International Journal of Plant & Soil Science

Volume 36, Issue 12, Page 537-543, 2024; Article no.IJPSS.128894
ISSN: 2320-7035

Molecular Diversity of Commercially Available Edible Oyster Mushroom by SSR Marker

Sanjay Kumar Bhariya [a*], Hemant Sahu [b], Vinay Premi [a] and Bamleshwar Sapre [a]

[a] Deaprtment of Molecular Biology and Biotechnology, College of Agriculture, I.G.K.V., Raipur (Chhattisgarh), India.
[b] Department of Genetics and Plant Breeding, College of Agriculture, I.G.K.V., Raipur (Chhattisgarh), India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2024/v36i125228

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128894

Original Research Article

Received: 19/10/2024
Accepted: 21/12/2024
Published: 28/12/2024

ABSTRACT

Oyster mushroom (*Pleurotus species*) are commercially cultivated worldwide. Oyster mushroom naturally found in rotten wood or agricultural lignocellulosic residues as saprophytic in nature. Due to the good amount of protein with low carbohydrates people prefer it in their daily diet. It can be alternate option of vegan protein with high lysine content up to 6-8% essential amino acid from fresh and dried sample. Our study aims to evaluated that availability of Simple sequence Repeat (SSR) PCR based marker for genetic diversity analysis on five different oyster mushrooms were also identified on the basis of polymorphism. The dendrogram obtained from UPGMA cluster

---

*Corresponding author: E-mail: sanjay.crin@gmail.com;

Cite as: *Bhariya, Sanjay Kumar, Hemant Sahu, Vinay Premi, and Bamleshwar Sapre. 2024. "Molecular Diversity of Commercially Available Edible Oyster Mushroom by SSR Marker". International Journal of Plant & Soil Science 36 (12):537-43. https://doi.org/10.9734/ijpss/2024/v36i125228.*

analysis of Jaccard's similarity values based on 7 SSR markers grouped 5 strains into two clusters species wise (*P. djamor*, *P. florida*, *P. ostreatus*, *P. pulmonarius* and *P. sajor-caju*) which confirm the reliability of PCR based molecular markers. diversity analysis also confirmed the grouping of genotypes as they were present in same group as revealed by SSR markers.

Keywords: Pleurotus species; genetic distance; SSR marker; genetic diversity; cluster analysis; polymorphisms.

1. INTRODUCTION

Genetic diversity plays a crucial role in the adaptability and survival of species, including economically and ecologically important fungi such as Pleurotus species, commonly known as oyster mushrooms (Tang et al., 2010). These species are widely cultivated due to their nutritional value, medicinal properties, and ability to grow on a variety of lignocellulosic materials, making them important for sustainable agricultural practices. In the world, 44 million tons of mushrooms are produced currently (FAOSTAT, 2023) a five-fold increase since 2000. The Asian continent accounts about 95% of global production, followed by European (3%), and American continental (1%) (Bijla and Sharma 2023). India is one of the world's top 6th mushroom-producing country, with 182000 MT produced year 2019 with 1.53% of the total production (FAO Stat, 2019). The highest oyster mushroom producing state is Odisha, followed by Maharashtra, Bihar, Haryana and Punjab. Even though Madhya Pradesh annual growth rate of mushroom production is 140.28% (metric tons) followed by Chhattisgarh 139.93%. The production of paddy straw in Chhattisgarh was approximately 9.14 million tones in year in 2018-19 (DES 2018-19). Chhattisgarh is one of the states in the country that procure paddy from the farmers at MSP so, it will also increase the area of rice production. And here, Climate is also suitable for production of all oyster mushrooms.

One of the most effective ways to assess genetic diversity is through molecular markers. Among these, Simple Sequence Repeats (SSRs), also known as microsatellites, are widely used due to their high polymorphism, reproducibility, and co-dominant inheritance. SSR markers target regions of repetitive DNA sequences scattered throughout the genome, making them powerful tools for studying genetic variation within and between populations of *Pleurotus species* (Kyung *et al*., 2009). Using SSR markers to evaluate the genetic diversity of *Pleurotus species* provides valuable insights into their population structure, evolutionary relationships, and breeding potential. This information is crucial for conservation efforts, improvement of strains for commercial production, and the identification of new strains with desirable traits such as enhanced yield, disease resistance, or specific bioactive compounds.

2. MATERIALS AND METHODS

All the materials were collected from mushroom division under department of plant pathology including *P. djamor*, *P. florida*, *P. ostreatus*, *P. pulmonarius* and *P. sajor-caju*. Seven SSR markers were identified and designed primers by using MISA-Web tool (Beier *et al*., 2017) from the downloaded genome sequence of *P. ostreatus* PC15 (AYUK00000000.1) for diversity analysis (Grigoriev *et al*., 2012 and Riley *et al*., 2014).

DNA extraction: Genomic DNA was extracted from the all mushroom samples by using Cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1990) with some modifications. CTAB buffer was prepared 2% CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA. 100 mg of fruiting body samples were prepared by grinding in a mortar and pestle. Ground samples was transferred in 2ml centrifuge tube homogenized each samples by adding 500 µl of CTAB Buffer. Mixed and thoroughly vortex for preparation of homogenized sample. And placed the tube in a 60°C water bath for 20 minutes. Then added an equal volume of chloroform/isoamyl alcohol (24:1). Samples were centrifuged for 15 minutes at 14,000 x g to separate the phases. The aqueous upper phase was transferred in 1.5 ml of centrifuge tubes and repeat thrice this extraction until the upper phase is cleared. Added equal volume of pre chilled isopropanol and incubate at 4°C for overnight to precipitate the DNA. Second day centrifuged the samples at 14,000 x g for 15 minutes and decant the supernatant without disturbing the pellet and subsequently washed with 500 µl 75% ethanol and dried it upto removal of ethanol residues. Pellet was dissolved in 20 µl TE buffer (10 mM Tris, pH 8, 1 mM EDTA).

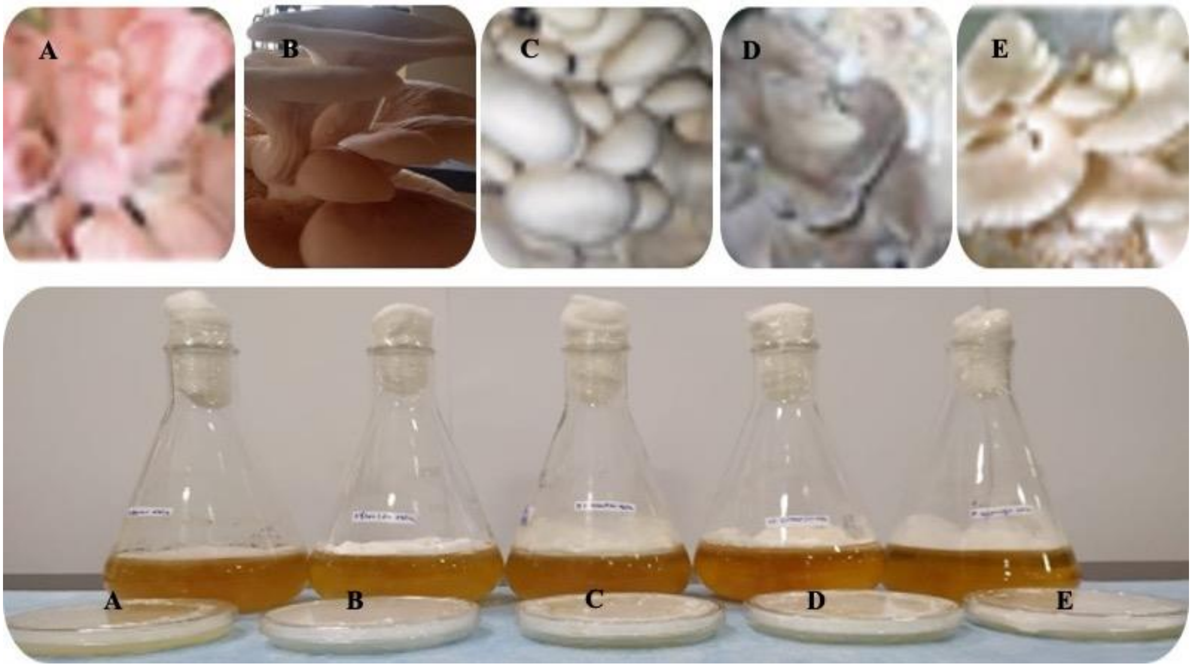

Fig. 1. Culture of different species of oyster mushroom: A. *P. djamor,* B*. P. florida*, C. *P. ostreatus*, D. *P.pulmonarius and* E*. P. sajor-caju*

Table 1. List of SSR primers for diversity analysis of *Pleurotus species*

S.No.	Primers	Sequence (5'-3')	TNB	TPB	P (%)	PIC	MI
1	PoM908	$(C)_{15}$	7	5	71.4	0.36	26.11
2	PoM909	$(AT)_{11}$	6	0	0	0.48	0
3	PoM910	$(ACG)_{6}$	17	5	29.4	0.41	12.17
4	PoM911	$(AG)_{6}$	16	5	31.2	0.39	12.16
5	PoM912	$(G)_{13}$	8	4	50	0.35	17.77
6	PoM913	$(G)_{13}$	12	5	41.6	0.41	17.19
7	PoM914	$(GGA)_{7}$	12	1	8.3	0.4	3.32

TNB= total numbers of band, TPB= Total polymorphic band, P= Polymorphism, PIC= Polymorphic information content, and MI= Marker index

SSR analysis: A SSR primer set of oligoes (Bioserve Pvt. Ltd. Bangalore) were screened purchased for the Molecular diversity analysis (Williams *et al.* 1990; Namita, P. S et al., 2013) using 5 diverse strain of oyster mushrooms. Out of these, 2 which gave sufficient polymorphism were used for assessing genetic diversity among genotypes.

The amplification reactions were carried out in a 25 µl volume containing 10X PCR buffer with $MgCl_2$, 10mM dNTPs (dATP, dCTP, dGTP and dTTP), 10 p mol/µl primer, 3 U/µl*Taq* DNA Polymerase (Bangalore Genei, India) and 50 ng/µl genomic DNA template. DNA amplifications were performed in duplicate in a DNA thermal cycler (Applied Biosystem, USA) programmed for a preliminary step of SSR amplification. DNA was denatured at 94°C for 5 minutes followed by 35 amplification cycles (94°C for 30 seconds; 35°C for 45 seconds; 72°C for 45 seconds) and a final extension step at 72°C for 7 minutes. The amplified products were resolved by electrophoresis in 2% Polyacrylamide gel run in 1X TBE buffer and amplified fragments were visualized by ethidium bromide staining. The resolved product was photographed under UV light using gel documentation Gel Doc^{TM} 5500 (Alpha InfoTech, USA; Namita, P. S et al., 2013).

Computation analysis: Genetic diversity analysis was done using NTSYS-pc Version 2.1 (Numerical Taxonomic System) software (Rohlf 2000) and similarity matrix was created to the cluster analysis of Unweighted Paired Group method using Arithmetic Averages (UPGMA) and dendrogram was constructed.

3. RESULTS AND DISCUSSION

The primary objective of this study was to assess the genetic diversity and group *Pleurotus sp.* based on molecular markers. These markers, which evaluate genome sequence composition, help to detect genetic variations among genotypes, enabling the effective use of genetic variability for crop improvement. Previous studies, such as those by Davierwala *et al*. (2000), Neeraja et al. (2002), and Saker et al. (2005), have highlighted the use of molecular markers to study genetic diversity and relationships among different crop cultivars. The use of SSR markers for species identification and molecular characterization has been well-documented, including in recent research on *Pleurotus* species in Seoul, Korea (Ryu et al., 2024). The genetic distances among five commercially cultivated oyster mushroom species are presented in Table 2.

Molecular diversity and polymorphism studies in five *Pleurotus species* were carried out through seven SSR primers. The highest Nei's genetic distance (0.73) was observed in *P. ostreatus*, *P. florida* and *P. pumlonarius* with all species except *P. djamor* and *P. sajor-caju*.The lowest genetic distance (0.44). The results revealed varying levels of genetic distance among the five Pleurotus species, ranging from low to high. Primers PoM912, PoM913, and PoM914 exhibited high genetic diversity, particularly between P. florida, P. sajor-caju, and P. florida. In contrast, primers PoM910 and PoM911 demonstrated a high degree of similarity between P. ostreatus and P. pulmonarius, followed by P. florida. Notably, primer PoM908 failed to produce any bands in the gel electrophoresis for all species (Fig. 2).

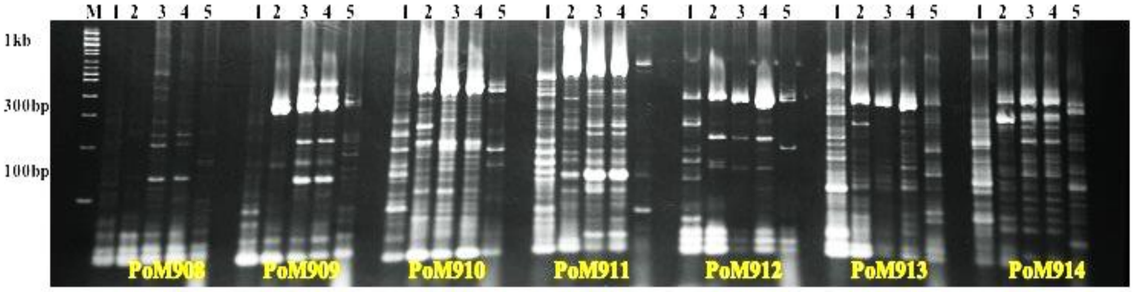

Fig. 2. Gel image of amplified product M: Ladder, 1. *P. djamor,* 2. *P. florida*, 3. *P. ostreatus*,4. *P. pulmonarius,* 5. *P. sajor-caju*and SSR markers PoM908, PoM909, PoM910, PoM911, PoM912, PoM913 and PoM914

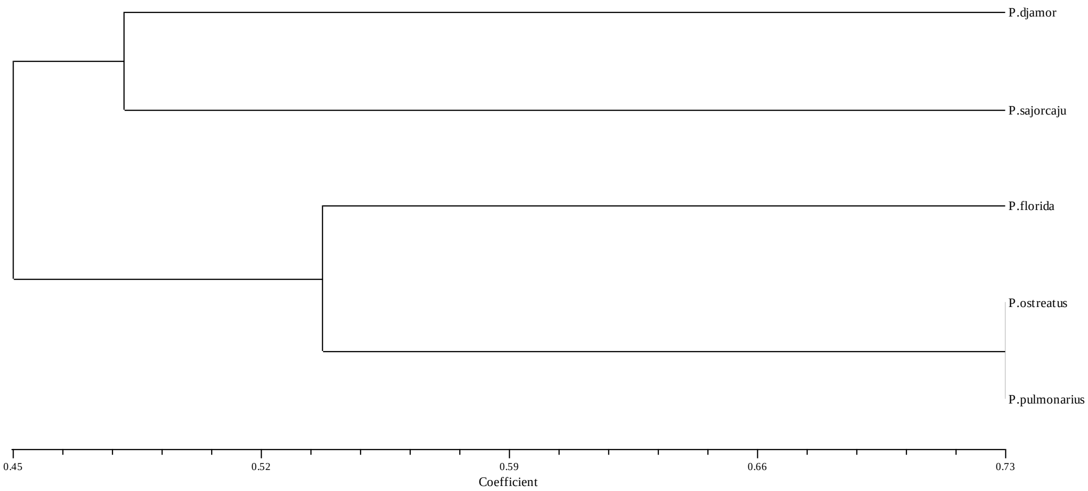

Fig. 3. Genetic diversity of species in the genus *Pleurotus* by UPGMA dendrogram. All data were analysed by using the NTSYS

Table 2. Similarity matrix of all five Pleurotus species based on SSR results

	P. djamor	***P. florida***	***P. ostreatus***	***P. pulmonarius***	***P. sajor-caju***
P. djamor	1.000				
P. florida	0.456	1.000			
P. ostreatus	0.392	0.582	1.000		
P. pulmonarius	0.430	0.494	0.734	1.000	
P. sajor-caju	0.481	0.519	0.481	0.418	1.000

3.1 Similarity Coefficient Analysis and Clustering

The relationships among Pleurotus species were estimated by a UPGMA cluster analysis of genetic similarity matrices. Similarity coefficient between different genotypes ranged from 0.392 to 1.00. Table 2 is given above:

Two major clusters were identified in the analysis. The first cluster comprised two genotypes, while the second cluster included three *Pleurotus species* (Fig. 1). Genetically similar accessions grouped together, with Group I exhibiting a 50% similarity coefficient across all five species, including two genotypes. Group II consisted of three genotypes with a 54% similarity. The similarity index revealed a high degree of similarity (73%) between *P. ostreatus* and *P. pulmonarius*, followed by *P. florida* and *P. ostreatus* (58.2%), and *P. florida* and *P. sajor-caju* (51.8%). Equal similarity (48.1%) was observed between *P. djamor* & *P. sajor-caju*. Various molecular markers, including RAPD, ISSR, and SRAP, have been used in Pleurotus diversity studies, but SSR markers were found to produce a higher frequency of polymorphisms due to their long repetitive loci. SSRs are therefore an efficient tool for genetic diversity analysis. Previous data has been described that *P. ostreatus*, *P. pulmonarius* and *P. florida* was phenotypically different but our results strongly supported that they have a close genetic relationship, compare to *P. djamor* an *P. sajor-caju* (Lin *et al*., 2022). The results will also aid in the identification and functional validation of hybrid strains, particularly those involving PoM912, PoM913, and PoM914 SSR markers.

4. CONCLUSION

The study highlights the effectiveness of SSR markers in assessing genetic diversity and clustering among *Pleurotus species*, revealing significant genetic variation and polymorphism. These findings provide valuable insights for molecular characterization and the development of improved hybrid strains. Based on the results, it is recommended to focus on further exploration of SSR markers (PoM912, PoM913, and PoM914) for their potential in identifying genetic variability and supporting hybrid strain development in *Pleurotus species*. Expanding the analysis to include more genotypes and additional molecular markers could enhance understanding of genetic relationships. Incorporating these findings into breeding programs may aid in developing high-performing, biotic and abiotic tolerant Pleurotus hybrids.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENT

Authors sincerely acknowledge the Department of Plant Pathology for providing the necessary materials for this study and thanks to the Nutritional Quality Lab of Plant Molecular Biology and Biotechnology, IGKV Raipur, for their essential support in conducting the analysis. Their contributions were pivotal to the successful completion of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Beier, S., Thiel, T., Münch, T., Scholz, U., & Mascher, M. (2017). MISA-web: A web server for microsatellite prediction. *Bioinformatics, 33*(16), 2583–2585.

Bijla, S., & Sharma, V. P. (2023). Status of mushroom production: Global and national scenario. *Mushroom Research*, 32(2), 91–98.

Davierwala, A. P., Chowdary, K. V., Kumar, S., Reddy, A. P. K., Ranjekar, P. K., & Gupta, V. S. (2000). Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. *Genetica, 108*(3), 269–284.

Directorate of Economics & Statistics, DAC&FW. (2018–2019). Rice: Area, production, and yield during 2018–19. Retrieved from https://www.tnagrisnet.tn.gov.in

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissues. *Focus, 12*, 13–15.

FAO Stat. (2019). Retrieved from http://www.fao.org/faostat/en/#data

Field, D., & Wills, C. (1998). Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 1647–1652.

Food and Agriculture Organization. (2023). FAOSTAT Statistics database 2023. Food and Agriculture Organization of the United Nations.

Grigoriev, I. V., Nordberg, H., Shabalov, I., Aerts, A., Cantor, M., Goodstein, D., et al. (2012). The genome portal of the Department of Energy Joint Genome Institute. *Nucleic Acids Research, 40*, D26–D32.

Katti, M. V., Ranjekar, P. K., & Gupta, V. S. (2001). Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Molecular Biology and Evolution, 18*, 1161–1167.

Li, Y. C., Korol, A. B., Fahima, T., Beiles, A., & Nevo, E. (2008). Microsatellites: Genomic distribution, putative functions, and mutational mechanisms: A review. *Molecular Ecology, 11*, 2453–2465.

Lin, P., Yan, Z. F., MooChang Kook, M. C., Chang-Tian Li, C. T., & Yi, T. H. (2022). Genetic and chemical diversity of edible mushroom *Pleurotus* species. *Biomedical Research International*, 1–13.

Ma, K. H., Lee, G. A., Lee, S. Y., Gwag, J. G., Kim, T. S., Kong, W. S., ... & Park, Y. J. (2009). Development and characterization of new microsatellite markers for the oyster mushroom (*Pleurotus ostreatus*). *Journal of Microbiology and Biotechnology, 19*(9), 851–857.

Namita, P. S., Sonah, H., Singh, S. P., & Sharma, T. R. (2013). Genetic diversity analysis of marigold (*Tagetes* sp.) genotypes using RAPD and ISSR markers. *Indian Journal of Agricultural Sciences, 83*(5), 484–490.

Neeraja, C. N., Sarla, N., & Siddiq, E. A. (2002). RAPD analysis of genetic diversity in Indian landraces of rice. *Journal of Plant Biochemistry and Biotechnology, 11*, 93–97.

Nei, M. (1987). Genetic distance and molecular phylogeny. In N. Ryman & F. Utter (Eds.), *Population genetics and fishery management* (pp. 193–223). University of Washington Press.

Richards, K. D., Goddard, M. R., & Gardner, R. C. (2009). A database of microsatellite genotypes for *Saccharomyces cerevisiae*. *Antonie Van Leeuwenhoek, 96*, 355–359.

Riley, R., Salamov, A. A., Brown, D. W., Nagy, L. G., Floudas, D., Held, B. W., et al. (2014). Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 9923–9928.

Ryu, J.-S., Park, B., van Peer, A. F., Na, K. S., & Lee, S. H. (2024). Quantitative trait loci analysis for molecular markers linked to agricultural traits of *Pleurotus ostreatus*. *PLoS ONE, 19*(8), e0308832.

Saker, M. M., Youssef, S. S., Abdullah, N. A., Bashandy, H. S., & El-Sharkawy, A. M. (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR, and AFLP. *African Journal of Biotechnology, 4*(9), 882–890.

Tang, L., Xiao, Y., Li, L., Guo, Q., & Bian, Y. (2010). Analysis of genetic diversity among Chinese *Auricularia auricula* cultivars using combined ISSR and SRAP markers. *Current Microbiology, 61*, 132–140.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128894