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GENETIC POLYMORPHISM AND PRODUCTION CAPACITY OF SOME ROMANIAN-NATIVE WILD ISOLATES OF *PLEUROTUS OSTREATUS*

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Abstract

Pleurotus ostreatus is a globally significant edible mushroom. Its ecological versatility and rapid growth make it essential for sustainable agriculture, waste valorization, and functional food production. The aim of this study is to explore the genetic variability and fruiting potential of seven Romanian-native wild isolates of P. ostreatus compared with some commercial strains of P. ostreatus, P. columbinus, and P. citrinopileatus from the RDIVFG Vidra collection. Genetic variability was assessed using the RAPD technique. Results showed a high degree of genetic divergence among isolates, highlighting intraspecific variability and clear genetic structuring within the analyzed P. ostreatus isolates. In mushroom farm testing for fruiting potential, the seven wild isolates of P. ostreatus were outperformed in mushroom production by the commercial P. ostreatus strains PoM-421 and PoM-77. However, differences were not very large in terms of quantity and, especially, quality. Among the wild isolates, the highest average yield was recorded at Po5C, 567.4 g of mushrooms per 2.6 kg of lignocellulosic substrate (21.82%). This value was only 20.4 g lower than the PoM-77 control, revealing a statistically insignificant difference.

Key words*: macromycetes, oyster mushroom, RAPD, wild isolates.*

INTRODUCTION

The genus *Pleurotus* includes numerous species of ligninolytic basidiomycetes with great ecological, nutritional, and biotechnological importance and with medicinal potential; the best-known and most cultivated of them being *Pleurotus ostreatus* (Jacq.) P. Kumm (Mastranzo-Pérez et al., 2025). The performance achieved in cultivating these mushrooms both in commercial and household systems is due to rapid mycelial growth, wide adaptability to substrates, efficient lignocellulose degradation, and the ability to convert low-value agricultural residues into biomass rich in high-quality proteins (Rusu et al., 2022; Juárez-Hernández et al., 2023; Zăgrean et al., 2024; Miśkiewicz et al., 2025). Advances in the application of breeding programs for *P. ostreatus* mushrooms, as well as for other cultivated species, have led to the introduction on the market of valuable commercial strains that meet the demand for higher yields and superior quality (Barh et al., 2019). However, commercial strains represent only a limited number of genotypes and often lose their performance after several consecutive subcultures and/or a long period of storage on the culture medium, causing reduced yield (Melanouri et al., 2022). A valuable aid can come from wild strains, which constitute important genetic material adapted to their ecosystems of origin and, as a result, require characterization and preservation. Collecting specimens of *P. ostreatus* basidiocarps grown in different areas from the spontaneous mycobiota and obtaining viable mycelial isolates from them, as in the case of other edible macrofungal species, provides the surplus genetic variability necessary for introducing or improving useful traits, with the aim of achieving higher yields and/or phenotypic traits demanded by production and market (Barh et al., 2019; Cruz-Villegas et al., 2024; Cai et al., 2025). The integration-preservation of these in the strain collection (fungal germplasm bank) and the evaluation of their biological and cultural potential constitute favorable premises for diversifying the cultivated assortment by increasing the number of new indigenous varieties or strains capable of utilizing the large

quantities of recyclable natural materials resulting from existing agro-forestry and livestock operations (Zăgrean et al., 2025). The classical taxonomy of fungi uses exclusively morphological, cultural, and biochemical characteristics for the identification and evaluation of such wild isolates from different geographical areas. Their discriminatory ability is not perfect, as it presents certain limitations. Most morphological and agronomic characteristics are quantitative traits that can be easily affected by environmental variation (Li et al., 2019). Nevertheless, these criteria are still widely used, being considered acceptable, and always constituting valuable, guiding complementary elements that support the determination of a precise systematic classification at the species/genus level, ultimately based on molecular genetics techniques. Molecular analysis is necessary to clarify the taxonomic and phylogenetic relationships within certain groups of *Pleurotus* (Cruz-Villegas et al., 2024). The molecular markers could directly reflect the genetic diversity at the DNA level. Among the molecular markers used to verify identity confusions of *P. ostreatus* strains and to assess genetic diversity at the species level are amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), and combinatorial tubulin based polymorphism (cTBP) (Popa et al., 2017; Li et al., 2019).

In the present study, seven wild isolates of *P. ostreatus* were obtained from various regions of Romania and evaluated for fruiting potential in comparison with two commercial strains of the same species. In parallel, the RAPD molecular technique was used to assess the genetic diversity and phylogenetic relationship among the wild strains, along with three additional commercial strains belonging to *P. ostreatus*, *P. columbinus*, and *P. citrinopileatus*, respectively. The aim of this study was to assess the fruiting performance and genetic diversity of these Romanian-native wild *P. ostreatus* strains in comparison with established commercial strains, in order to identify new genotypes with valuable agronomic potential for cultivation and strain improvement.

MATERIALS AND METHODS

Biological material. Specimens of *P. ostreatus* mushrooms were collected from the spontaneous mycobiota of natural habitats located in different areas of Romania, specifically in four counties and Bucharest: Po1Br (forest, Brebu, Prahova Co.), Po2T (forest, Tigveni, Argeș Co.), Po3M (forest, Mogoșoaia, Ilfov Co.), Po4V (meadow, Vidra, Ilfov Co.), Po5C (forest, Comana, Giurgiu Co.), Po6M (forest, Mogoșoaia, Ilfov Co.), Po7B (park, sector 5, Bucharest).

Preliminary identification and taxonomic classification based on morphological characteristics of the collected mushroom specimens were carried out in accordance with the data provided by the studied specialized literature (Sălăgeanu & Sălăgeanu, 1985; Șesan & Tănase, 2004; Kuo, 2017).

From the collected carpophores, seven wild mycelial isolates were obtained using the tissue culture method: tissue blocks of 2-3 mm, aseptically excised from the inner part of the cap trama, were cultured on Petri dishes with malt extract agar medium (MEA) containing 17 g/L malt extract broth (VVR Chemicals) and 20 g agar, pH 6.5 and incubated in the dark at 24-26°C for 14-16 days. The obtained wild isolates were clonally multiplied on MEA Petri plates and subsequently propagated in the laboratory, with the resulting mycelium intended partly for preservation in the Collection and partly for use in evaluation and characterization work based on *in vitro* morphophysiological and cultural criteria (Zăgrean & Șovărel, 2025 - previous study). Other mycelial subcultures of the wild *P. ostreatus* isolates were intended for this assessment of intraspecific genetic variability at the molecular level and fruiting potential in the testing mushroom growing unit.

In order to evaluate the fruiting capacity of the seven wild isolates, two commercial strains from the RDIVFG Vidra Collection, namely PoM-421 and PoM-77, were used for comparison.

Seeding spawn has been prepared on a granular support: pre-boiled wheat grains (moisture 65%) and fined with 6% $CaSO_4$ and 2% $CaCO_3$ were distributed in 400 g portions into 500 ml Erlenmeyer flasks and sterilized in an autoclave for 90 minutes at 123°C. The flasks with sterilized granular substrate were inoculated in a

laminar flow hood using pure cultures on agar medium from the mycelia of the experimental isolates. After inoculation, they were incubated at 24-26°C in the dark for 16-18 days until the total colonization of the granular support.

Substrate preparation, inoculation and incubation. The culture substrate was prepared from chopped wheat straw and ground corn cobs, moistened to saturation with water overnight. The excess water was removed, and $CaSO_4$ (6%), $CaCO_3$ (2%) and natural nutritional supplements consisting of wheat bran, cornmeal and sunflower seed cake (3% w/w, each) were added. The homogenized lignocellulosic material, adjusted to 65-68% moisture, was divided into 1.3 kg portions in polypropylene-autoclavable bags fitted with cotton plugs, which were then sterilized for 90 minutes at 123°C. After cooling, they were inoculated under a laminar flow hood with the experimental strains' spawn at a rate of 5% w/w. Subsequently, the bags with inoculated substrate (6 bags for each strain) were placed for incubation at 24-26°C, in the absence of light. At the end of the incubation period (3-4 weeks), the bags with colonized substrate were transferred into PVC culture vessels (4 liters) and moved to the test mushroom growing room to verify their fruiting capacity. The individual replicates of each variant were arranged in a completely randomized design.

Mushroom cultivation. To initiate the formation of mushroom primordia, the temperature in the growing room was reduced to 15-16°C; relative humidity was maintained at 92-94%, light was provided for 8 hours daily at 600-700 lux, and CO_2 concentration was kept below 1000 ppm by introducing fresh air. During the production period, the air temperature was maintained at 16-19°C, the CO_2 level under 1200 ppm, relative humidity was lowered to 82-85%, and light was provided for 10 hours daily at 700-800 lux. The yield of mushrooms was evaluated for the first harvest flush.

Statistical analysis. Experiments were carried out in triplicate (1 repetition = 2 bags x 1.3 kg substrate/bag = 2.6 kg substrate) and results were expressed as means ± standard deviation (SD). The data were analysed by analysis of variance (ANOVA) using Microsoft Office Excel 2010. Checking the significance of the differences between variants was done based on limit differences (DL 5%, DL1%, DL 0,1%) and by Duncan′s multiple range test at $p < 0.05$.

Molecular analysis. The seven autochthonous *P. ostreatus* strains were analysed compared to three authenticated reference strains: *P. ostreatus* PoM-421, *P. citrinopileatus* Pc95, and *P. columbinus* Pc98. For studying the genetic polymorphism of the Romanian-native *P. ostreatus* wild strains, Random Amplified Polymorphic DNA (RAPD) analysis was performed using oligonucleotide primers from Carl Roth GmbH + Co. KG (Karlsruhe, Germania) (Table 1).

Table 1. Primers used for RAPD analysis

Primer	Sequence	Annealing temperature
Roth 01	5'-CTG GCC CTT C-3′	34 °C
Roth 02	5'-TGC CGA GCT G-3'	
Roth 06	5'-GGT CCC TGA C-3′	
Roth 09	5′-GGG TAA CGC C-3′	
Roth 13	5′-CAG CAC CCA C-3′	

*Legend: A- adenine; C- cytosine; G- guanine; T- thymine.

Amplification was performed in a final reaction volume of 20 µL per sample. The reaction mixture included 1X reaction buffer with $MgCl_2$, 0.2 mM deoxynucleotides, 5 pmol/µL of primer, 0.4 U of Dream Taq DNA Polymerase (Thermo-Scientific LSG) and 20 ng template DNA. The amplification program initiated with a denaturation step at 94ºC for 5 min, followed by 45 cycles of 1 min at 94ºC for denaturation, 45 s at 34ºC for primer annealing, 90 s at 72ºC for extension, then a final step of elongation for 7 min at 72ºC. The PCR products were analysed in 1.2% agarose gel supplemented with ethidium bromide, in 0.5 X Tris-Borate-EDTA (TBE) as migration buffer (VWR International GmbH). To estimate the size of the amplicons, the samples were compared with HyperLadder 100 pb, and 1Kb (Meridian BioScience) respectively. Samples were migrated by horizontal gel electrophoresis at 70 V for 2 hours and 30 min. The electrophoretic profiles were visualized under UV light using the BioDoc-It Imaging System (Ultra-Violet Products Ltd.).

The molecular fingerprints generated by the electrophoretic profiles of RAPD reactions were visually compared. Amplicons having the same length were considered a locus and scored in a binary matrix, where each PCR product was recorded as present and coded as 1, or absent as 0, across genotypes. The data were used to

generate a phylogenetic tree using the TreeCon software (version 1.3b) for Windows based on the Lei & Li (1979) genetic distance estimation method and the Unweighted Pair-Group Method using arithmetic Averages (UPGMA) clustering analysis. The Shannon-Weaver index (H') was also calculated within the *P. ostreatus* group to evaluate the intraspecific genetic diversity, as well as for the whole set of analysed strains to evaluate the interspecific genetic diversity.
Therefore, the Shannon-Weaver (H') index per locus (Spellerberg & Fedor, 2003) was computed using the following formula:

$$H'_{locus} = -(p_{1,i}\, ln\, +\, p_{0,i}\, ln\,)$$

where:
$p_1 = n_1$ / total number of loci;
n_1 = number of present loci;
ln = natural log;
$p_0 = n_0$ / total number of loci;
n_0 = number of absent loci;
$0 < H'_{locus} < \ln(2)$.

While the total H' and mean H' were as follows:

$$H'_{total} = \sum_{i=1}^{n} [\, -(p_{1,i}\, ln\, +\, p_{0,i}\, ln\,)]$$

$$\bar{H}' = \frac{H'_{total}}{n}$$

where:
i = locus;
n = number of total detected loci;
$H'_{total} \leq n \times \ln(2)$.

The Polymorphic Information Content (PIC), which quantifies how informative each locus is for distinguishing among genotypes, was also calculated for all polymorphic loci generated by the five RAPD primers. This analysis provided an estimate of the overall discriminatory power of the marker set to differentiate the studied *Pleurotus* spp. and *P. ostreatus* strains (Singh et al., 2014). The PIC value was computed using the formula:

$$PIC = 1 - p^2 - q^2$$

where:
p = frequency of band presence (binary encoded as 1);
q = frequency of band absence (binary encoded with 0);
Within the *P. ostreatus* group, the Percentage of Polymorphic Bands (PPB) were also evaluated as follows:

$$PPB = \frac{\text{Number of polymorphic bands}}{\text{Total number of loci}} \times 100$$

These indices were used collectively to characterize the genetic variability revealed by the RAPD profiles and to determine the discriminatory efficiency of the marker system across the examined *Pleurotus* genotypes.

RESULTS AND DISCUSSIONS

All seven wild strains of *P. ostreatus* and the two commercial ones supported primordia formation and mushroom production under the conditions of our experiment. The the time required for complete colonization of the substrate (incubation period), time elapsed from the inoculation of the culture substrate until the visible appearance of fruiting primordia (earliness), yield, and biological efficiency are important parameters of the cultivation cycle of *P. ostreatus* in particular and of edible mushrooms in general, and they are strongly dependent on the interaction between the culture substrate and the tested strain/species (Hoa et al., 2015; Melanouri et al., 2022; Timm et al., 2025). In our study, incubation period and earliness varied among the strains tested. Almost all the tested strains showed statistically significant individual differences ($p < 0.05$) regarding the time of substrate colonization (Table 2).

Table 2. The incubation period and earliness in cultivation of a variety of *P. ostreatus* strains - 7 wild strains and 2 commercial ones

Strain	Strain type	Incubation* (days)	Earliness* (days)
Po1Br	Wild	23.83 ± 1.32 c	30.17 ± 1.15 a b c
Po2T	Wild	29.33 ± 1.26 g	41.33 ± 1.04 g h
Po3M	Wild	26.17 ± 1.53 f	38.83 ± 1.04 g h
Po4V	Wild	24.00 ± 0.50 c	33.17 ± 0.76 d e
Po5C	Wild	24.17 ± 0.29 c d	31.83 ± 1.76 c d
Po6M	Wild	24.83 ± 0.58 d e	34.17 ± 0.76 e f g
Po7B	Wild	24.00 ± 0.50 c	33.67 ± 0.76 d e f
PoM-421	commercial	20.83 ± 1.15 a	28.67 ± 0.29 a
PoM-77	commercial	22.83 ± 0.29 b	29.83 ± 1.26 a b

*Mean values with standard deviations from 3 replicates.
Mean values of each parameter within the same column, not sharing common letters, are significantly different ($p < 0.05$)

Table 2 shows that when cultivated on a substrate of wheat straw and corn cobs supplemented with wheat bran, corn meal, and sunflower seed cake, the two commercial strains of *P. ostreatus* colonized the substrate faster

than the wild ones, namely in 20 days (PoM-421) and 22 days (PoM-77), with significant differences ($p < 0.05$). Among the wild strains, the fastest colonization was reported for the strain Po1Br (23 days), followed closely, with a non-significant difference, by Po4V, Po7B and Po5C (24 days). They showed good adaptability and rapid mycelial colonization, suggesting high nutrient utilization efficiency and active lignocellulolytic enzyme systems (Cai et al., 2025). Po2T colonized the entire substrate the latest (29 days), with a significant delay compared to the others. Results of the present study are in accordance with the findings of Melanouri et al. (2022), who, in a study on the cultivation of *P. ostreatus* and *Pleurotus eryngii* on 10 agro-industrial residues and the influence of the strain/species-substrate interaction, obtained complete colonization of *P. ostreatus* strains in between 16 and 38 days.

Intraspecific variability also decisively influenced the earliness of the seven wild strains of *P. ostreatus*, with the appearance of primordia occurring between 30 and 41 days after spawning: first primordia appeared in isolate Po1Br (30 days), followed by the others Po5C (32), Po4V (33 days), Po6M and Po7B (34 days), with Po3M (39 days) and Po2T (41 days) being later (Tabel 1). The commercial strains formed primordia earlier than the wild-type ones. The first primordia appeared in the PoM-421 strain 29 days after spawning, followed by PoM-77 (30 days), this possibly correlates with their faster ability to colonize the lignocellulosic substrate and the corresponding shortening of the incubation period.

Consistent results regarding the effect determined by genetic factors on the earliness of *P. ostreatus* strains have also been reported by others. The study by Koutrotsios et al. (2017) on the cultivation of 16 commercial and wild *P. ostreatus* strains on pasteurized wheat straw revealed a wide variation in earliness, with earliness time ranging from 21 to 71 days.

Bekiaris et al. (2025) obtained results slightly different from ours: by cultivating 5 strains of *P. ostreatus* on substrates obtained from agro-industrial by-products and agricultural waste enriched with various supplements, they reported the staggered appearance of their primordia between 14 and 18 days after spawning.

All experimental wild isolates developed healthy basidiomata, predominantly forming tiered clusters, with normal individual morphology typical for the species *P. ostreatus*: large, fleshy, shell-shaped, slightly concave caps, with margins usually entire and turned up in the young stage; cap cuticle colored gray with darker shades on some (Po4V and Po7B), lighter on others, and with small radial striations (Po1Br, Po3M, Po6M, and PoM-421); nude hymenophore, with long gills on the underside of the cap, decurrent, cream-beige in color; stem with an excentric, rarely lateral insertion, medium short, slightly curved, fused towards the base in clusters (Figure 1).

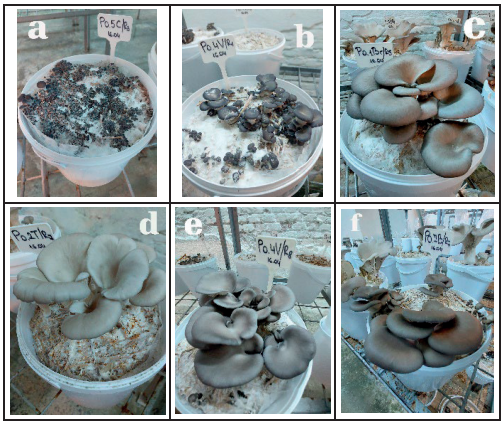

Figure 1. Wild strains of *P. ostreatus* cultivated in plastic bags/vessels: primordia (a, b) and basidiocarps ready for harvest (c-f)

Intraspecific variability also had a considerable effect on mushroom yield and biological efficiency of the nine strains of *P. ostreatus* tested. In Tables 3 and 4 it can be seen that the highest production of the first flush of mushrooms was recorded for the commercial strain PoM-421, with an average of 612.9 g of mushrooms per 2.6 kg of substrate (2 culture bags/vessels), a value corresponding to a harvest yield of 23.57%, surpassing the other commercial strain, PoM-77, designated as the control, by a non-significant surplus of 25.1 g (+0.96%). The control PoM-77 ranked second with a production of 587.8 g, equivalent to a yield of 22.61% (22.61 g of fresh mushrooms harvested per 100 g of culture substrate, or more illustratively, 22.61 kg of mushrooms per 100 kg of culture substrate).

Table 3. Mushroom yield and biological efficiency in a variety of 7 wild isolates and 2 commercial strains of *Pleurotus ostreatus* (1st flush)

Strain	Strain type	Yield*		Biological efficiency (BE)
		(g)	(%)	(%)
Po1Br	wild	482.9 ± 10.76	18.57 ± 0.42	56.28 ± 1.25
Po2T	wild	394.5 ± 17.99	15.17 ± 0.70	45.98 ± 2.11
Po3M	wild	464.1 ± 7.66	17.85 ± 0.30	54.09 ± 0.92
Po4V	wild	439.7 ± 12.91	16.91 ± 0.50	51.24 ± 1.51
Po5C	wild	567.4 ± 15.55	21.82 ± 0.60	66.13 ± 1.81
Po6M	wild	410.3 ± 18.77	15.78 ± 0.72	47.82 ± 2.19
Po7B	wild	390.5 ± 16.75	15.02 ± 0.44	45.52 ± 1.95
PoM-421	commercial	612.9 ± 21.38	23.57 ± 0.82	71.43 ± 2.48
PoM-77	commercial	587.8 ± 20.16	22.61 ± 0.78	68.51 ± 2.38

*Mean values with standard deviations from 3 replicate
Yield (g): weight of fresh mushrooms (g) per 2.6 kg of cultivated substrate (2 bags, each with 1.3 kg substrate)
Yield (%): average weight of fresh mushrooms (g)/2.6 kg of cultivated substrate x 100
Biological efficiency (BE%): average weight of fresh mushrooms (g)/weight of dry substrate (g) × 100

Table 4. The influence of strain on the yield in a variety of 7 wild isolates and 2 commercial strains of *Pleurotus ostreatus* (1st flush)

Strain	Strain type	Average yield*			Significance of difference
		(g)	%	±d (g)	
Po1Br	wild isolate	482.9	82.15	-104.9	ns
Po2T	wild isolate	394.5	67.11	-193.3	0
Po3M	wild isolate	464.1	78.95	-123.7	ns
Po4V	wild isolate	439.7	74.80	-148.1	ns
Po5C	wild isolate	567.4	96.52	-20.4	ns
Po6M	wild isolate	410.3	69.80	-177.5	0
Po7B	wild isolate	390.5	66.43	-197.3	0
PoM-421	commercial strain	612.9	104.27	25.1	ns
PoM-77 (control)	commercial strain	587.8	100	-	-

*Yield (g): weight of fresh mushrooms (g) per 2.6 kg of cultivated substrate (2 bags, each with 1,3 kg substrate)

DL 5% 149.48
DL 1% 205.88
DL 0.1% 283.45

Among the wild isolates, Po5C achieved the highest average yield, namely 567.4 g of fresh mushrooms per 2.6 kg of substrate (21.82%), just 20.4 g less than the control PoM-77, a statistically non-significant difference. It was followed, in decreasing order of yield harvested in the first flush, by isolates Po1Br with 482.9 g of mushrooms (18.57%), Po3M with 464.1 g of mushrooms (17.85%), and Po4V with 439.7 g of mushrooms (16.91%), also with non-significant negative differences compared to the control. The lowest mushroom productions were recorded for the strains Po2T with an average of 394.5 g of mushrooms (15.17%) and Po7B with 390.5 g of mushrooms (15.02%), with significant negative differences of -193 g and -197.3 g of mushrooms, respectively, compared to the control PoM-77.

In general, substrates that gave higher yields also gave higher values of BE (Melanouri et al., 2022). The experimental results of the present study showed that the highest BE values were recorded in the commercial strains PoM-421 (71.43%) and PoM-77 (68.51%), while among the wild types, Po5C (66.13%) stood out, followed by Po1Br (56.28%) and Po3M (54.09%). These wild strains have proven to have a better conversion efficiency of lignocellulosic substrate, which makes them recommended for future testing.

Based on the molecular analysis of the tenth *Pleurotus* strains studied, a total of 79 loci were distinguished after five RAPD amplifications. Using the Roth 01 primer, 15 loci were differentiated (Figure 2), whereas with the ROTH 02 primer, 20 loci (Figure 3).

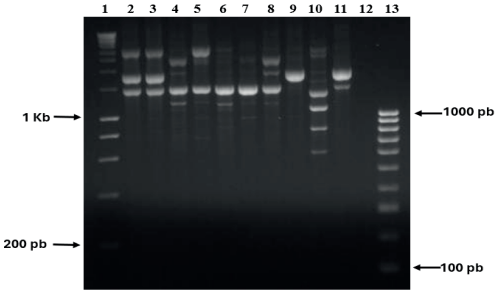

Figure 2. The RAPD electrophoretic profile obtained with the Roth01 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

When using the Roth 01 primer, the electrophoretic profiles of the Po1Br and Po2T strains proved to be identical. However, some variations can be noted in the electrophoretic patterns of the remaining *P. ostreatus* strains, even though certain amplicons are shared among specific loci. However, a substantial difference was noticed between *P. ostreatus* strains and *P. columbinus* Pc98, compared to *P. citrinopileatus* Pc95, based on their electrophoretic profiles, the latter having a completely different pattern from the rest of the strains studied (Figure 2).

P. citrinopileatus Pc95 showed a distinct electrophoretic pattern from the *P. ostreatus* strains and *P. columbinus* Pc98, also when RAPD was performed with the Roth 02 primer (Figure 3).

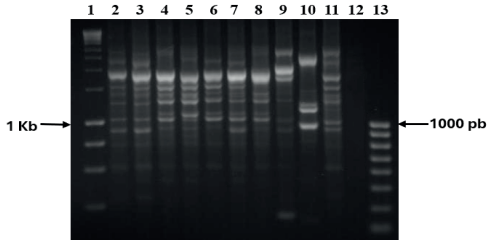

Figure 3. The RAPD electrophoretic profile obtained with the Roth02 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

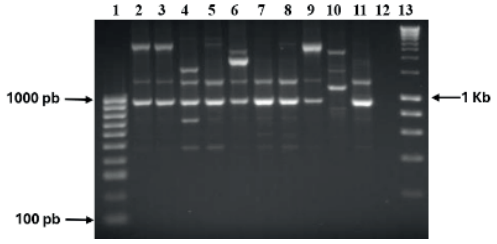

Figure 4. The RAPD electrophoretic profile obtained with the Roth09 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

The electrophoretic profiles generated with the Roth 02 primer indicate that Po1Br and Po2T strains share a nearly identical pattern and display high similarity with the Po6M strain. Apart from some variations in the intensity of individual bands, the *P. ostreatus* Po3M, Po4V, Po5C and Po7B strains also display almost identical electrophoretic patterns among them when using the Roth 02 primer. While the *P. ostreatus* reference strain (Po-421) differed significantly from the rest of the native *P. ostreatus* strains.

Analysing the RAPD electrophoretic patterns obtained with the Roth 06 primer, it was observed that Po1Br and Po2T strains have no detectable amplicons across all loci. The *P. ostreatus* Po3M, Po4V, Po5C, Po6M, Po7B strains, and the P. *columbinus* Pc98 reference strain, displayed a remarkable similar pattern, revealing the same diagnostic loci. *P. ostreatus* PoM-421 reference strain showed a comparable pattern with the previously mentioned strains, but lacking one of the common amplicons. Regarding the *P. citrinopileatus* Pc95 reference strain, it displayed a completely distinct pattern, sharing no common loci with the other strains examined.

When using the Roth 09 primer, the electrophoretic profiles of the amplification products from the Po1Br and Po2T strains proved to be very similar to those of the P." *O. ostreatus* PoM-421 reference strain, the recorded differrences being in terms of the intensity of the bands obtained. Likewise, the Po4V, Po6M and Po7B strains showed some similarities among them. In contrast, the Po3M and Po5C strains differed considerably between themselves and from the rest of the strains of the same species (Figure 4).

As in the previous RAPD reactions, the P. *citrinopileatus* Pc95 strain showed a discordant note in terms of the electrophoretic profile when using the Roth 09 primer. A single amplicon from this strain shared a similar size with an amplification product from Po5C strain. In contrast, *P. columbinus* Pc98 presented an electrophoretic profile similar to the native *P. ostreatus* strains Po4V, Po6M and Po7B (Figure 4).

When using the RAPD primer Roth13, the electrophoretic profiles of the Po1Br and Po2T strains proved to be identical. Some similarities can also be observed between the electrophoretic profiles of the PoM-421 and Po7M strains, with some differences between the loci for which amplification products were obtained. Substantial differences, compared to the rest of the *P. ostreatus* strains, were noted in the Po3M and Po4V strains, which revealed distinctive electrophoretic patterns (Figure 5).

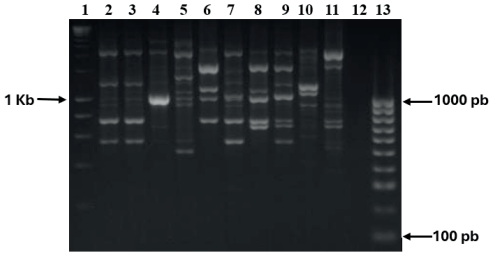

Figure 5. The RAPD electrophoretic profile obtained with the Roth13 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

P. columbinus Pc98 strain shared some similarities with *P. ostreatus* PoM-421 and

Po7M. However, the electrophoretic profile of *P. citrinopileatus* Pc95 varied noticeably from that of the other studied strains.

Compiling the results from RAPD analysis, the phylogeny of the studied *Pleurotus* spp. strains was determined. The electrophoretic profiles were converted into a binary matrix and processed in TreeCon using UPGMA clustering based on the Nei-Li coefficient, with 100 iterations. The molecular fingerprints generated by the RAPD primers allowed the construction of a phylogenetic tree for the ten *Pleurotus* spp. strains included in the genetic study (Figure 6).

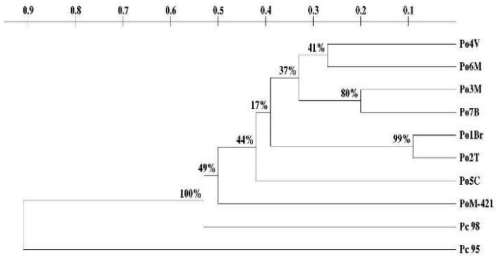

Figure 6. Dendrogram revealing the phylogeny of the studied *Pleurotus* spp. strains based on RAPD analysis with five oligonucleotide primers

The dendrogram revealed no deviations from the expected taxonomic relationship among the *P. ostreatus* strains, with the Romanian native strains clustering together with the reference strain PoM-421. The *P. columbinus* Pc98 strain was grouped closely with this cluster, whereas the *P. citrinopileatus* Pc95 strain appeared more distantly related (Figure 6).

According to the genetic distance values, Po1Br and Po2T exhibit a distance below 0.1, indicating an extremely high genetic similarity (Sharma et al., 2018). Similarely, Po1Br and Po2T, Po3M and Po7B, Po4V and Po6M, together with Po5C show genetic distance values below 0.45 among themselves, suggesting a relatively recent common origin, consistent with their Romanian provenance, despite originating from different locations. The reference strain PoM-421 remained grouped together with the autochthonous *P. ostreatus* strains, showing a moderately low distance from *P. columbinus* Pc98.

Among the five RAPD markers used, the Roth 13 primer revealed the highest number of loci (23), followed by Roth 02 with 20 distinctive amplicons, thereby increasing more substantially the resolution of the genetic variability analysis.

The combined diversity metrics derived from the RAPD dataset indicate that the ten *Pleurotus* strains exhibit a high level of overall genetic variability across the 79 detected loci. This is reflected in the total H' value of 36.97 and the mean H' of 0.468 (Table 5), that the genetic variation is broadly distributed across many loci, and that the presence/absence patterns are relatively heterogenous. In contrast, when the analysis was restricted to the subset of *P. ostreatus*, the total H' index decreased to 26, while the mean H' dropped to 0.329 (Table 5). This reduction is understandable, as 28 loci were found monomorphic in this group. The remaining 51 polymorphic loci contributed to the observed diversity, although with lower average entropy per locus, indicating more unevenly distributed bands.

Table 5. Integrated diversity metrics

Group\Index	Total H'	Mean H'	Polymorphic loci	PIC	PPB
P. ostreatus group	26.00	0.329	51	0.329	64.56%
All ten strains	36.97	0.468	79	0.468	

The Shannon-Weaber diversity index indicates that the *P. ostreatus* group possesses a moderate genetic variability, whereas the full set of *Pleurotus* strains shows a broad and more evenly distributed diversity.

The mean PIC value of 0.466 obtained for the full set of ten strains suggests that the dataset contains rich and well-distributed genetic information across the ten *Pleurotus* strains. Moreover, a PIC value of such magnitude confirms that the identified loci exhibit moderately high presence/absence diversity, and to each locus this variation is evenly distributed, therefore, increases the entropy and discriminatory power of the marker. However, the mean PIC value of 0.329 found in *P. Ostreatus* group reflects a moderate level of marker informativeness, still providing useful discriminatory power to distinguish the strains within this group.

The PPB value of 64.56% indicates that approximately two-thirds of the RAPD loci (more precisely, 51 of the total 79 loci) are polymorphic within the *P. ostreatus* group. This means that most markers detect some level of genetic variation among the *P. ostreatus* strains,

while the remaining 28 loci are monomorphic and reflect shared genomic markers with *P. citrinopileatus* Pc95 and *P. columbinus* Pc98 strains.

These results all together confirm that the analysed strains possess considerable genetic heterogeneity, and the RAPD marker system used is suitable for assessing the diversity within *Pleurotus* spp.

CONCLUSIONS

In our study, all tested *P. ostreatus* strains, both the seven wild strains and the two commercial ones, supported the formation of primordia and mushroom production when cultivated on a substrate of wheat straw and corn cobs supplemented with wheat bran, cornmeal, and sunflower seed cake.

The group of wild strains showed a well-defined intraspecific variability, which influenced the expression of several important cultivation cycle parameters: substrate colonization capacity, timing of mushroom emergence and their morphological characteristics, yield, and biological efficiency. The two commercial strains of *P. ostreatus* showed better results in these parameters than the wild-type ones because they were obtained through research within applied selection and breeding programs, with the main objective of achieving mushroom yields that are superior in both quantity and quality.

Although the seven wild strains were surpassed in mushroom production by the commercial strains they were compared with in this trial, the differences were not very large, both quantitatively and, more importantly, qualitatively. They developed healthy mushrooms, predominantly forming tiered clusters, with normal individual morphology typical for the species *P. ostreatus*. Among the wild isolates, Po5C strain achieved in the 1st flush the highest yield, namely 567.4 g of fresh mushrooms per 2.6 kg of substrate (21.82%), just 20.4 g less than the control commercial strain PoM-77, a statistically non-significant difference. It was followed by the Po1Br with 482.9 g of mushrooms (18.57%), Po3M with 464.1 g of mushrooms (17.85%), and Po4V with 439.7 g of mushrooms (16.91%), also with non-significant negative differences compared to the commercial strain. These wild strains have proven to have good efficiency in converting lignocellulosic substrates, which makes them recommended for future testing and application.

The RAPD technique used for the molecular analysis of *Pleurotus* mushrooms grouped the indigenous strains of *P. ostreatus* all together at 0.5 genetic distance from the commercial reference strain Po-421. The phylogenetically closer strains are Po1Br and Po2T, and to a lesser extent the strains Po3M and Po7B, respectively, Po4V and Po6M. *P. columbinus* Pc98 proved to be phylogenetically closer to *P. ostreatus* strains, while *P. citrinopileatus* Pc95 was shown to be the most genetically distant from all the other strains.

Although the phylogenetic tree reveals the clustering and the genetic distance among the studied *Pleurotus* spp., suggesting similar strains, the diversity indices show an overall variability in the whole group.

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