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Inter and intraspecific genetic diversity (RAPD) among three most frequent species of macrofungi (*Ganoderma lucidum*, *Leucoagricus* sp. and *Lentinus* sp.) of Tropical forest of Central India

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ABSTRACT

In present study seven RAPD primers were used to access the diversity within and among twelve populations of three mushroom species *Ganoderma lucidum*, *leucoagaricus* sp. and *Lentinus* sp. Total of 111 bands were scored by 7 RAPD primers in 30 accessions of three mushroom species collected from different sampling sites of central India. Total 111 bands were generated using seven primers which were F-1, OPG-06, OPC-07, OPD-08, OPA-02, OPD-02, OPB-10. All 111 bands were polymorphic in nature (100%). Therefore, it revealed that the used primers had sufficient potency for population studies and 30 accessions had higher genetic differences among each other. In best of the knowledge, this is the first report, which accesses the genetic diversity between three mushroom species (*Gd Ganoderma lucidum*, *Lg Leucoagaricus* sp., *Ls Lentinus*). The polymorphic percentage ranged from 3.60 to 23% within twelve populations, while polymorphic percentage among group was 40.56, among population within groups was 41.12 and within population was 18.32. This indicated that the genetic diversity within the population was very low, but slightly higher in the populations of three species. Among three groups representing *Gd*., *Lg* and *Ls*, Among populations within groups shown highest percentage of variation (*Pv* = 41.12) while within populations, the lowest percentage of variation (18.32) was observed. This result also support that the highest genetic variation was present among groups in comparison to among the population within a species and lowest genetic variation was observed within the population.

1. Introduction

Utilization of wild mushrooms as a food source started with prehistoric man. Human as a hunter collected wild fungi of forest that serve as important source of nutrition during the long period. There are many edible mushrooms i.e. Agarics, Volvarias, Polypores and tubers fungi that have been used as ethno-botanical food by the tribal of forest regions. These are obviously non toxic as these have been in intimate human consumption by native and tribal, since ancient past [1]. Mushrooms offer significant vital health benefits, including antioxidants, cholesterol-lowering properties, anti-hypertensive, anti-inflammatory, liver protection, as well as anti-diabetic, anti-viral, and anti-microbial properties [2]

Lentinus tigrinus and *G. lucidium* are proved anticholesterolmic [3]. *Lentinus edodes* has been used to enhance vigour, sexuality, energy and as an anti aging agent. Lentinan sulphate obtained from *Lentinus* species inhibits HIV [4]. *Lentinus sajor-caju* can easily be recognized in the field by its large, thin, whitish to cream pileus with pale brown disc, lacking or with small squamules at the center, short stipe with annulus or annular ridge [5] *Lentinus* sp. is high source of protein, carbohydrate and low amounts of fat and possess high quantities of micronutrients (vitamins and carotenoids) and minerals (P, K, Mn, Ni, and Fe) with strong antioxidant properties [6].

Ganoderma lucidum is considered to be a natural medicine that promotes longevity and maintains the vitality of human beings. *G. lucidum* is well known as traditional medicine used against cancer, viral and bacterial infection, diabetes, and liver injury. Among its

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activities, its anticancer properties have been the most interesting studies. It revealed cytotoxic activity of suppressed inflammatory breast cancer [7–10]. The genus *Leucoagaricus* has been well studied in Europe. However, species diversity of *Leucoagaricus* in Asia remains poorly known.

Now a day's molecular techniques are becoming very important for the taxonomic and phylogenetic relationship studies among different fungi [11]. By the use of DNA based techniques like Random Amplified Polymorphic DNA Polymorphism (RAPD), Amplified Fragments length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP) or DNA sequence analysis (nSSU and mtSSU), limitation of identification of mushroom strains based on a few morphological characters can be overcome.

Any of the molecular methods mentioned above could be combined with morphological methods to make identification of fungal species reliable [12]. The random amplified polymorphic DNA (RAPD) is a convenient method to detect genetic diversity [13]. This method has been particularly successful when applied to check the strains of mushrooms with different origins [12]. RAPD have been used to examine material from the genera *Agaricus*, *Coprinus* and *Lentinula* [14]. Genetic Diversity Characterization of *Pleurotus* strains by Random Amplified Polymorphic DNA Fingerprinting has been performed by various workers [15,16]. Fruiting body observations provide information about the fungi on the surface. In addition, evolutionary relationships cannot be determined accurately through morphology alone [17].

2. Materials and methods

2.1. Study sites

Forest areas of four districts Anuppur, Rewa, Shahdol and Umaria of Central India were main study sites of present study (Fig. 1). Sampling sites for mushroom collection of these four study sites are shown in the map.

2.2. Samples collection and identification

Extensive survey of the sampling sites of forest areas of four districts Anuppur, Rewa, Shahdol and Umaria was done in rainy season (2012–13). Ecological features, macroscopic studies and mushroom field test were performed. Specimens were preserved in dried as well as in wet form. Samples were identified to their respective families, genera and species by consulting literature [18–22]. Help of mushroom guide "The great encyclopedia of mushrooms" [23] "Eye Witness Handbooks Mushroom" [24] was also taken. Help of experts in taxonomy of mushroom was also taken when ever required.

2.3. Genomic DNA isolation

DNA isolation and RAPD Analysis of Mushrooms was done by the following protocol [25,26]. Total three species, 12 populations and 30 accessions were used for genetic diversity study for RAPD analysis 0.50 g of dried fruiting bodies of all 30 mushroom samples were cut into small pieces and were soaked in 1 ml buffer. After that all pieces were incubated at 65 °C for 2 h. After incubation the samples were homogenised using pestle and mortar.15 ml of Lysis buffer was added [25]. The tubes were incubated at 65 °C for 1 h in a water bath with intermittent mixing. Centrifuge at 1000 rpm for 15 min to separate out the unlysed cells. Supernatant was transferred to a fresh 30 ml centrifuge tube carefully. Equal volumes of chloroform was added and mixed well. Centrifuge this at 10,000 rpm for 15 min. The aqueous layer was pipette out into the fresh 30 ml centrifuge tube without taking the interface. Equal volumes of isopropanol and 1/10th volumes of 3 M sodium acetate was added and mixed well. Then left at room temperature to stand for 5–10 min. Centrifuge at 10,000 rpm for 15 min and the supernatant was discarded. The pellet was washed with 500 μl of 70% ethanol. The pellet air dried and suspended in 500 μ1 of 1X Tris–EDTA buffer. To remove PCR inhibitors, further the DNA sample was purified by Column purification.

2.4. Column purification

The column was placed in collection tube, 400 μl of equilibration buffer was added to the column and centrifuged at 10,000 rpm for 1 min. Collected buffer was discarded. 400 μl of equilibration buffer was added to the DNA samples, mixed and loaded into the column (This step was repeated till the DNA sample was completed). Flow

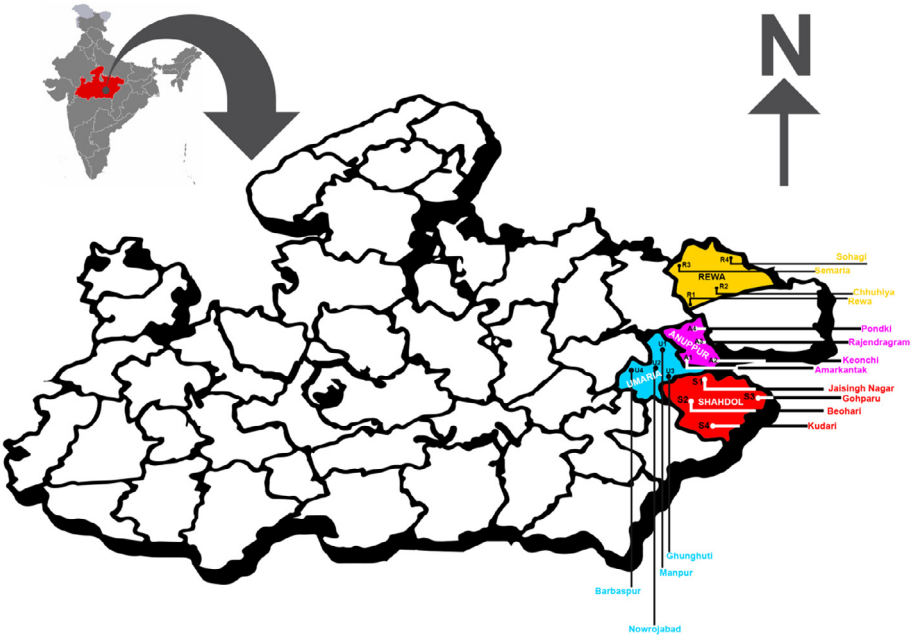

Fig. 1. Map showing sampling sites of Central India for study of mushroom diversity.

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