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(RESEARCH ARTICLE)

Research on the carbohydrate and protein content of basil genotypes from the Buzău Region, Romania

Mădălin Vasile CHIUOARU, Lenuța CHIRA, Daniel NAGY, Gabriela BUTNARIU and Ligia ION *

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania.

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Abstract

The aromatic character of each type of basil is determined by the genotype and depends on the major chemical compounds formed mainly by the chemical compounds monoterpenes and essential oils. phenylpropanoids. The essential oil has antimicrobial, antifungal and insect repellent, anticonvulsant, hypnotic and antioxidant activities. Research has shown that the *Basil Aromatic of Buzău* genotype showed a significantly positive difference in terms of carbohydrate content, according to ANOVA analysis. The other genotypes (L1, L7, L8), although they showed positive differences, these were not large enough to be statistically significant. This analysis supports the choice of the Basil *Aromatic of Buzău* genotype for crops oriented towards a high carbohydrate content. The protein content values were influenced by the genotype and the environmental conditions in the years in which the research was carried out. The *Aromatic basil of Buzău* genotype recorded the highest protein content, and its values significantly exceeded the DL, which confirms that the differences from other genotypes are statistically significant.

Keywords: *Ocimum basilicum*; Genotypes; Carbohydrate; Protein; Statistical Analysis

1. Introduction

The plant Ocimum basilicum originates from ancient Persia, Pakistan and India, as well as other areas in Asia. Basil grows optimally in a temperate climate, having a low sensitivity to low temperatures and preferring the Mediterranean regions. It grows spontaneously in Asia, Africa and the warm areas of North America. In tropical climatic conditions, the plant develops over a perennial period of several years, while in temperate areas, it behaves as an annual species. [11], [8]. Basil has antiseptic properties for the intestines, kidneys, febrifuge, antifungal, galactogogue, diuretic, antiemetic and anti-inflammatory for both the kidneys and intestines, etc. It destroys bacteria in the body, especially intestinal ones and those in the kidneys and lungs. [10], [12], [6] It acts against parasitic fungi that affect the body, reduces fever, eliminates abdominal pain and nausea, while stimulating milk secretion in women, etc. Among the conditions treated in animals are kidney diseases, cystitis, including hemorrhagic cystitis, and gastrointestinal problems. Basil is recognized as an important storehouse of beneficial substances, also having applicability in modern medicine.

Basil contains important vitamins, namely: vitamin A (protective role for the eyes and mucous membranes, helps maintain healthy skin), vitamin K (plays an important role in blood clotting and helps strengthen bones). Vitamins B1, B2, B3, C and E are also present in basil. Species of the genus Ocimum do not show harmful effects, as long as they are used correctly in therapeutic treatments. Therefore, in case of overdose, poisoning may occur, which often presents with symptoms of contact dermatitis. Due to its uterotonic properties, the oil is not recommended for use during pregnancy, but it can be applied during massage (1-2 drops added to a massage oil). [4], [1], [3], [7].

* Corresponding author: Ligia ION

Basil has been used in traditional medicine in South Asia for over three millennia. Over time, it has acquired the status of a multifunctional medicinal plant, recognized and validated in contemporary studies by researchers and doctors. In the last decade, the multiple therapeutic effects of basil have been confirmed: adaptogenic, antimicrobial, anti-inflammatory, cardio- and hepatoprotective, immunomodulatory, metabolic anticancer, antioxidant, radioprotective, antidiabetic, antispastic. [2], [5], [9].

2. Material and methods

The plant material used in the study consists of basil genotypes from southern Romania, in sufficient numbers to conduct a meaningful study of the growth characteristics of basil genotypes (Table 1.) included in the study:

Table 1 Plant material basil plant biometrics for the 16 genotypes studied (sutras/source original)

Nr cart.	Genotypes studied	Average height of the plant (cm)
1.	Aromatic Basil from Buzau	75.50
2	Purple basil Serafim	77.10
3	Sweet basil L1	62.20
4	Lemon basil L2	73.30
5	Greek basil	30.21
6	Basil with salad leaves L4	84.40
7	Grand Vert Basil L5	60.30
8	Persian basil	52.48
9	Spicy Globe Basil L7	37.51
10	African basil L8	118.00
11	Holy basil var. Krishna L9	78.10
12	Macedonian basil	60.85
13	Dwarf basil Smarald	29.40
14	Dark Opal red basil	56.56
15	Siam Queen Basil	51.48
16	Basil cinnamon	110.50

2.1. Operational method

The determination of carbohydrate content in the basil varieties studied was carried out by the colorimetric method (phenol–sulfuric acid method).

Principle of the phenol–sulfuric acid method (colorimetric method for total carbohydrates). Carbohydrates in the plant extract are subjected to dehydration in a concentrated acid medium (sulfuric acid). Under the action of sulfuric acid, sugars are converted into furfural or its derivatives. These compounds react with phenol, forming a yellow–orange colored complex. The color intensity is directly proportional to the carbohydrate concentration and is determined spectrophotometrically at λ = 490 nm. (Figure 1.1.)

2.2. The working steps were

Sample preparation – the plant material (basil leaves) was dried, ground, then the carbohydrate was extracted in distilled water or 80% ethanol.

- Addition of phenol (5% solution).
- Addition of concentrated sulfuric acid to hydrolyze and dehydrate carbohydrates.
- Formation of the colored complex (the reaction was allowed to proceed for 10–20 min).

- Reading the absorbance on the spectrophotometer at 490 nm.
- Calculation of the carbohydrate content based on a standard curve obtained with a standard glucose solution.

Working principle of the Kjeldahl method (total nitrogen → total protein determination)

For the determination, the dried samples were digested in concentrated sulfuric acid with a catalyst; the organic nitrogen was converted to ammonium; the ammonium was then distilled as NH_3 after alkalization and then titrated. The nitrogen content is converted to protein by a conversion factor (usually 6.25 for total protein, but for plants 5.7 can also be used — the method is specified in the paper).

2.3. Materials and Reagents

- Concentrated H_2SO_4 (98%)
- Catalyst (K_2SO_4 + copper or mercury, or selenium sulfate)
- 40% sodium hydroxide (NaOH) for alkalization
- 4% borate solution (or collection solution for distillation)
- N HCl (or standardized H_2SO_4) for titration
- Phenolphthalein indicator or preferred titration solution

2.4. Sample preparation

- Harvest leaves from all 16 basil genotypes studied (same phenological stage), dry at 60–70 °C to constant weight.
- Grind dry in a pestle and homogenize.
- Record the mass of the dry sample (0.5–1.0 g, depending on the N content and sensitivity of the method).

2.5. Procedure (typical protocol)

In the digestion tube/erlenmeyer flask, 0.5–1.0 g of dry sample + 10–15 mL of concentrated H_2SO_4 + 1–2 g of K_2SO_4 + 0.5 g of catalyst (or amounts according to the kit) were placed.

- The digestion was carried out in a block at 375–420 °C until the solution became clear (2–3 hours depending on the sample).
- After cooling, the digest was transferred to the distillation flask (fill with water if necessary).
- 40% NaOH was added to make the reaction basic and release NH_3.
- NH_3 was distilled and captured in a borate solution (or H_3BO_3).
- The solution containing NH_4^+ was titrated with 0.1 N HCl until the indicator color changed.
- Perform the same steps for the blank sample (digest without sample) and standard (e.g. ammonium sulfamate) for control.

2.6. Calculation

Nitrogen (mg) = (V_sample − V_blank) × N_HCl × 14.007 × 1000

- (V in L, N_HCl normality; 14.007 = atomic mass of N in mg/mmol)
- % Nitrogen = (mg N / sample mass (mg)) × 100
- % Protein = % Nitrogen × conversion factor (typically 6.25)
- Example: if %N = 0.5 → %protein = 0.5 × 6.25 = 3.125%

The data resulting from the measurements were statistically analyzed using ANOVA software.

3. Results and discussion

Table 1.2. presents the results related to the carbohydrate content recorded in the different basil genotypes during the 3 years of study. They demonstrate that the highest values of the average of the 3 years of study were for the *Aromatic basil of Buzău* variety 2.62 (g/100g) followed by the *Greek Basil* genotype with values of 2.07 (g/100g), followed by the *African Basil L8* genotype with values of 2.05 (g/100g). The lowest values were recorded for the *Siam Queen Basil* genotype with values of 1.23 (g/100g), followed by the *Persian Basil* genotype with values of 1.32 (g/100g). But as can be seen from the average for the study years and per plant, it is observed that the weakest year with low carbohydrate

content values was 2022, which leads to the conclusion that environmental conditions again constitute a limiting factor in terms of carbohydrate content.

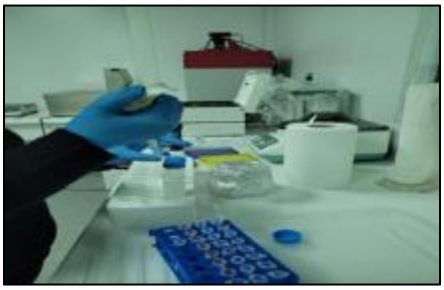

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Figure 1 Carbohydrate content - work stages

Table 2 Unifactorial analysis regarding the carbohydrate content of basil genotypes analyzed in the three years of experience (g/100g) (sursa/source: original)

Nr. Cart.	Genotypes studied	Average carbohydrate content (g/100g)	Report Medigen %	Difference Medigen	Estimation
1.	Genotypes studied	2.63	145.68	0.82	* * *
2.	Aromatic Basil from Buzau	1.88	104.10	0.07	
3.	Purple basil Serafim	1.98	109.92	0.18	
4.	Sweet basil L1	1.89	104.65	0.08	
5.	Lemon basil L2	1.83	101.33	0.02	
6.	Greek basil	1.58	87.60	-0.22	
7.	Basil with salad leaves L4	1.63	90.38	-0.17	
8.	Grand Vert Basil L5	1.32	73.19	-0.48	o
9.	Persian basil	2.06	114.08	0.25	
10.	Spicy Globe Basil L7	2.05	113.80	0.25	
11.	African basil L8	1.87	103.54	0.06	
12.	Holy basil var. Krishna L9	1.84	102.02	0.04	
13.	Macedonian basil	1.56	86.36	-0.24	
14.	Dwarf basil Smarald	1.86	103.27	0.06	
15.	Dark Opal red basil	1.24	68.61	-0.56	o o
16.	Siam Queen Basil	1.65	91.48	-0.15	

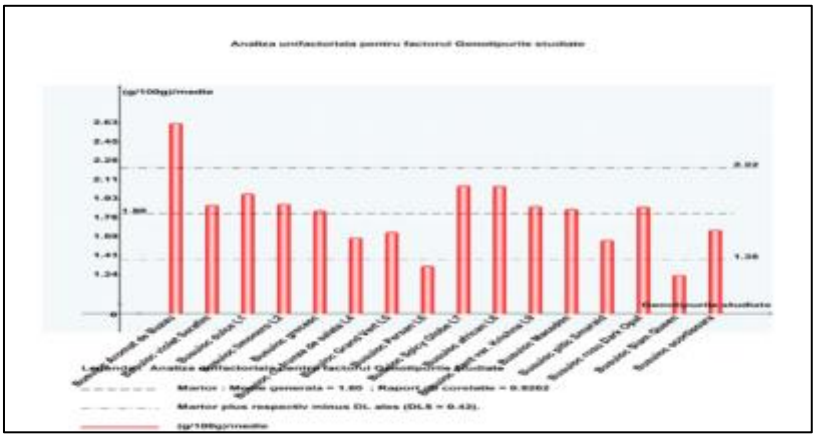
3.1. Overall average = 1.80

The unifactorial analysis regarding the carbohydrate content of the basil genotypes studied in the three years of experience, with the software (the Anova statistical program), in table 1.2., demonstrated that the most significant positive differences are found in the *Aromatic Basil of Buzău* genotype of 0.82, followed by *Sweet Basil L1* with 0.18 and *Spicy Globe Basil L7* with 0.25, which had the same result as *African Basil L8*.

Table 3 Unifactorial analysis regarding the carbohydrate content of basil genotypes analyzed in the three years of experience (g/100g) (sutras/source: original)

SOURCE OF THE VARIANT	VARIANT	GRADE of LIBERTy	CORRECTED DISPERSION	FACTOR FISHER	CRITICAL FACTOR			
	(SPA)	(L)	(S2)	(F calcul)	10 %	5 %	1 %	0.1 %
BETWEEN GROUPS (SYSTEMATICS)	6.31	15	0.4207	19.3000	1.6480	1.9000	2.4800	3.3100
WITHIN GROUPS (RESIDUAL)	1.05	48	0.0218					
TOTAL	7.36	63		DIFERENCE LIMIT (DL)	0.3500	0.4193	0.5592	0.7300

Using the Anova software, which is an analysis tool, the results in table 1.2. were analyzed and we can see in table 1.3., from a statistical point of view, we can differentiate the influence of the first factor, the influence of the second factor, as well as the combined influence and the interaction of the two factors, where the Limit Difference (LD) at 5% is 0.4193%, at 1% is 0.5592% and at 1% is 0.7300%.



(sutras/*source*: original)

Figure 2 Unifactorial analysis regarding the carbohydrate content of basil genotypes analyzed in the three years of experience (g/100g)

The *Armatic Basil* genotype of Buzău showed a positive difference of 0.82, a value that is much higher than the DL (limit difference) at 5% = 0.4193%, which means that the difference is statistically significant. Consequently, this genotype showed a significantly higher carbohydrate content than the average of the genotypes analyzed. For the *Sweet Basil L1* genotype, the difference was 0.18 – lower than the DL, so it is not statistically significant. The *Spicy Globe Basil L7* and *African Basil L8* genotypes with differences of 0.25 were also recorded below the significance threshold of 0.4193%, so they are also not statistically significant. The DL (Limit Difference) at 5% = 0.4193% constitutes the threshold below which the differences between genotypes are not considered significant at a confidence level of 95%. Only values exceeding this limit can be interpreted as real and significant differences between genotypes.

În conclusive doar genotype de *Basil Aromatic* of Buzău a prezentat o diferență semnificativ pozitivă în ceea ce privește conținutul de carbohidrați, conform analizei ANOVA. Celelalte genotipuri (L1, L7, L8), deși au manifestat diferențe pozitive, acestea nu au fost suficient de mari pentru a fi semnificative statistic. Această analiză sprijină alegerea genotipului *Basil Aromatic of Buzău* pentru culturi orientate către un conținut ridicat de carbohidrați.(grafic 1.2.)

The table below (table 1.4.) summarizes data on the protein content of basil genotypes researched during 2021-2023.

Regarding protein content, their values were mentioned in table 1. 4. The highest values of this protein content were recorded in the *Aromatic of Buzău* genotype with the highest value in 2021 with 3.29 (g/100g), followed by 2022 with a value of 3.22 (g/100g) and with an average value of the study years of 3.10 (g/100g). Another genotype with high protein content values was *Serafim Violet Basil*, which recorded average values of 2.41 (g/100g) followed by the *African Basil L8* genotype with average protein content values of 1.90 (g/100g). The lowest protein values were recorded for the *Sweet Basil L1* genotype with an average value of 1.09 (g/100g), followed by the *Siam Queen Basil* genotype with an average value of 1.22 (g/100g). The protein content values were influenced by the genotype and the environmental conditions of the years in which the research was conducted.

From a statistical point of view, using the Anova software, the difference in the average value of the protein content (g/100g) is significantly positive for the *Aromatic Basil of Buzau*, compared to the *Sweet Basil L1*, according to table 1.4.

The *Aromatic of Buzău* genotype recorded the highest protein content, and its values significantly exceeded the DL, which confirms that the differences from other genotypes are statistically significant. Purple *Basil Serafim* and *African Basil L8* recorded an average protein content that was probably significant compared to those with very low values. *Sweet Basil L1* and *Siam Queen* were at the lower end, with substantially lower values, and the differences from the higher genotypes exceeded the DL, so they are significant.

Table 4 Results regarding the protein content (g/100g) of the basil genotypes analyzed in the three years of experience

Nr cart.	Genotypes studied	Protein content (g/100g)/2021	Protein content (g/100g)/2022	Protein content (g/100g)/2023	Average protein content values per genotype/3 years of study (g/100g)
1.	Aromatic Basil from Buzau	3,29	3,02	2,99	3.10
2.	Purple basil Serafim	2,58	2,42	2,25	2.42
3.	Sweet basil L1	1,14	1,01	1,12	1.09
4.	Lemon basil L2	1,59	1,42	1,51	1.51
5.	Greek basil	1,74	1,63	1,64	1.67
6.	Basil with salad leaves L4	1,92	1,85	1,36	1.71
7.	Grand Vert Basil L5	1,45	1,32	1,31	1.36
8.	Persian basil	1,55	1,32	1,42	1.43
9.	Spicy Globe Basil L7	1,79	1,65	1,56	1.67
10.	African basil L8	1,92	1,95	1,85	1.91
11.	Holy basil var. Krishna L9	1,75	1,67	1,52	1.65
12.	Macedonian basil	1,65	1,61	1,52	1.59

13.	Dwarf basil Smarald	1,91	1,47	1,68	1.69
14.	Dark Opal red basil	1,75	1,63	1,51	1.63
15.	Siam Queen Basil	1,22	1,32	1,12	1.22
16.	Basil cinnamon	1,37	1,31	1,25	1.31

(sutras/source: original)

The unifactorial ANOVA analysis of protein content for the basil genotypes studied in the period 2021–2023 revealed statistically significant differences. The *Aromatic basil of Buzău* genotype recorded the highest average value (3.10 g/100g), with a significant difference compared to the other genotypes, exceeding DL = 0.3320%. In contrast, the *Sweet Basil L1* (1.09 g/100g) and *Siam Queen* (1.22 g/100g) genotypes recorded the lowest values, with significant negative differences compared to the other genotypes.

Table 5 Results regarding the protein content (g/100g) of the basil. genotypes analyzed in the three years of experience

Nr. Cart.	Genotypes studied	Average protein content (g/100g)	Report MedGen %	Difference MedGen	Estimation
1.	Aromatic Basil from Buzau	3.10	183.55	1.41	* * *
2.	Purple basil Serafim	2.42	143.14	0.73	* * *
3.	Sweet basil L1	1.09	64.54	-0.59	o o o
4.	Lemon basil L2	1.51	89.26	-0.18	
5.	Greek basil	1.67	98.88	-0.01	
6.	Basil with salad leaves L4	1.71	101.25	0.02	
7.	Grand Vert Basil L5	1.36	80.53	-0.32	
8.	Persian basil	1.43	84.67	-0.25	
9.	Spicy Globe Basil L7	1.67	98.73	-0.02	
10.	African basil L8	1.91	112.94	0.22	
11.	Holy basil var. Krishna L9	1.72	101.99	0.03	
12.	Macedonian basil	1.59	94.29	-0.09	
13.	Dwarf basil Emarald	1.69	99.92	0.00	
14.	Dark Opal red basil	1.63	96.51	-0.05	
15.	Siam Queen Basil	1.22	72.24	-0.46	o o
16.	Basil cinnamon	1.31	77.56	-0.37	o

(sutras/source: original)

The one-way analysis (Anova) on the protein content of the 16 basil genotypes is summarized in Table 1.6.

Table 6 Results regarding the protein content (g/100g) of the basil genotypes analyzed in the three years of experience

SOURCE OF THE VARIANT	**VARIANT**	**GRADE of LIBERTY**	**CORRECTED DISPERSION**	**FACTOR FISHER**	**CRITICAl FACTOR**			
	(SPA)	(L)	(S2)	(F calculat)	10 %	5 %	1 %	0.1 %
BETWEEN GROUPS (SYSTEMATICS)	14.06	15	0.9374	68.5976	1.6480	1.9000	2.4800	3.3100
IN INTERIORUL GRUPELOR (REZIDUALA)	0.66	48	0.0137					
TOTAL	14.72	63		DIFERENCE LIMIT (DL)	0.2771	0.3320	0.4427	0.5780

(sutras/source: original)

Table 6 summarizes data on the sugar content of the 16 basil genotypes under research in the period 2021-2023.

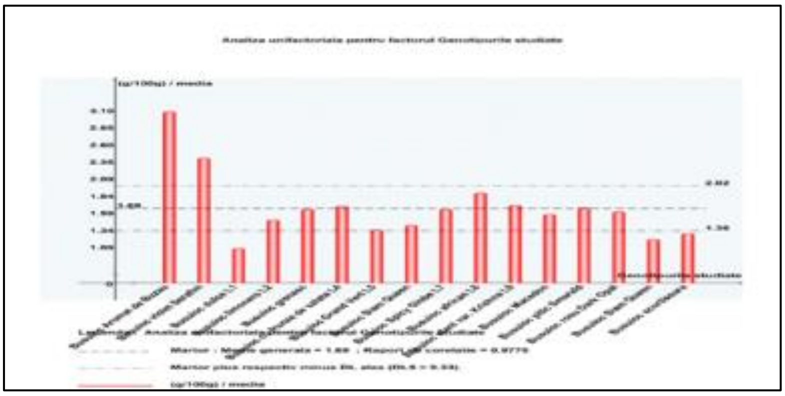


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Figure 3 Unifactorial analysis of protein content (g/100g) of basil genotypes analyzed inthe three years of experience

4. Conclusion

In conclusion, only the *Aromatic of Buzău Basil* genotype showed a significant positive difference in terms of carbohydrate content, according to the ANOVA analysis. The other genotypes (L1, L7, L8), although they showed positive differences, these were not large enough to be statistically significant. This analysis supports the choice of the *Aromatic of Buzău Basil* genotype for crops oriented towards a high carbohydrate content.

The protein content values were influenced by the genotype and the environmental conditions in the years in which the research was carried out. The *Aromatic basil of Buzău* genotype recorded the highest protein content, and its values significantly exceeded the DL, which confirms that the differences compared to other genotypes are statistically significant. *Serafim Violet Basil* and *African Basil L8* recorded an average protein content that was probably significant compared to those with very low values. *Sweet basil L1* and *Siam Queen* were located at the lower end, with substantially lower values, and the differences from the higher genotypes exceed the DL, so they are significant.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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