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DNA FINGERPRINTING OF COMMERCIAL MUSHROOMS BY ISSR AND SSR MARKERS FOR GENETIC DISCRIMINATION

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ABSTRACT

Research for new improved varieties, with high increase in yields and quality with disease resistance is required today to make mushroom production highly productive.Increasing the yield and quality of crops as well as resistance to diseases are the primary goals for mushroom breeders and mushroom research. Breeders now use DNA molecular markers to identify and select specific genes to locate superior traits. Thus, utilizing molecular markers to explore traits from wild isolates can expand the genetic base of the cultivated mushrooms. In current research eleven commercial mushrooms samples namely 1) A. bisporus (Button), 2) *A. bisporus* (Portobello), 3) *P. eryngii* (King Oyster), 4) *L. edodes* (shiitake), 5) H. tessellatus (Brown Shimeji), 6) H. tessellatus (White Shimeji), 7) F. velutipes (Enoki), 8) *P. ostreatus* (Oyster), 9) *P. djamor* (Pink Oyster), 10) *C. indica* (Milky), and 11) *P. florida* (Florida Oyster) were used. The ISSR and SSR markers were used for discrimination of the selected mushroom samples. The results of ISSR markers construe that some samples might originate from a single strain. The SSR amplified alleles can be used as marker for identification of mushrooms. The information on the genetic relatedness presented here will be useful for breeding programmers for detection of duplicate sample and selection of parent strains to avoid inbreeding depression.

KEYWORDS: Mushroom, ISSR, SSR, DNA Fingerprinting

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INTRODUCTION

Since ancient time mushrooms have been used as food, especially in the eastern countries and recognized as natural and healthy foods [1]. As time progress there was increase in awareness about mushrooms as they are rich in proteins and fibers contain although contain low amount of calories and fat [2]. Mushrooms are rich sources of nutraceuticals and their bioactive properties are already reported [3-5]. Earlier studies demonstrated antioxidant potential and antitumor, antibacterial, antiviral and haematological activity [6, 7]. But due to lack of clearly distinguishable characters, mushroom samples are very difficult to discriminate and hence create problems in sample protection and improvement [8]. The determination of genotypic identity is important for biological analysis about population, structure and evolutionary pathways among species [9]. For long, different DNA markers along with morphological traits have been used for the determination of genetic variations at molecular level [10]. The genetic diversity of mushrooms has been determined earlier using molecular markers like random amplified polymorphic DNA (RAPD) [11-13]. Although ISSR and SSR markers can be highly variable within a species and have an advantage over RAPDs as they utilize longer primers and more stringent annealing temperatures [14, 15]. Environmental factors along with the close genetic relation between species make their isolation and identification difficult. Sometimes commercial varieties from different companies reflect lack of genetic diversities [16]. Thus, the use of ISSR and SSR to determine genetic diversity is promising to develop new varieties with resistance to diseases or adaptation to climate changes [17]. Variability of microsatellite regions and easy application has made ISSR and SSR marker a very useful tool for most systematic and ecological evaluations [18]. In this study the ISSR and SSR molecular markers have been exploited in genetic studies of the edible mushrooms.

MATERIALS AND METHODS

Sample Collection

Eleven samples of mushrooms, 1) *A. bisporus* (Button), 2) *A. bisporus* (Portobello), 3) *P. eryngii* (King Oyster), 4) *L. edodes* (shiitake), 5) *H. tessellatus* (Brown Shimeji), 6) *H. tessellatus* (White Shimeji), 7) *F. velutipes* (Enoki), 8) *P. ostreatus* (Oyster), 9) *P. djamor* (Pink Oyster), 10) *C. indica* (Milky) and 11) *P. florida* (Florida Oyster) were collected from a local market in Bangalore.

DNA Extraction

DNA extraction was performed as reported[19] with following modifications. Fresh mushroom samples (50 mg) were grinded with homogenization buffer (50 mM Tris, 10 mM EDTA and 50 mM glucose) and extraction buffer (100 mM Tris, 10 mM EDTA, 250 mM NaCl and 1% SDS, pH= 8.0) with ProteinaseK and incubated in dry bath at 60^0 C for 1 h. After incubation equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) mixture was added and centrifuged at 8,000 rpm for 6 min. The previous step was repeated and the supernatant was collected. The DNA was precipitated using equal volumes of chilled isopropanol and incubated at 4 ^{0}C for 30 min. Centrifuged at 10000 rpm for 5 min and DNA pellet was dissolved in TE buffer.

PCR Reaction

The PCR was performed in 25 µl reaction containing 100 ng DNA, 3 U of Taq DNA polymerase, 2.5 mM $MgCl_2$, 1.5 µl of 2.5 mM dNTP mix (Chromous Biotech, Bangalore) and 100 pmol of primers (Eurofins Genomics, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min) followed by 35 cycles of amplification (94°C for 30 sec, 50°C for 30 sec and 72°C for 45 sec) and the final elongation step (72°C for 5 min).

Gel electrophoresis and Data Analysis

The amplified product was subjected to electrophoresis on 1.5 % agarose gel containing ethidium bromide in 1x TAE buffer at 70 V for 1 h. The DNA bands were observed on a Gel Doc system and the photographed. The DNA fingerprint was analyzed based on

the presence or absence of individual bands. The dendrogram was constructed for frequency similarity based on UPGMA (Unweighed Pair Group Method with Arithmetic averages).

RESULTS AND DISCUSSION

The quantity of isolated DNA was checked by Nanodrop ND 1000 spectrophotometer. The qualitative estimation of the DNA on 0.8% agarose gel gave single, sharp and distinct bands devoid of any smear. Thus, genomic DNA of good quality without any degradation was successfully isolated from all the eleven samples. Among the ISSR primers tested (Table 1), only two primers produced distinguishable bands. ISSR 4 produced a total 15 bands ranging from 200 bp- 600 bp (Figure 1). The most polymorphic pattern was produced in the case of ISSR 6 which produced a total 44 bands ranging from 100 bp- 1000 bp (Figure 2). Since, ISSR 6 gave the best banding pattern it was used for interpretation of the phylogenetic relationship. A cluster analysis with frequency similarity was performed for ISSR 6 (Figure 3). The dendrogram separated the eleven putative mushrooms into two major clusters. Cluster 1 was further subdivided in which sample 11, 3, 2 and 1 were grouped together which in turn was connected to sample 4 and 9. The Cluster 2 was subdivided in which samples 10, 8, 7 and this 5 formed one group which was connected to sample 6. All the strains were successfully differentiated and showed a high similarity coefficient between the strains, implying that they might originate from a single maternal strain. The results of our study also showed high similarity between {(1, 2, 3 and 11) (5, 7, 8 and 10)} which might originate from a single maternal strain (Table 2).

A total of three SSR markers were used in this study out of which SSR 3 produced the maximum number of bands and the bands were observed for all the eleven samples (Figure 4). Hence, SSR3 was used for establishing phylogenetic relationship among the mushroom samples. The SSR 3 amplified 38 alleles, ranging in size from approximately 50 bp to 800 bp. The number of alleles per sample ranged from 1 to 7. The cluster analysis with frequency similarity performed for the SSR 3 markers formed two clusters (Figure 5). Cluster 1 being the bigger cluster and cluster two being the smaller cluster. Cluster 1 was sub divided into three smaller clusters consisting of samples 8, 2, 11, 4 and 6; 10 and 7; 5 and 1 respectively. The cluster two being the smaller cluster consisted of only two samples i.e. 9 and 3. The frequency similarity was summarized in Table 3. The lowest and highest frequency similarity coefficient for SSR3 was found to be 0.06 and 0.31 respectively. All the allele showed polymorphism and there was no single monomorphic band for any allele. The 50 bp allele was observed in most number of samples (2, 3, 4, 5, 7, 8, 9, 10 and 11) followed by 250 bp allele which was detected in samples 2, 3, 8, 9 and 10.

Table 1
ISSR and SSR Primers

Primers	Sequence (5'→3')	Tm (°C)
ISSR1	ACACACACACACACACG (17)	52.8
ISSR2	AGAGAGAGAGAGAGAGC (17)	52.8
ISSR3	CTCTCTCTCTCTCTCTG (17)	52.8
ISSR4	CACACACACACACACAA (17)	50.4
ISSR5	GAGAGAGAGAGAGAGAC (17)	52.8
ISSR6	GTGTGTGTGTGTGTGTC (17)	52.8
SSR 1 F	TCGATTGTCAGATTGTTGGA (20)	53.2
SSR 1 R	CGGAGAAGCAGTTGGTTG (18)	56.0
SSR 2 F	TGCGTTTGCTCGGTTAAT (18)	51.4
SSR 2 R	CGCTACTACGTCGATCCG (18)	58.2
SSR 3 F	ACTGAGCCTTCAGCACCA (18)	56.0
SSR 3 R	CATAGGGACGACAGCGAG (18)	58.2

Figure-1
DNA Fingerprint for ISSR 4

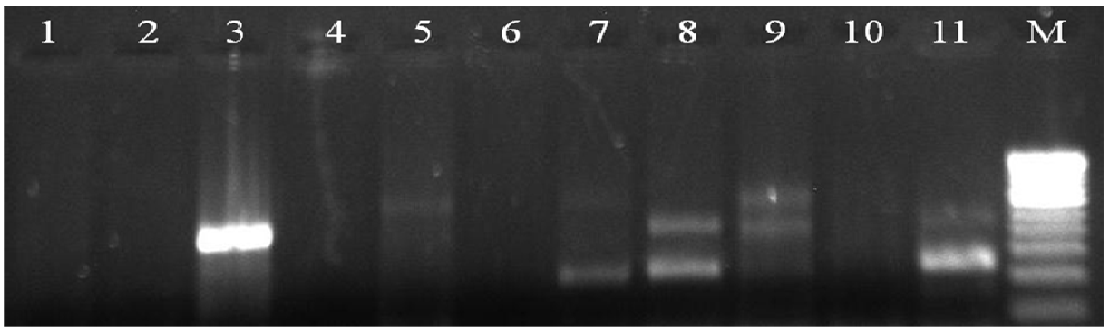

Figure-2
DNA Fingerprint for ISSR 6

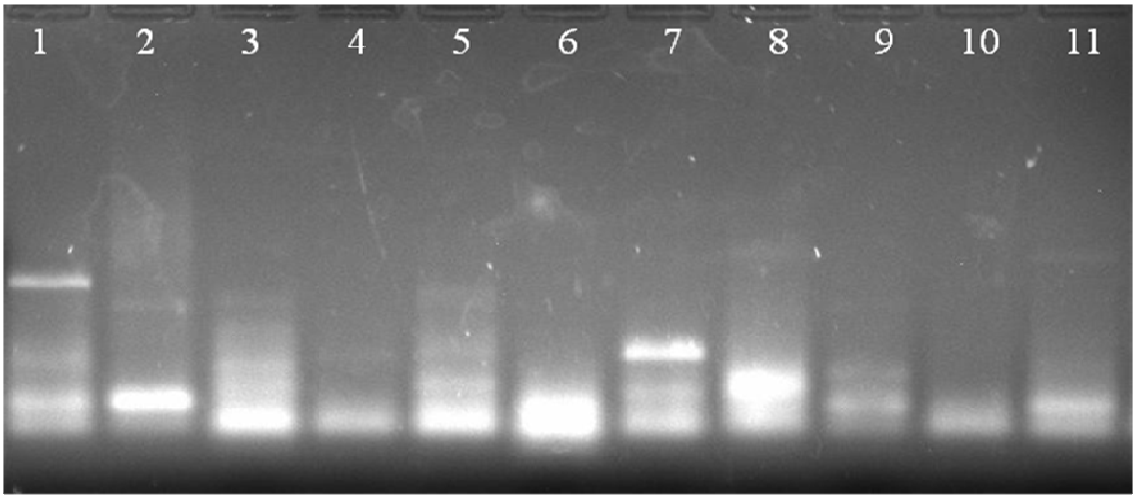

Figure-3
Dendrogram for ISSR 6

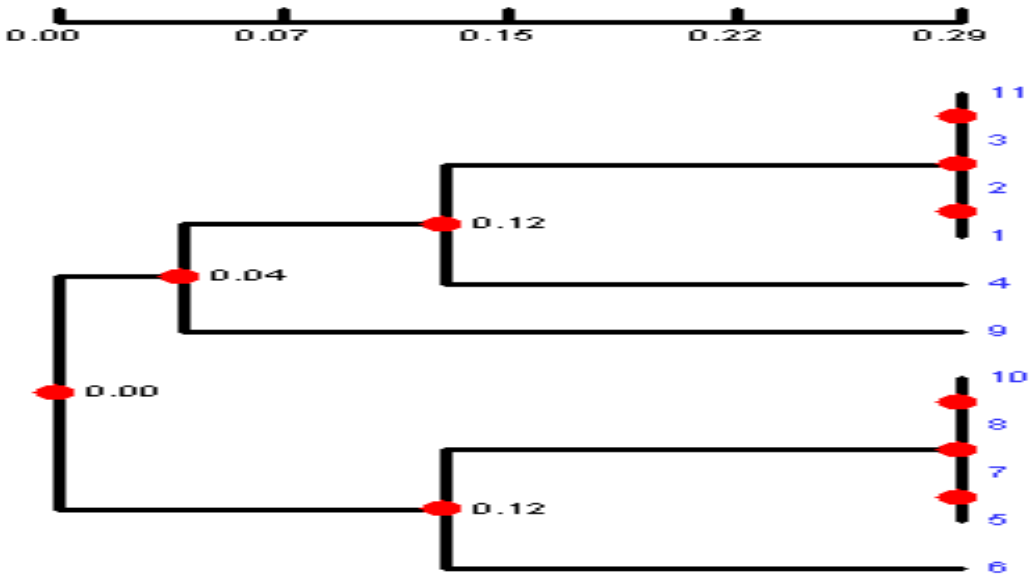

Table-2
Similarity matrix for ISSR 6

	1	2	3	4	5	6	7	8	9	10	11
1	100	-	-	-	-	-	-	-	-	-	-
2	100	100	-	-	-	-	-	-	-	-	-
3	100	100	100	-	-	-	-	-	-	-	-
4	66.67	66.67	66.67	100	-	-	-	-	-	-	-
5	33.33	33.33	33.33	66.67	100	-	-	-	-	-	-
6	0	0	0	33.33	66.67	100	-	-	-	-	-
7	33.33	33.33	33.33	66.67	100	66.67	100	-	-	-	-
8	33.33	33.33	33.33	66.67	100	66.67	100	100	-	-	-
9	66.67	66.67	66.67	33.33	66.67	33.33	66.67	66.67	100	-	-
10	33.33	33.33	33.33	66.67	100	66.67	100	100	66.67	100	-
11	100	100	100	66.67	33.33	0	33.33	33.33	66.67	33.33	100

Figure-4
DNA Fingerprint for SSR 3

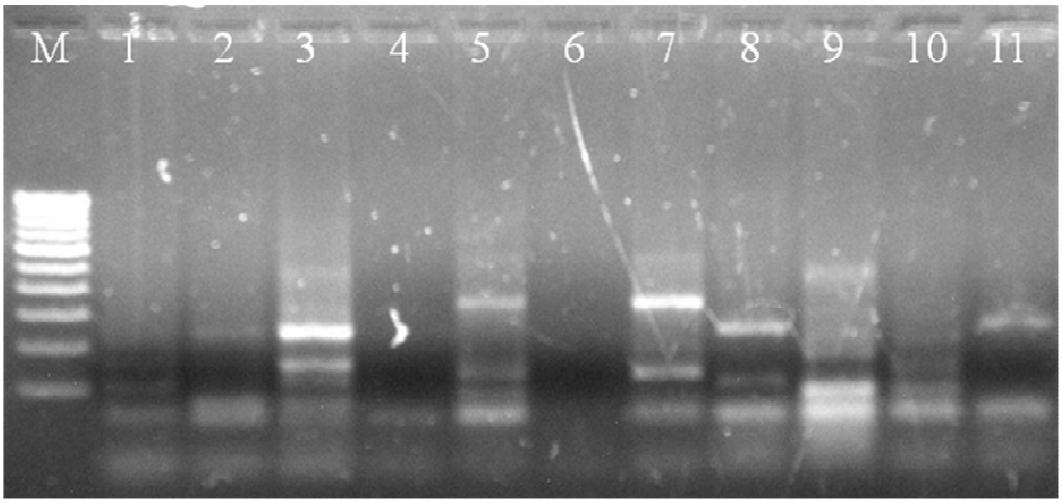

Figure-5
Dendrogram for SSR 3

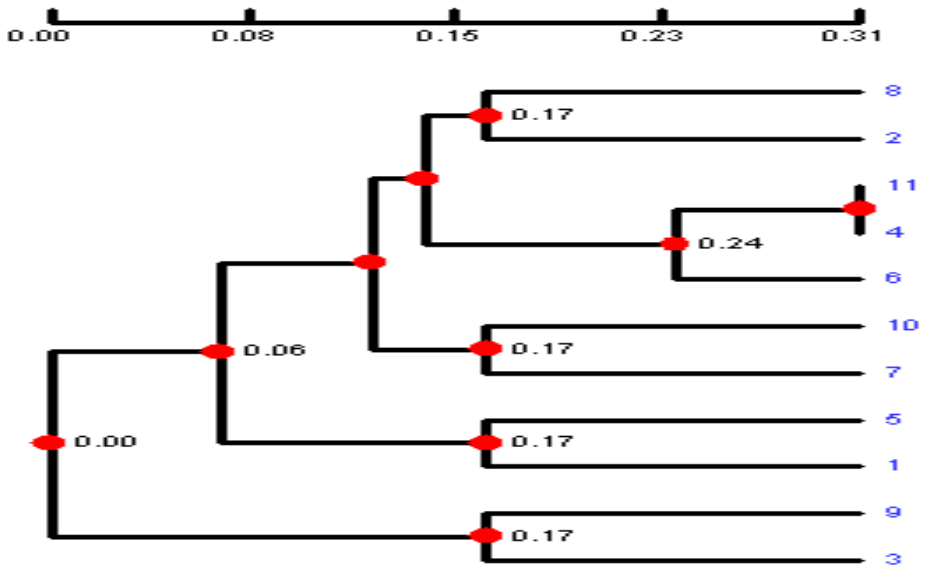

Table-3
Similarity matrix for SSR 3

	1	2	3	4	5	6	7	8	9	10	11
1	100	-	-	-	-	-	-	-	-	-	-
2	57.14	100	-	-	-	-	-	-	-	-	-
3	28.57	42.86	100	-	-	-	-	-	-	-	-
4	57.14	71.43	14.29	100	-	-	-	-	-	-	-
5	71.43	57.14	57.14	57.14	100	-	-	-	-	-	-
6	71.43	57.14	0	85.71	42.86	100	-	-	-	-	-
7	28.57	42.86	42.86	71.43	57.14	57.14	100	-	-	-	-
8	57.14	71.43	42.86	71.43	57.14	57.14	42.86	100	-	-	-
9	28.57	42.86	71.43	42.86	57.14	28.57	42.86	71.43	100	-	-
10	28.57	71.43	42.86	71.43	28.57	57.14	71.43	71.43	42.86	100	-
11	57.14	71.43	14.29	100	57.14	85.71	71.43	71.43	42.86	71.43	100

CONCLUSION

The ISSR and SSR analysis in this study has proven to be useful in discrimination of the mushroom samples. The results of ISSR 6 showed high similarity between some samples which interpret that these samples might originate from a single strain. The SSR 3 amplified alleles (50 bp and 250 bp) can be used as marker for identification of these samples. The genetic data collected during this work will guide the choice of genotypes to cross according to their lineage belonging or their level of diversity. Genetic mapping of the mushroom genome will help in understanding their complex traits such as yield, size, colour, flavour and shelf-life. Consequently, the results of the present study can be seen as a starting point for future research to determine the level of intra and inter specific/generic diversity in mushroom.

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