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Research on genes identification for antiseptic action in some basil genotypes

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Abstract

The studies undertaken by the present work refer to 21 domestic and foreign genotypes, in which a screening was done with 8 molecular markers, for the investigation of the genome in terms of highlighting the gene that controls the antiseptic action and the resistance of plants to biotic factors.

The collection of basil genotypes is not very large in Romania. There are only 150 autochthonous basil genotypes. The genomic DNA isolated from the basil genotypes, *"Aromat de Buzau", "BZ 1", "Hof Igal 2", "Gea", "LBRS 2", "LBVS 1",* etc. was used to implement of RADP molecular markers to highlight the gene that controls antiseptic action. In order to determine, the DNA samples extracted from young leaves by the methods of Lodhi et al. 1994, modified by Pop et al., 2003 and Doyle & Doyle, 1990, were quantified/measured, amplified, and the amplification products (RAPDs) were electrophoretic ally separated in agarose gel. The research aimed to highlight the ObGAPDH gene in basil genotypes taken in the study using 8 specifically designed molecular markers.

Keywords: Ocimum Basilico; Genotypes; Pathogen; Molecular Markers; Samples

1. Introduction

Ocimum is a genus of aromatic plants and perennial and annual shrubs belonging to the Liliaceae family, comprising nearly 200 genera and 3,200 species. The number of species in the *Ocimum* genus is uncertain due to several taxonomic difficulties. Therefore, it could have 30–160 species. *Ocimum basilicum L.,* or sweet basil, is an important species known for its medicinal properties and essential oil [15], [16]. Its flower has bilateral symmetry, with five petals and five sepals; the stem is erect, branched, solid, and hairy; the seeds are oval-shaped and black; the leaf is simple and opposite, with epidermal glands containing aromatic oil [12]. In recent years, *Ocimum basilicum* has been extensively studied for its various activities [4] [3].

In silico molecular docking is a rapidly developing field for understanding and predicting possible modes of interaction between a ligand and a target biomolecule [8, 9, 5, 11, 1], while molecular dynamics simulations have presented a plausible way to estimate the dynamic and energetic stability of a protein-ligand complex [1, 7, 17, 6, 13, 16, 10]. Therefore, a docking analysis of the main constituents of Ocimum basilica, linalool and estragole, with alpha-amylase and lipase together with their inhibitors was planned.

The lipase inhibitory activity of plant extracts was estimated according to a reported protocol [14]. The DPPH free radical scavenging potential of polar and nonpolar extracts from Ocimum basilica leaves and flowers was determined according to the reported test [2] PCR which is used to rapidly make millions or billions of copies of a specific DNA sample, amplifying it exponentially in a series of temperature change cycles, thus allowing scientists to take a very small sample of DNA and amplify it to a quantity large enough to be studied. PCR technology was pioneered in 1983 by

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American biochemist Kary Mullis at Cetus Corporation. The PCR process is now an indispensable procedure used in medical laboratory research in a wide variety of applications, including biomedical and forensic research. There are several PCR methods, but all are based on thermal cycling. These expose the reactants to repeated temperature changes to allow different reactions to occur—in particular, the melting and enzymatic replication of DNA.

2. Material and methods

2.1. Plant material consists of 21 genotypes of basil

The research was conducted over three growing seasons, from 2021 to 2024, and involved measurements of the phenotypic characteristics of different basil genotypes, namely growth rate at 15 days, plant height, average bush diameter, average shoot length, and average number of shoots per plant. Other characteristics studied include the green mass weight of basil for the 16 genotypes studied over the three years allocated to the research. Other research addressed the average *values of the germination period* for the 16 genotypes studied over the three years allocated to the research.

Table 1 Plant material basil plant biometrics for the 16 genotypes studied

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	Limonero 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 Emarald	29.40
14	Dark Opal red basil	56.56
15	Siam Queen Basil	51.48
16	Basil cinnamon	110.50
17	BZ 1	60.22
18	Hofigal 2	71.13
19	Gea	81.11
20	LBVS 1	60.32
21	LBVS 2	62.22

(sutras/source: original)

After the DNA isolation, the amount and purity of the DNA was determined for the 21 varieties studied. These determinations were made using the Nano Drop ND 33 000 device, with the Eppendorf Bio Photometer. The amount of DNA was expressed in ng/all and the purity was highlighted by realizing the ratio between two wavelengths, respectively 260/280.

After the phenotypic determinations were made, genotypic determinations followed, starting with the isolation of DNA from the basil genotypes studied. The DNA was quantified using a nanodrop and then revealed in a 2% electrophoresis gel. An amplification kit from Applied Biosystems with primers specific to the *Ocimum Basilicum species* was used to perform PCR.

Following the optimization of amplification conditions, a PCR reaction was performed in a temperature gradient. Thus, a DNA sample was amplified at eight different temperatures: 51°C; 51.8°C; 53.1°C; 54.9°C; 57.4°C; 59.3°C; 60.5°C; 61°C (table 2). The PCR amplification products were migrated in 2% agarose gel and visualized under UV transilluminator. Considering the amplicon profile, the optimal primer hybridization temperature was selected. The amplification reaction was performed as follows: 20 all of reaction mixture was pipetted into each 0.2 all tube and 5 all of DNA was added, respectively 5 all of deionized H2O in the case of the negative control. The samples were vortexed and gently centrifuged, then placed in the PCR machine. The PCR machine was set according to the program in table 1.

Table 2 PCR reaction scheme to optimize amplification conditions

	Temperature / Timp
Denaturise initial	95 ºC, 10 min.
Cyclura	Denaturise: 95 ºC, 30 sec.
	Alimera primer: 51- 61 ºC, 30 sec.
	Extender: 72 ºC, 1 min.
Nr. Cyclura	35
Extensae final	72 ºC, 15 min.

(sutras/source: original)

3. Results and discussion

Validation of amplicon identity is necessary to verify that the amplified DNA corresponds to the chosen target sequence. Agarose gel electrophoresis is a simple method for verifying the size of PCR products. However, sequencing the amplicons and comparing the results with those in databases is the most effective method of verifying the results obtained by PCR. The reaction products obtained by conventional PCR were sequenced after purification with the Wizard PCR Preps DNA Purification System (Promega). The markers and primers used for PCR amplification were labeled with Big Dye (ABI PRISM® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems).

Results regarding the quantification of genomic DNA at nanodrop for the studied basil genotypes (2021-2023) are summarized in Table 3.

Table 3 Results regarding the quantification of genomic DNA at nanodrop for the studied basil genotypes

Nr. Cart.	**Genotype**	**Genomic and concentration (ng/ all)**	**Report abs. 260/280 1.98**	**Report abs. 260/230 2,01**	**Absorption D260 24.532**
1.	Aromatic Basil from Buzau	1226.6	1.98	2.01	24.532
2.	Purple basil Serafim	2108.2	1.96	2.02	42.164
3.	Sweet basil L1	1630.2	1.97	1.98	32.603
4.	Lemon basil L2	4847.3	1.7	1.64	96.945
5.	Greek basil	259.3	1.71	1.12	5.185
6.	Basil with salad leaves L4	429.7	1.71	0.97	8.593
7.	Grand Vert Basil L5	870.6	1.71	1.05	17.411

8.	Persian basil	1481.2	1.86	1.43	29.625
9.	Spicy Globe Basil L7	164	1.71	0.98	3.279
10.	African basil L8	442.8	1.72	1.21	8.856
11.	Holy basil var. Krishna L9	479.5	1.67	1.01	9.59
12.	Macedonian basil	1478.7	1.82	1.36	29.575
13.	Dwarf basil Emarald	261.6	1.82	1.38	5.231
14.	Dark Opal red basil	224.2	1.34	1.71	4.485
15	Siam Queen Basil	794.6	1.82	1.26	15.892
16	Basil cinnamon	222.4	1.25	1.23	12.852
17	BZ 1	462.2	1.52	1.12	4.241
18	Hofigal 2	481.1	1.61	1.22	3.123
19	Gea	391.5	1.45	1.14	4.472
20	LBVS 1	473.4	1.71	1.21	4.732
21	LBVS 2	444.2	1.62	1.11	3.751

(sutras/source: original)

Table 3 shows the results of genomic DNA quantification by nanodrop for the basil genotypes studied. The amount of genomic DNA varied greatly from genotype to genotype, with the highest value recorded for the *Limonero basil* genotype, with a value of 4847. The lowest values were recorded for the *Sweet Basil* genotype with values of 1630.2 ng/µl and the *Persian Basil* genotype with values of 1481.2 ng/all. The lowest values were recorded for the *Spicy Globe basil* genotypes with values of 164 ng/all, the *Dark Opal red basil* genotype with values of 224.2 ng/all, followed by the *Greek basil* genotype with values of 259.3 ng/all and *Basil cinnamon* 222.4 ng/all

The final DNA solution is prepared according to the specific preparation method, either by resuspending the DNA sediment after its precipitation with alcohols (ethanol or isopropanol) in the presence of salts. The genetic material, DNA, was stored (at -20ºC) in TE solution, but it can also be stored in sterile deionized water. In the present experiment, the sediment was resuspended in 100 all TE pH 8.0.

Table 4 Results of the unifactorial analysis for the factor regarding the quantification of genomic DNA at nanodrop, for the genotypes studied

SOURCE OF THE VARIANT	**VARIANT**	**GRADE of Liberty**	**CORRECTED DISPERSION**	**FISHER FACTOR**	**CRITICAL FACTOR**			
	(SPA)	(L)	(S2)	(F calculate)	10 %	5 %	1 %	0.1 %
BETWEEN GROUPS (SYSTEMATICS)	5232179.07	14	373727.0763	0.5877	1.6620	1.9200	2.5200	3.3600
WITHIN GROUPS (RESIDUAL)	28618013.96	45	635955.8657					
TOTAL	33850193.02	59		DIFERENCE LIMIT (DL)	1894.6883	2271.3704	3033.7568	3969.8231

(sutras/source: original)

Thus, Table 4. presents the univariate analysis between the factors of variation regarding the quantification of genomic DNA at nanodrop for the studied basil genotypes, which shows us a limit difference of 5% at 2271.3704, 1% at 3033.7568, and at 0.1% of 3969.8231, meaning the influence of factors on the resulting characteristics.

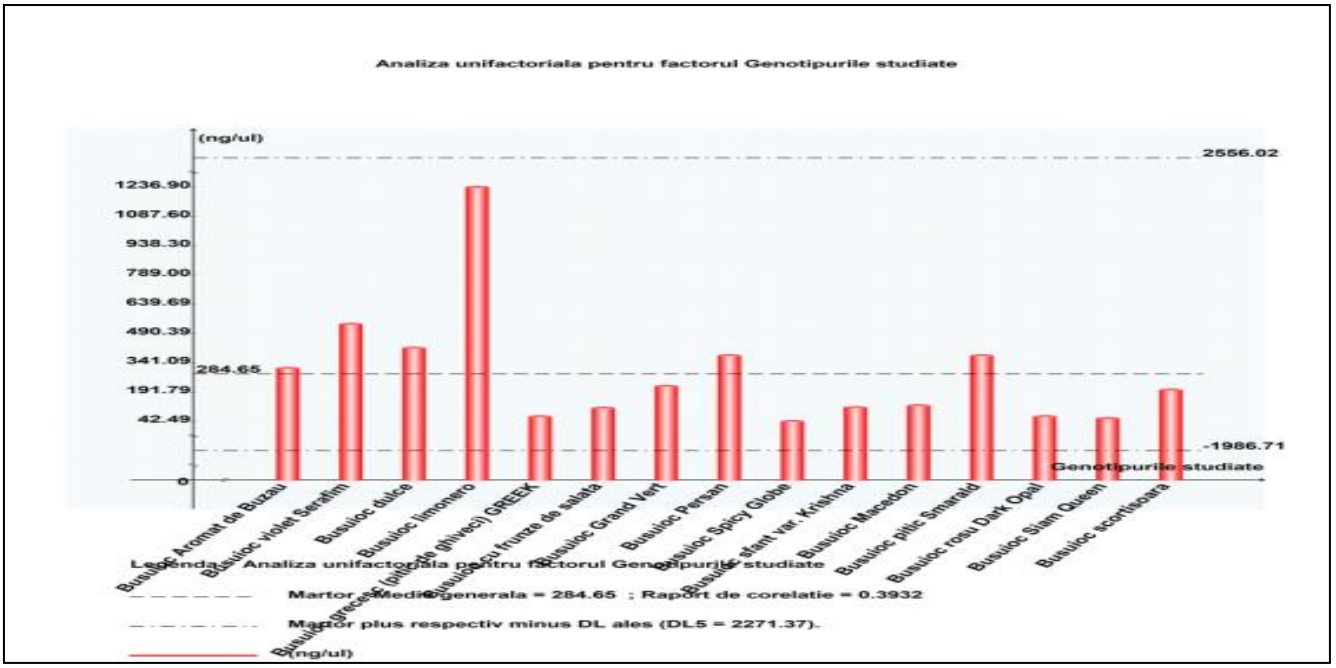

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Figure 1 Results of the unifactorial analysis for the factor regarding the quantification of genomic DNA at nanodrop, for the genotypes studied

After DNA extraction, the samples were quantified spectrophotometrically using the Eppendorf Bio Photometer in the first part of the experiment and the Nanodrop NA 33 000 in the second part of the experiment. The spectrophotometric method is based on the fact that most biological substances have a characteristic absorption rate in the ultraviolet radiation range. Thus, the absorption rate of 260 nm corresponds to nucleic acids, that of 280 nm to proteins, and 230 nm to various contaminants. The optical density was measured at the absorption rates A 260 nm and A 280 nm, calculating the ratio between the two absorptions. (Figure1.)

The univariate ANOVA analysis of the amount of genomic DNA extracted from different *Ocimum basilicum* genotypes revealed large variations between genotypes. The highest value was recorded in the *Basil Limonero* genotype (4847.3 ng/all), followed by *Basil violet Serafim* (2108.2 ng/all), *Sweet basil* (1630.2 ng/all), and *Basil person* (1481.2 ng/all). In contrast, the lowest values were found in *Spicy Globe basil* (164 ng/all), *Dark Opal red basil* (224.2 ng/all), and *Greek basil* (259.3 ng/all).

However, only the difference between the *Limonero basil* genotype and the other genotypes exceeded the Limit Difference (LD) at $p < 0.05$ of 2271.3704 ng/all, indicating a statistically significant difference only in this case. Therefore, *Basil Limonero* stands out as having a significantly superior genetic capacity in terms of genomic DNA accumulation or stability, which may indicate a higher potential for biotechnological or genetic applications.

Regarding the quantification of genomic DNA, the results were quite uneven, with values ranging from 4847.3 ng/µl for the "*Basil Limonero*" variety to 164 ng/µl for the "*Basil Spicy Globe*" variety.

Table 5 Primers tested at Og-USAMVB Hort invest

Nr Cart	Primers	Sequence (5'-3')
1.	Og A07	GAG ACG GGT A
2.	ObGA08	ATG ACG TAG G
3.	Og A09	CGG TAA CGC C
4.	ObGA10	CTG ATC GCA C
5.	Og A11	GAA TGC CCG T

6.	ObGA12	CTG CAC CCA C
7.	Og A13	ATG TGA CCG T
8.	ObGB04	CGA CAG GTG A

(sutras/source: original)

However, given that small amounts of DNA in the order of 10–20 ng/µl are sufficient for SSR marker analysis, it is important to know the initial values in order to determine the necessary dilutions.

For molecular marker analysis, the reaction mixture was prepared under a laminar flow hood under sterile conditions. A control sample containing all components of the reaction mixture except DNA was also taken into account. In order to amplify the samples obtained in the first part of the experiment, eight Deca nucleotide primers were used, each primer having 10 nucleotides (Table 5.) They were chosen from among those that presented polymorphic bands. These primers arrive at the laboratories in lyophilized form, each with a specific recipe for dilution when used.

3.1. Results obtained in the case of amplification of DNA extracted using the Doyle and Doyle method

Of the primers studied, five primers (ObGA07, ObGA08, ObGA11, ObGA13, ObGA18, and ObGB04) amplified bands in only one variety, and two of the primers, ObGA09 and ObGA10, did not amplify any fragments in any of the varieties (Figure 1.6), and the amplified bands were unclear, making it impossible to assess them accurately.

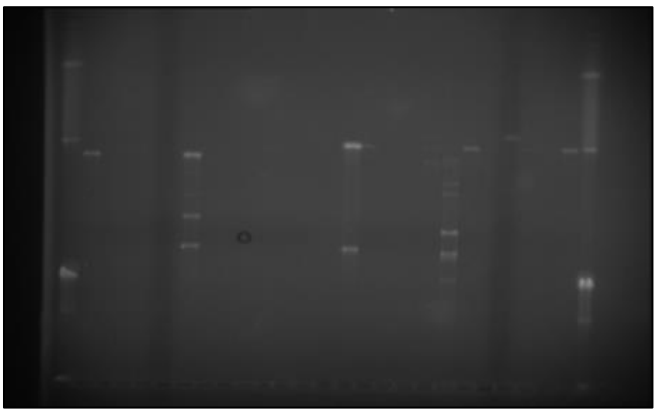

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Figure 2 Results concerning the bands obtained from three varieties of the test primers Og

There could be an explanation related to the DNA samples, which did not have the necessary quantity or purity to produce polymorphism with the primers tested, even though they are universal and should have produced results. The polymerase used was Aplite Polymerase (Stoffel) and not Promega, and the amplification power may have been lower. (Figure 2.)

3.2. Analysis and processing of results obtained by RAPD

The results refer to a total of 8 samples, of which 2 revealed polymorphism between genotypes, while the other 6 presented monomorphic bands, with a total of 75 bands generated, of which 66 were polymorphic (Table 6).

Table 6 RAPD data obtained from image analysis (2023)

Nr. Cart.	**Primer**	**Total number of lanes Lanes/primer**	**No. of polymorphic bands Bands/primer**	**No. of monomorphic bands Bands/primer**	**% polymorphism**
1.	*Og* A07	12	11	1	99.6
2.	*Og* A08	9	8	1	88.8
3.	*Og* A09	7	6	1	85.7

4.	*Og* A10	7	5	2	71.4
5.	*Og* A11	11	10	1	92.8
6.	*Og* A12	10	9	1	93.3
7.	*Og* A13	8	8	0	100
8.	*Og* B04	11	10	1	90.9

(sutras/source: original)

In the laboratory, band detection was performed using the Faliscan program, by comparing them with a standard DNA, with a size between 100 and 4000 bp. All fragments resulting from amplification with polymorphic primers were between 200 and 2000 bp in length, with most between 300 and 1200 bp (in the first amplification case).

3.3. Interpretation of results

Interpretation of the results obtained by the RAPD method involves the genomic DNA isolation protocol, the PCR reaction, the amplification program, and image acquisition using the Alpha Imager 2200 program. The smaller the genetic distance values in the table (binary matrix), the more closely related the genotypes are. These primary data were entered into the RAP Distance 1.04 program, which calculated the genetic distances based on the Jaccard coefficient (Table 7.). The formula used to calculate the Jaccard coefficient (Jij) is as follows:

$$Jiji = Cin/ (in + Nj – Cin)$$

where: Cin - represents the number of identical bands presented in the two genotypes; nj - represents the total number of bands identified in genotype I and j, respectively.

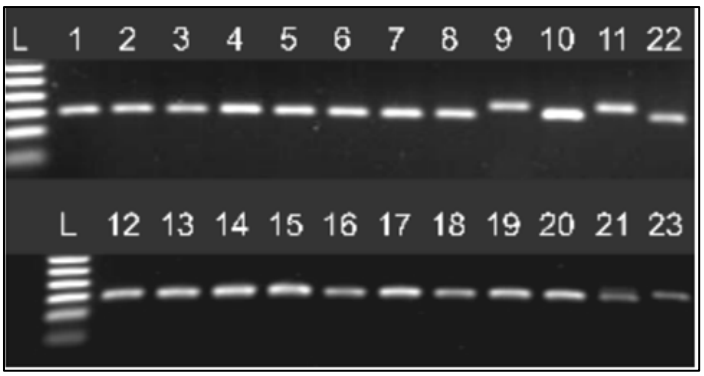
Table 7 Results regarding the genetic distance of basil genotypes

Nr cart.	**Genotypes**	**Gene distance (come) Marker *Og* A07**	**Gene distance (come) Marker *Og* B04**
1.	Aromat de Buzău basil	1/04	1/42
2.	BZ 1	1/04	1/42
3.	Hof Igal 2	1/04	1/42
4.	Gea	1/04	1/42
5.	LBRS 2	1/12	1/62
6.	LBVS 1	1/12	1/62
7.	Serafim basil	1/12	1/62
8.	Sweet basil	1/04	1/42
9.	Basil Limonero	1/12	1/62
10.	Greek basil L3	1/12	1/62
11.	Basil Frunze de salat L4	1/21	1/69
12.	Basil Grand Vert L5	1/21	1/69
13.	Persin basil	1/21	1/69
14.	Basil Spicy Globe L7	1/21	1/69
15.	African basil	1/21	1/69
16.	Basil Krishna L9	1/21	1/69
17.	Basil Macedon	1/21	1/69
18.	Basil Piti Emarald	1/21	1/69

19.	Dark Opal basil	1/21	1/69
20.	Basil Siam Queen	1/21	1/69
21.	Basil Cinnamon	1/21	1/69

(sutras/source: original)

Table 7. shows the results of the gene distance calculation for the two markers that showed the highest percentage of polymorphism, ObGA07 and Og B04. Both molecular markers establish the presence of the ObGAPDH gene, but the significance of its expression covers more distant characters for foreign genotypes.



(sutras/*source*: original)

Figure 3 Results concerning the bands obtained from three varieties of the test primers Og

Among the studied primers, five amplified bands in only one variety Hofigal 2, (ObGA07, ObGA08, ObGA11, ObGA13, ObGA18 and ObGB04), and 2 of the primers: ObGA09 and ObGA10 did not amplify any fragment in any of the varieties of basil (figure 3.), and the amplified bands had poor clarity, not being able to make a fair assessment of them. There could be an explanation related to the DNA samples not having the quantity or purity required to give polymorphism with the primers tested, although they are universal and should have given results, the polymerase used was Aplite Polymerase (Stoffel) and not Promega, and the amplification power may have been lower.

4. Conclusion

In the laboratory, the bands were detected using the Faliscan program, by comparing them with a standard DNA sample, with a size between 100 and 4000 bp. All the fragments resulting from the amplification with the polymorphic primers were between 200 and 2000 bp in length, most of them between 300 and 1200 bp (in the first case of amplification). The lower the values of the genetic distances in the table (binary matrix), the more closely related the genotypes are. These primary data were entered into the RAPD distance 1.04 program which calculated the genetic distances based on the Jaccard coefficient. Rresults regarding the calculation of the genetic distance for the two markers that showed the highest percentage of polymorphism ObGA07 and Og B04. Both molecular markers establish the presence of the ObGAPDH gene, but the significance of its expression covers more distant characters for foreign genotypes. The choice of primers proved quite difficult, out of 8 primers used in total, only 2 gave distinct polymorphic bands, among them: ObGA07 and ObGB04.

It turned out that the RAPD technique is a simple and fast method for revealing genetic polymorphism in basil genotypes according to the Doyle Doyle protocol or the CTAB method.

The RAPD technique is extremely sensitive to working conditions (apparatus, reagents, solution concentrations, their pH, etc.). Optimizing the RAPD protocol can be an extremely difficult problem, changing the reaction components or the program used can have unpredictable consequences.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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