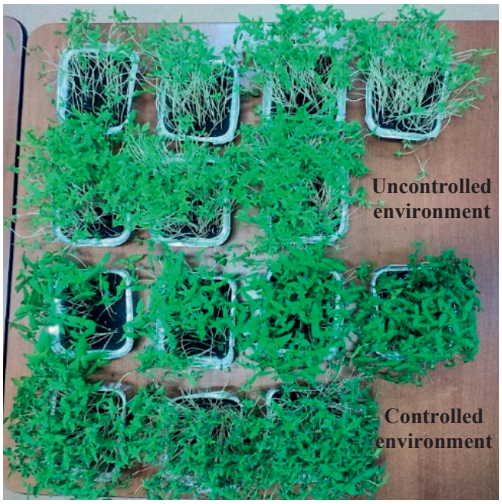

Figure 4. Hemp microgreens - experimental variants

Productivity indicators, CCI and Color parameters

The microgreens were harvested at the age of 14 days, from germination to harvest (AIHP, 2022), at the emergence of the second pair of true leaves (BBCH 11) (Mishchenko et al., 2017).

Fresh biomass was determined using a three-decimal analytical balance, weighing all plants in the tray, and the results were expressed in grams per 100 cm², respectively in grams per 100 microgreens.

Dry biomass was determined after drying the microgreens in an oven at 50°C until a constant weight was reached.

Leaf area was determined for all plants in the tray using the LI-3100C area meter, LI-COR (Lincoln, NE, USA), and the results were reported in cm² per 100 cm², respectively in cm² per 100 microgreens.

Microgreens length. For each experimental variant, the length was measured using a ruler, with 45 microgreens being considered.

The content of assimilatory pigments was determined using the CCM-200Plus (Chlorophyll Content Meter) purchased from Opti-Science Company, with 20 readings taken for each experimental variant, and the results expressed in CCI (Chlorophyll Content Index).
For ***color parameters*** determination, the MiniScan XE Plus apparatus produced from HunterLab, Reston, VA, USA, was used. The studied parameters were L, a, and b. L represents lightness to darkness (100 to 0), a represents redness to greenness (0 to 100 = red and −80 to 0 = green), and b represents yellowness and blueness (0 to 70 = yellow; −100 to 0 = blue).

Biochemical analyses
Total soluble solids (TSS) content was evaluate using a Refractometer Zeiss. The results were expressed in percentage °Brix (%) according to OECD standards, 2018.
The ***pH*** was measured by the potentiometric method with a laboratory pH meter, the results being expressed in units of pH (Irimia, 2013).
Ascorbic acid (vitamin C) content was determined with a Reflectoquant, a dispositive that measures light reflected from the test strip. The determination range is between 25 and 450 mg/L ascorbic acid and the results are expressed in mg/100 g fresh product (Irimia, 2021).
Titratable acidity (TA) was determined by titrimetric method. Microgreens hemp samples were homogenized with distilled water and titrated with 0.1 NaOH until reaching of 8.1 pH. The results were expressed in meq/100 g fresh product (OECD, 2018).
Biochemical analyses were made in triplicate according to the standards, averages being statistically analyzed.

Statistical analyses
The statistical analysis involved conducting an analysis of variance (ANOVA) to assess the significance of differences among the obtained results. Tukey's honestly significant difference (HSD) test was applied to compare the means of different groups, but only for those data points that showed significance at the ANOVA test with a confidence level of 95%. Results were reported as means with corresponding standard errors (Gomez and Gomez, 1984; Jităreanu, 1999; Leonte and Simioniuc, 2018).

RESULTS AND DISCUSSIONS

The production results obtained for hemp microgreens included determinations of fresh and dry matter, as well as leaf area index, with all values reported per 100 cm² and per 100 microgreens (Table 1).
Regarding the influence of the growing environment, microgreens grown in the climate-controlled chamber exhibited the highest productivity indicators, with significant differences compared to the uncontrolled environment, regardless of the reporting method.

Table 1. Productivity indicators for the hemp microgreens based on the growing environment and seeding density

Experimental factor	Fresh matter (g/100 cm^2)	Dry matter (g/100 cm^2)	LAI (cm^2/100 cm^2)	Fresh matter (g/100 microgreens)	Dry matter (g/100 microgreens)	LAI (cm^2/100 microgreens)
Growing environment						
CE	9.77 ± 0.04	1.11 ± 0.01	281.68 ± 0.77	7.54 ± 0.05	0.85 ± 0.02	237.87 ± 5.13
UCE	8.16 ± 0.29	0.81 ± 0.03	209.01 ± 3.02	5.25 ± 0.20	0.52 ± 0.02	138.16 ± 2.40
Significance	*	*	*	*	*	*
Seeding density						
D40	4.18 ± 0.06 c	0.47 ± 0.02 d	152.03 ± 5.50 e	10.46 ± 0.14 a	1.17 ± 0.05 a	380.07 ± 13.76 a
D80	5.30 ± 0.18 c	0.52 ± 0.02 d	152.11 ± 6.48 e	6.62 ± 0.22 bc	0.65 ± 0.02 b	190.14 ± 8.10 b
D120	8.20 ± 0.20 b	0.85 ± 0.01 c	231.49 ± 0.59 d	6.84 ± 0.17 b	0.71 ± 0.01 b	192.90 ± 0.49 b
D160	9.08 ± 0.60 b	0.99 ± 0.06 c	268.70 ± 8.98 c	5.67 ± 0.37 cd	0.62 ± 0.03 bc	167.94 ± 5.61 bc
D200	11.33 ± 0.30 a	1.23 ± 0.03 b	286.89 ± 1.94 bc	5.66 ± 0.15 cd	0.61 ± 0.02 bc	143.44 ± 0.97 cd
D240	11.81 ± 0.51 a	1.25 ± 0.05 b	302.18 ± 9.39 ab	4.92 ± 0.21 de	0.52 ± 0.02 c	125.91 ± 3.91 d
D280	12.85 ± 0.44 a	1.43 ± 0.04 a	324.00 ± 2.07 a	4.59 ± 0.16 e	0.51 ± 0.02 c	115.71 ± 0.74 d
Significance	*	*	*	*	*	*

Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2; LAI - Leaf Area Index).

Data reported per 100 cm² revealed that the highest values for all three productivity indicators (fresh matter, dry matter, and leaf area index) were obtained at the highest density (280 microgreens/100 cm²). A direct correlation between seeding density and

productivity indicators can be observed, with the latter increasing with density.

The density of 40 microgreens/100 cm² resulted in the highest values of fresh and dry matter, as well as leaf area index compared to the other experimented densities, with statistically significant differences, for data reported per 100 microgreens.

The highest values of productivity indicators, reported per 100 cm², were recorded for the combination of the controlled environment and a density of 280 microgreens/100 cm² (12.86 g/100 cm² f.m., 1.51 g/100 cm² d.m., 326.33 cm²/100 cm² LAI), followed by the interaction between the uncontrolled environment and a density of 280 microgreens/100 cm² (11.64 g/100 cm² f.m., 1.15 g/100 cm² d.m., 284.98 cm²/100 cm² LAI), as shown in Table 2. High values were also obtained for the interactions between the controlled environment and a density of 240 microgreens/100 cm² (11.99 g/100 cm² f.m., 1.35 g/100 cm² d.m., 319.39 cm²/100 cm² LAI), as well as with a density of 200 microgreens/100 cm² (11.70 g/100 cm² f.m., 1.34 g/100 cm² d.m., 311.06 cm²/100 cm² LAI).

Reported per 100 microgreens, the most significant values of productivity indicators were obtained in the controlled environment at the lowest densities (CE x D40, CE x D80, CE x D120, and CE x D160), with the best results belonging to the combination CE x D40 (15.00 g/100 microgreens f.m., 1.75 g/100 microgreens d.m., 584.81 cm²/100 microgreens).

Table 2. Productivity indicators for the hemp microgreens based on the interaction between growing environment and seeding density

Experimental factor	Fresh matter (g/100 cm²)	Dry matter (g/100 cm²)	LAI (cm²/100 cm²)	Fresh matter (g/100 microgreens)	Dry matter (g/100 microgreens)	LAI (cm²/100 microgreens)
CE X D40	6.00 ± 0.30 gh	0.70 ± 0.06 de	233.92 ± 16.62 efg	15.00 ± 0.74 a	1.75 ± 0.15 a	584.81 ± 41.54 a
CE X D80	6.88 ± 0.03 fg	0.69 ± 0.01 e	207.02 ± 4.87 fg	8.60 ± 0.04 b	0.86 ± 0.01 b	258.77 ± 6.09 b
CE X D120	9.02 ± 0.18 cdef	0.98 ± 0.03 cd	276.50 ± 5.86 bcde	7.52 ± 0.15 bc	0.82 ± 0.03 bc	230.42 ± 4.88 bc
CE X D160	9.92 ± 0.34 bcde	1.20 ± 0.02 bc	297.51 ± 11.40 abc	6.20 ± 0.21 cd	0.75 ± 0.01 bcd	185.94 ± 7.13 cd
CE X D200	11.70 ± 0.11 abc	1.34 ± 0.00 ab	311.06 ± 4.14 abc	5.85 ± 0.06 cd	0.67 ± 0.00 bcde	155.53 ± 2.07 de
CE X D240	11.99 ± 0.20 ab	1.35 ± 0.00 ab	319.39 ± 12.68 ab	5.00 ± 0.08 d	0.56 ± 0.00 cde	133.08 ± 5.28 de
CE X D280	12.86 ± 0.01 a	1.51 ± 0.03 a	326.33 ± 9.73 a	4.59 ± 0.00 d	0.54 ± 0.01 de	116.55 ± 3.47 e
UCE X D40	2.36 ± 0.20 i	0.23 ± 0.02 f	70.13 ± 5.84 h	5.91 ± 0.49 cd	0.58 ± 0.05 cde	175.33 ± 14.59 cde
UCE X D80	3.72 ± 0.33 hi	0.36 ± 0.03 f	97.20 ± 8.56 h	4.64 ± 0.41 d	0.45 ± 0.04 e	121.50 ± 10.70 de
UCE X D120	7.38 ± 0.58 efg	0.72 ± 0.06 de	186.47 ± 6.48 g	6.15 ± 0.48 cd	0.60 ± 0.05 bcde	155.39 ± 5.40 de
UCE X D160	8.23 ± 1.12 defg	0.79 ± 0.11 de	239.89 ± 7.90 def	5.15 ± 0.70 d	0.49 ± 0.07 de	149.93 ± 4.94 de
UCE X D200	10.95 ± 0.61 abcd	1.11 ± 0.06 bc	262.72 ± 1.41 cde	5.48 ± 0.31 d	0.55 ± 0.03 cde	131.36 ± 0.70 de
UCE X D240	11.64 ± 0.98 abc	1.15 ± 0.10 bc	284.98 ± 15.46 abcd	4.85 ± 0.41 d	0.48 ± 0.04 e	118.74 ± 6.44 e
UCE X D280	12.83 ± 0.88 a	1.35 ± 0.09 ab	321.66 ± 8.16 ab	4.58 ± 0.31 d	0.48 ± 0.03 e	114.88 ± 2.91 e
Significance	*	*	*	*	*	*

Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm²); LAI - Leaf Area Index).

The values of microgreens length in the uncontrolled environment were higher compared to those in the climate-controlled chamber, because of reduced and uneven light intensity (Figure 5). Density influenced the length of microgreens, as observed from Figure 5, with the most pronounced elongation observed at the highest density of 280 microgreens/100 cm² (11.57 cm), indicating a direct correlation between length and density across the entire experiment.

The length of microgreens varied according to the combination of the growing environment with seeding density, with values showing an increasing trend from CE x D40 (6.67 cm) to UCE x D280 (12.48 cm), as shown in Figure 6.

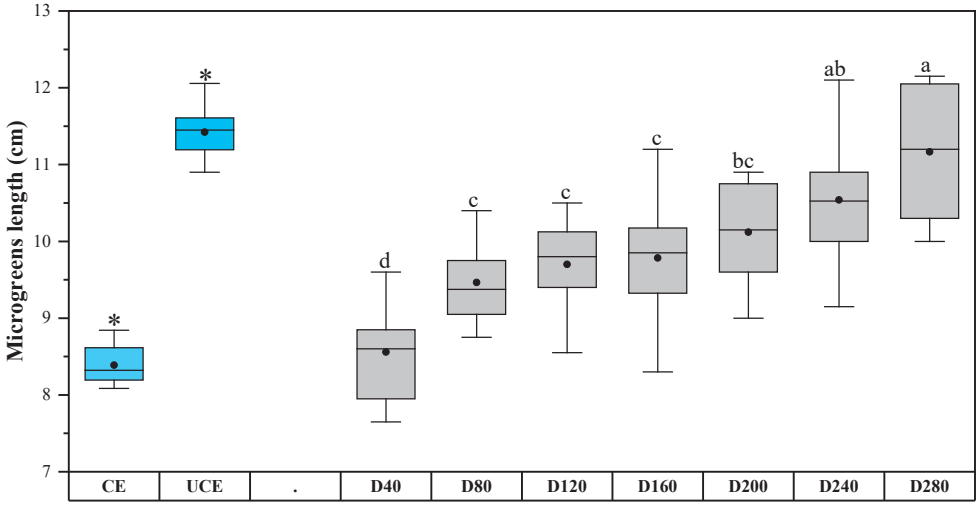

Figure 5. The influence of growing environment and seeding density on hemp microgreens length

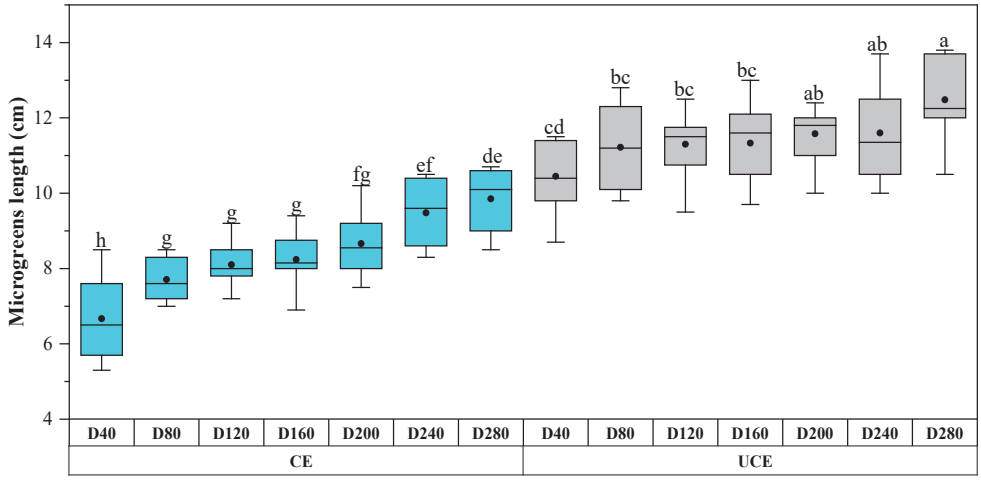

Figure 6. The influence of the combination of growing environment and seeding density on hemp microgreens length

The highest content of assimilatory pigments (expressed as Chlorophyll Content Index) was observed in hemp microgreens from the climate-controlled chamber, with the controlled environment registering values 30.8% higher than the uncontrolled environment (Table 3).

The lowest density of hemp microgreens (D40) recorded the highest content of assimilatory pigments (10.5 CCI), with the content decreasing as the seeding density increased (Table 3).

Regarding the color parameters (L lightness-darkness, a redness-greenness and b yellowness-blueness), they recorded significant values for the growing environment, but insignificant for the density factor (Table 3).

Table 3. CCI and color parameters for the hemp microgreens based on the growing environment and seeding density

Experimental factor	CCI	L	a	b
Growing environment				
CE	10.36 ± 0.12	27.41 ± 0.79	-4.77 ± 0.15	10.08 ± 0.27
UCE	7.16 ± 0.05	31.49 ± 1.15	-5.56 ± 0.22	12.31 ± 0.41
Significance	*	*	*	*
Seeding density				
D40	10.55 ± 0.17 a	30.12 ± 1.46 ns	-5.55 ± 0.24 ns	11.69 ± 0.53 ns
D80	9.76 ± 0.21 b	30.00 ± 1.18 ns	-5.47 ± 0.23 ns	11.64 ± 0.36 ns
D120	9.32 ± 0.18 b	29.15 ± 0.33 ns	-5.54 ± 0.22 ns	11.36 ± 0.20 ns
D160	8.31 ± 0.13 cd	29.63 ± 1.23 ns	-4.98 ± 0.30 ns	11.01 ± 0.40 ns
D200	8.34 ± 0.12 c	29.24 ± 1.96 ns	-5.19 ± 0.29 ns	11.13 ± 0.60 ns
D240	7.66 ± 0.16 de	29.12 ± 0.85 ns	-4.50 ± 0.28 ns	10.76 ± 0.33 ns
D280	7.58 ± 0.11 e	28.93 ± 1.16 ns	-4.94 ± 0.11 ns	10.78 ± 0.29 ns
Significance	*	ns	ns	ns

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); CCI - Chlorophyll Content Index; L - lightness-darkness; a - redness-greenness; b - yellowness-blueness.

The content of assimilatory pigments was influenced by the combination of growing environment and seeding density, with the highest values observed in the controlled environment at the lowest density (CE x D40 - 13.1 CCI). From this point, the trend is downward to 6.7 CCI, the value recorded at UCE x D280 (Table 4). Among the analyzed color parameters (L lightness–darkness, a redness–greenness and b yellowness–blueness), L recorded insignificant values, while parameters a and b had significant values (Table 4).

Table 4. CCI and color parameters for the hemp microgreens based on the interaction between growing environment and seeding density

Experimental factor	CCI	L	a	b
CE X D40	13.13 ± 0.26 a	30.89 ± 0.68 ns	-6.01 ± 0.21 d	11.80 ± 0.41 abc
CE X D80	11.96 ± 0.33 b	27.57 ± 1.48 ns	-5.29 ± 0.29 bcd	10.73 ± 0.46 abcde
CE X D120	11.18 ± 0.34 b	27.02 ± 0.68 ns	-4.92 ± 0.20 abcd	10.13 ± 0.24 bcde
CE X D160	9.67 ± 0.27 c	26.87 ± 2.23 ns	-4.41 ± 0.17 abc	9.40 ± 0.49 cde
CE X D200	9.60 ± 0.21 cd	28.09 ± 1.41 ns	-4.82 ± 0.21 abcd	10.29 ± 0.48 abcde
CE X D240	8.54 ± 0.23 de	25.43 ± 1.04 ns	-3.80 ± 0.25 a	8.92 ± 0.37 e
CE X D280	8.44 ± 0.21 ef	26.03 ± 0.84 ns	-4.15 ± 0.16 ab	9.25 ± 0.30 de
UCE X D40	7.90 ± 0.19 efg	29.34 ± 2.50 ns	-5.09 ± 0.32 abcd	11.59 ± 0.79 abcd
UCE X D80	7.51 ± 0.21 efgh	32.42 ± 1.43 ns	-5.65 ± 0.19 cd	12.54 ± 0.35 a
UCE X D120	7.41 ± 0.19 fgh	31.28 ± 0.85 ns	-6.16 ± 0.31 d	12.58 ± 0.40 a
UCE X D160	6.89 ± 0.16 gh	32.37 ± 0.95 ns	-5.55 ± 0.47 bcd	12.61 ± 0.42 a
UCE X D200	7.02 ± 0.19 gh	30.40 ± 2.55 ns	-5.55 ± 0.41 bcd	11.97 ± 0.73 ab
UCE X D240	6.72 ± 0.18 h	32.81 ± 1.22 ns	-5.20 ± 0.51 abcd	12.60 ± 0.66 a
UCE X D280	6.67 ± 0.11 h	31.84 ± 1.57 ns	-5.73 ± 0.18 cd	12.29 ± 0.35 ab
Significance	*	ns	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); CCI - Chlorophyll Content Index; L - lightness-darkness; a - redness-greenness; b - yellowness-blueness

The growing environment influenced the results of biochemical analyses in hemp microgreens, with the highest values of total soluble solids (3.91) and vitamin C content (49.52 mg/100 g fresh product) recorded in the controlled environment. In contrast, pH values (6.36) and titratable acidity (0.40 meq/100 g fresh product) were higher in the uncontrolled environment. Except for pH, where the differences were insignificant, the other differences were statistically significant (Table 5).

The density of 280 microgreens/100 cm^2 resulted in the highest vitamin C content (45.00 mg/100 g fresh product) and titratable acidity (0.48 meq/100 g fresh product), as well as the lowest pH value (6.22).

Total soluble solids recorded the highest value at a density of 240 microgreens/100 cm^2, followed by a density of 160 microgreens/100 cm^2 with a value of 3.62°Bx, while the highest pH value (6.39) was determined at a density of 120 microgreens/100 cm^2, according to Table 5.

For the experimented densities as well, only the differences related to pH were insignificant, while the differences for the rest of the biochemical analyses were statistically significant.

Table 5. Biochemical analyses of hemp microgreens based on the growing environment and seeding density

Experimental factor	TSS (°Bx)	pH	Vitamin C (mg/100 g fresh product)	TA (meq/100 g fresh product)
Growing environment				
CE	3.91 ± 0.01	6.25 ± 0.11	49.52 ± 0.36	0.25 ± 0.01
UCE	3.67 ± 0.01	6.36 ± 0.23	27.69 ± 0.14	0.40 ± 0.01
Significance	*	ns	*	*
Seeding density				
D40	3.90 ± 0.03 a	6.33 ± 0.10 ns	38.33 ± 0.22 c	0.24 ± 0.01 e
D80	3.62 ± 0.02 c	6.36 ± 0.16 ns	41.50 ± 0.43 b	0.24 ± 0.00 e
D120	3.62 ± 0.02 c	6.39 ± 0.18 ns	40.50 ± 0.72 b	0.27 ± 0.00 d
D160	3.92 ± 0.02 a	6.27 ± 0.22 ns	38.08 ± 0.22 c	0.38 ± 0.01 c
D200	3.78 ± 0.03 b	6.32 ± 0.16 ns	35.33 ± 0.51 d	0.29 ± 0.00 d
D240	3.97 ± 0.03 a	6.29 ± 0.22 ns	31.50 ± 0.29 e	0.40 ± 0.00 b
D280	3.70 ± 0.00 bc	6.22 ± 0.14 ns	45.00 ± 0.14 a	0.48 ± 0.01 a
Significance	*	ns	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); TSS - Total soluble solids; TA - Titratable acidity.

The highest content of total soluble solids (4.33°Bx) was obtained by the variant with 200 microgreens/100 cm² grown in the climate-controlled chamber. Measurements indicated the highest pH in the UCE x D120 combination (6.56), while the lowest value was recorded by CE x D160 (6.03). The highest vitamin C content (58.00 mg/100 g fresh product) was identified at a density of 160 microgreens/ 100 cm² grown in the controlled environment, followed by the variant with 80 microgreens/100 cm² in the controlled environment (57.17 mg/100 g fresh product) with significant differences compared to the other experimental variants (Table 6).

Table 6. Biochemical analyses of hemp microgreens based on the interaction between growing environment and seeding density

Experimental factor	TSS (°Bx)	pH	Vitamin C (mg/100 g fresh product)	TA (meq/100 g fresh product)
CE X D40	3.87 ± 0.03 cd	6.46 ± 0.00 ns	47.50 ± 0.29 c	0.11 ± 0.00 i
CE X D80	3.57 ± 0.03 fg	6.38 ± 0.11 ns	57.17 ± 0.73 a	0.11 ± 0.00 i
CE X D120	3.43 ± 0.03 gh	6.22 ± 0.11 ns	46.83 ± 1.30 c	0.29 ± 0.01 f
CE X D160	4.17 ± 0.03 b	6.03 ± 0.16 ns	58.00 ± 0.29 a	0.36 ± 0.01 de
CE X D200	4.33 ± 0.03 a	6.19 ± 0.05 ns	42.50 ± 0.29 d	0.18 ± 0.01 h
CE X D240	3.97 ± 0.03 c	6.20 ± 0.17 ns	42.17 ± 0.17 d	0.34 ± 0.01 e
CE X D280	4.00 ± 0.00 c	6.30 ± 0.16 ns	52.50 ± 0.29 b	0.39 ± 0.01 cd
UCE X D40	3.93 ± 0.03 cd	6.19 ± 0.20 ns	29.17 ± 0.17 g	0.36 ± 0.02 de
UCE X D80	3.67 ± 0.03 ef	6.33 ± 0.21 ns	25.83 ± 0.17 h	0.36 ± 0.01 de
UCE X D120	3.80 ± 0.00 de	6.56 ± 0.24 ns	34.17 ± 0.17 f	0.26 ± 0.00 g
UCE X D160	3.67 ± 0.03 ef	6.50 ± 0.27 ns	18.17 ± 0.17 i	0.40 ± 0.00 c
UCE X D200	3.23 ± 0.03 i	6.44 ± 0.28 ns	28.17 ± 1.17 gh	0.42 ± 0.01 c
UCE X D240	3.97 ± 0.03 c	6.38 ± 0.26 ns	20.83 ± 0.44 i	0.45 ± 0.01 b
UCE X D280	3.40 ± 0.00 h	6.14 ± 0.13 ns	37.50 ± 0.29 e	0.57 ± 0.01 a
Significance	*	ns	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); TSS - Total soluble solids; TA - Titratable acidity.

Similarly, other experiments were carried out in order to determine the content of vitamin C in microgreens belonging to other species. Thus, in a study realized by Xiao et al. (2019), Chinese cabbage microgreens had a vitamin C content of 18.9 mg/100 g FW, while in the study conducted by De la Fuente et al. (2019) different values of vitamin C content have reported for mustard microgreens (30.67 mg/100 g FM), radish (45.43 mg/100 g

FM), broccoli (50.99 mg/100 g FM) and kale (56.14 mg/100 g FM).
In our research, compared to the values above, the vitamin C content of hemp microgreens, in the controlled environment and with different seeding densities, varied between 42.17 mg and 58.00 mg/100 g fresh product (FP).

CONCLUSIONS

The growing environment had a significant influence on the productivity indicators, the highest values of the fresh matter, the dry matter and the leaf area index being found in microgreens grown in the controlled environment, by directing the light, temperature and humidity factors, regardless the reporting method.
The use of high seeding densities positively influenced the productivity indicators, fresh matter, dry matter and leaf area index, recording the highest values at the highest density experienced.
The results showed that assessed physico-chemical quality of hemp microgreens (total soluble solids, pH, titratable acidity) depends on growth conditions and density. Also, as it appears from the conducted study, an adequate management of environmental factors (light, temperature, humidity) and technological factors, such as seeding density leads to higher accumulations of vitamin C in hemp microgreens.
Thus, the special antioxidant properties and productivity indicators of hemp microgreens represent another step forward in terms of knowing the multifunctionality of industrial hemp and represent a starting point for deepening the physico-chemical research on this niche, as a demand due to the modern trend of consumers to have a diversified diet beneficial to the human body.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Horticultural Research Center of IULS and ARDS Secuieni - Neamt.

REFERENCES

Bahadoran, Z., Mirmiran, P., Hosseinpanah, F., Hedayati, M., Hosseinpour-Niazi, S., Azizi, F. (2011). Broccoli Sprouts Reduce Oxidative Stress in Type 2 Diabetes: A Randomized Double-Blind Clinical Trial. *Eur. J. Clin. Nutr., 65(8), 972–977*. DOI: 10.1038/ejcn.2011.59.

Bhaswant, M., Shanmugam, D. K., Miyazawa, T., Abe, C., Miyazawa, T. (2023). Microgreens - A Comprehensive Review of Bioactive Molecules and Health Benefits. *Molecules, 28, 867.*

Caracciolo, F., Vecchio, R., Lerro, M., Migliore, G., Schifani, G., Cembalo, L. (2019). Natural versus enriched food: Evidence from a laboratory experiment with chewing gum. *Food Res. Int., 122, 87–95*.

Caracciolo, F., El-Nakhel, C., Raimondo, M., Kyriacou, M. C., Cembalo, L., De Pascale, S., Rouphael, Y. (2020). Sensory Attributes and Consumer Acceptability of 12 Microgreens Species. *Agronomy, 10, 1043.* doi:10.3390/agronomy10071043.

Colantuoni, F., Cicia, G., Del Giudice, T., Lass, D., Caracciolo, F., Lombardi, P. (2016). Heterogeneous preferences for domestic fresh produce: Evidence from German and Italian early potato markets. *Agribusiness, 32, 512–530*.

De la Fuente, B., López-García, G., Máñez, V., Alegría, A., Barberá, R., Cilla, A. (2019). Evaluation of the bioaccessibility of antioxidant bioactive compounds and minerals of four genotypes of *Brassicaceae* microgreens. *Foods, 8, 250*.

Delian, E., Chira, A., Bădulescu, L., Chira, L. (2015). Insights into microgreens physiology. *Scientific Papers. Series B, Horticulture, vol. LIX.*

Di Bella, M. C., Niklas A., Toscano, Ş., Picchi, V., Romano, D., Lo Scalzo, R., Branca, F. (2020). Morphometric Characteristics, Polyphenols and Ascorbic Acid Variation in Brassica oleracea L. Novel Foods: Sprouts, Microgreens and Baby Leaves. *Agronomy, 10(6), 782*.

El-Nakhel, C., Pannico, A., Graziani, G., Kyriacou, M. C., Giordano, M., Ritieni, A., De Pascale, S., Rouphael, Y. (2020). Variation in macronutrient content, phytochemical constitution and in vitro antioxidant capacity of green and red butterhead lettuce dictated by different developmental stages of harvest maturity. *Antioxidants, 9, 300*.

Ghoora, M. D., Babu, D. R., Srividya, N. (2020). Nutrient composition, oxalate content and nutritional ranking of ten culinary microgreens. *Journal of Food Composition and Analysis, 91, 103495.*

Gomez, K. A., Gomez, A. A. (1984). *Statistical procedures for agricultural research*. New York, John Wiley & Sons.

Gupta, A., Sharma, T., Singh, S. P., Bhardwaj, A., Srivastava, D., Kumar, R. (2023). Prospects of microgreens as budding living functional food: Breeding and biofortification through OMICS and other approaches for nutritional security. *Front. Genet. 14:1053810*. doi: 10.3389/fgene.2023.1053810.

Irimia, L. M. (2013). *Controlul și expertiza calității legumelor, fructelor și produselor derivate.* Editura „Ion Ionescu de la Brad", Iași.

Irimia, L. M. (2021). *Manual de practică, specializarea horticultură, volumul II.* Editura „Ion Ionescu de la Brad", Iași.

Jităreanu, G. (1999). *Tehnica experimentală (Agricultural experimental technique)*. Editura „Ion Ionescu de la Brad", Iași.

Keutgen, N., Hausknecht, M., Tomaszewska-Sowa, M., Keutgen, A. J. (2021). Nutritional and Sensory Quality of Two Types of Cress Microgreens Depending on the Mineral Nutrition. *Agronomy, 11, 1110*. https://doi.org/10.3390/agronomy11061110.

Leonte, C., Simioniuc, V. (2018). *Metode și tehnici utilizate în cercetarea agronomică (Methods and techniques used in agronomic research)*. Editura „Ion Ionescu de la Brad", Iași.

Lobiuc, A., Vasilache, V., Oroian, M., Stoleru, T., Burducea, M., Pintilie, O., Zamfirache, M. M. (2017). Blue and red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. *Molecules, 22 (12), 2111*. https://doi.org/10.3390/molecules22122111.

Maftei (Hrițcu), A., Munteanu, N., Stoleru, V., Teliban, G. C. (2018). Studies of seeds germination as a technical stage in the production of microgreens. *Scientific papers, Horticulture, 61(2), 71-74*. ISSN: 1454-7376.

Mishchenko, S., Mokher, J., Laiko, I., Burbulis, N., Kyrychenko, H., Dudukova, S. (2017). Phenological growth stages of hemp (*Cannabis sativa* L.): codification and description according to the BBCH scale. *Žemės Ūkio Mokslai, 24(2), 31–36.*

OECD (2018). *OECD fruit and vegetables scheme. Guidelines on objective tests to determine quality of fruit and vegetables, dry and dried produce*.

Pannico, A., El-Nakhel, C., Graziani, G., Kyriacou, M. C., Giordano, M., Soteriou, G. A., Zarrelli, A., Ritieni, A., De Pascale, S., Rouphael, Y. (2020). Selenium biofortification impacts the nutritive value, polyphenolic content, and bioactive constitution of variable microgreens species. *Antioxidants, 9, 272*. doi:10.3390/antiox9040272.

Pannico, A., Kyriacou, M. C., El-Nakhel, C., Graziani, G., Carillo, P., Corrado, G., Ritieni, A., Rouphael, Y., De Pascale, S. (2022). Hemp microgreens as an innovative functional food: Variation in the organic acids, amino acids, polyphenols, and cannabinoids composition of six hemp cultivars. *Food Res Int. 161:111863.* doi: 10.1016/j.foodres.2022.111863.

Paraschivu, M., Cotuna, O., Sărățeanu, V., Durău, C., Păunescu, R. A. (2021). Microgreens - current status, global market trends and forward statements. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, vol. 21, issue 3.*

Popa, L. D., Vlăduț, N. V., Buburuz, A. A., Trotuș, E., Matei, Gh., Ungureanu, N., Isticioaia, S. F. (2021). *Cânepa (Cannabis sativa L.) - de la cultivare la valorificare*. Editura Universitaria Craiova. ISBN 978-606-14-1727-8.

Rouphael, Y., Colla, G., De Pascale, S. (2021). Sprouts, Microgreens and Edible Flowers as Novel Functional Foods. *Agronomy, 11, 2568*. https://doi.org/10.3390/agronomy11122568.

Rusu, I. E. (2021). *Utilizarea compușilor activi din făina de cânepă în produsele de panificație*. Teză de doctorat USAMV Cluj.

Schayot, C. T. (2021). Hemp microgreen mineral content, cannabinoids, total phenolics and antioxidants. *LSU Master's Theses 5464*.

Sehrish, A., Majeed, I., Zongo, E., Ayub, H., Rasul, H., Rahim, M. A., AL-Asmari, F. (2023). A review on various extraction and detection methods of bio-functional components from microgreens: food applications and health properties. *International Journal of Food Properties, 26:2, 3082-3105*. DOI:10.1080/10942912.2023.2272564.

Teliban, G. C., Pavăl, N. E., Patraș, A., Iavorschi, M., Stoleru, V., Lobiuc, A. (2023). Light modulated phenolic synthesis in *Chenopodium quinoa* microgreens as a potential biotechnological tool. *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry, 24(4), 347-355*.

Viskovic, J., Zheljazkov, V. D., Sikora, V., Noller, J., Latkovic, D., Ocamb, C. M., Koren, A. (2023). Industrial Hemp (*Cannabis sativa* L.) Agronomy and Utilization: A Review. *Agronomy, 13, 931.*

Wu, F., Xu, X. (2019). Sprouted Grains-Based Fermented Products. In Sprouted Grains. *AACC International Press, pp. 143–173.* DOI:10.1016/B978-0-12-811525-1.00007-53102A.

Xiao, Z., Codling, E. E., Luo, Y., Nou, X., Lester, G. E., Wang, Q. (2016). Microgreens of Brassicaceae: Mineral composition and content of 30 varieties. *Journal of Food Composition and Analysis, 49:87-93.*

Xiao, Z., Rauscha, S., Luoa, Y., Sunc, J., Yud, L., Wangd, Q., Chenc, P., Yud, L., Stommel, J. R. (2019). Microgreens of *Brassicaceae*: Genetic diversity of phytochemical concentrations and antioxidant capacity. *LWT volume 101, 731-737*.

Zhang, Y., Xiaob, Z., Agera, E., Konga, L., Tana, L. (2021). Nutritional quality and health benefits of microgreens, a crop of modern agriculture. *Journal of Future Foods, vol. 1, issue 1, 58-66.*

***Arizona Industrial Hemp Program (2022). *Hemp Microgreens and Hemp Greens Producer Guidance*.