



DIVERSITY ANALYSIS OF ECTOMYCORRHIZAL GENUS *RUSSULA* USING RAPD MARKERS

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Abstract: It is very difficult to distinguish among the species of ectomycorrhizal genus on the basis of phenotypic characters. Random Amplified Polymorphic DNA (RAPD) technology is rapid and sensitive technique used to estimate relationship between closely and more distantly related species of various genus of ectomycorrhiza. Eight RAPD markers were used to analyze diversity of selected seven genotypes of *Russula* collected from North eastern zone of India. Phylogenetic analysis clustered seven genotypes in to two major groups. Group 1 composed of genotypes 5 and 6 found to share homology. Group 2 is composed of five genotypes: 1,2,3,4 and 7. Of these genotypes 1, 2 and 3 share near homology and genotype 4 was found to be more similar to this sub group. Genotype 7 was found to be separated from all five and Genotype 6 was found to be distantly related to genotype 1, 2 and 3. As species of *Russula* genus are known to have various antimicrobial and antioxidant activities so it is important to identify the species of this genus. These results may serve as reference information for researchers.

Key words: Ectomycorrhiza; Random Amplified Polymorphic DNA; *Russula*

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INTRODUCTION

Ectomycorrhizae are probably the most diverse type of mycorrhizae. Around 750 worldwide species of mycorrhizal mushrooms comprises the genus *Russula*. Mushrooms are regarded as edible and even highly desirable in many areas of the world. Species of *Russula* are among the most popular. *Russula* is a genus of Basidiomycota, belonging to Russulales. The biodiversity of edible *Russula* in India has still not been well investigated due to a lack of involved experts and pure cultures of *Russula* species. A large number of species of this genera are known for their antimicrobial and antioxidant activities and thus having medicinal importance (Mercan et al., 2006; Ji-kai Liu, 2007; Turkoglu et al., 2007; Jain and Pande, 2013). Traditional method of identifying species by phenotypic characters is now gradually being replaced by protein or DNA profiling because of several limitations of morphological data (Grades and Bruns 1993; Eberhardt, 2002; Binder et al., 2005; Buyck et al., 2008; Upadhyay et al., 2010). The emergence of phylogenetic mycology as a paradigm for fungal biology studies has been greatly accelerated by numerous advancement in phylogenetic methods, especially in the area of molecular systematic. In recent years, DNA profiling through RAPD (Random Amplified Polymorphic DNA) techniques has been used for analysis of diversity and identification of duplicate within large germplasm population, phylogenetic relationship of breeding, rational designing of breeding programmes, management of genetic resources and for assessing genetic fidelity of tissue culture raised plants (Upadhyay et al., 2010). Evidently, RAPD technology is rapid and sensitive technique

which can be used to estimate relationship between closely and more distantly related species of *Russula*.

EXPERIMENTAL

Sample collection: Fruiting bodies of different *Russula* genotypes were collected from forest of North eastern zone of India. On the basis of morphological and physiological characters, seven genotypes of *Russula* have been identified. The collected tissue samples were dried in hot air oven.

Genomic DNA extraction: To extract the genomic DNA, the dried tissue samples were ground in liquid nitrogen, then added to 400 ml of lysis buffer and mixed well. The mixture was incubated at 65°C for 1 h followed by phenol-chloroform extraction. DNA was precipitated by cold 95% ethanol and washed with 70% ethanol before air drying. The DNA pellets were resuspended in TE buffer containing RNaseA (100 mg/ml). DNA of all selected seven genotypes was then quantified using spectrophotometer.

PCR amplification and data analysis: Eight RAPD markers were used to analyze diversity of all selected seven genotypes of *Russula* using PCR (Polymerase Chain Reaction) amplification. PCR were carried out in 25 ml reaction mixture containing 2.5 ml of 5' PCR buffer, 100 ml each of dATP, dGTP, dTTP and dCTP, Taq DNA polymerase decamer random primer and 50 ng of genomic DNA. Amplification reaction performed in DNA Mini Thermal Cycler and the sequential steps were: 1 cycle 3 min at 94°C, 2 min at 52°C and 3 min at 72°C followed by 38 cycle of 1 min at 94°C, 2 min at 40°C and 2min at 72°C. The last 10 min, extension at 72° C was carried out. 10 ml of amplification products along with PCR loading buffer were loaded in 1.0% agarose gel containing 1 mg/ ml EtBr. PCR products were analyzed on 1.5% agrose gel and visualized under UV light. The gel was observed under gel documentation and photograph was taken. The DNA bands of the different *Russula* species were scored manually and then Phylogenetic analysis was done using SPSS-13 software and dendogram was constructed with electrophoresis generated banding pattern from PCR amplification.

RESULTS AND DISCUSSION

Ectomycorrhizal species are easy to distinguish from other genera species but it is very difficult to distinguish among the ectomycorrhizal species on the basis of phenotypic characters. Using RAPD markers one can easily estimate relationship between closely and more distantly related species of *Russula*. Seven species of *Russula* (*Russula cynoxatha*, *Russula erythropus*, *Russula padulosa*, *Russula mustellina*, *Russula sardonina*, *Russula aeruginea*, *Russula emetica*) were identified on the basis of their morphological and physiological characters. Out of eight primers used, only two produced clear and scorable amplification products in selected *Russula* genotypes (Table 1 and 2).

Table 1. Primers showed positive results of RAPD analysis.

Primer	<i>Russula cynoxatha</i>	<i>Russula erythropus</i>	<i>Russula padulosa</i>	<i>Russula mustellina</i>	<i>Russula sardonina</i>	<i>Russula aeruginea</i>	<i>Russula emetica</i>
5'GCTGGTCCG-3'	+	-	-	-	+	-	+
5'GACGTAGG-3'	+	+	+	+	+	+	+

Table 2. Polymorphic products generated during RAPD analysis.

Primer	No. of amplified products	No. of polymorphic products	Size range (kb)
5'GCTGGTCCG-3'	4	4	0.4-3.0
5'GACGTAGG-3'	10	9	0.1-2.5

Phylogenetic analysis reveals that all the selected seven genotypes can be clustered in to two major groups. Group 1 composed of genotypes 5 and 6 found to be located on single cluster, reflecting homology of genomes. Group 2 is composed of five genotypes: 1, 2, 3, 4 and 7. Of these genotypes 1, 2 and 3 share near homology and genotype 4 was found to be more similar to this sub group. Genotype 7 was found to be separated from all five. Genotype 6 was found to be distantly related to genotype 1, 2 and 3 in evolutionary terms (Figure 1). These results may serve as reference information

for researchers. The result of RAPD analysis showed that there was diversity within species, which otherwise could not be detected by morphological studies. The methods used in this work have permitted recognition of remarkable differences, which are not evident at morphological level between ecological variant isolates of *Russula* species. Similar observations were also recorded while studying the vegetative compatibility group variation of isolates of *Colletotrichum dematium*, *Acremonium cucurbitacearum* and *Fusarium solani*, respectively (Correll *et al.*, 1993; Vicente *et al.*, 1999; Alymanesh *et al.*, 2009).

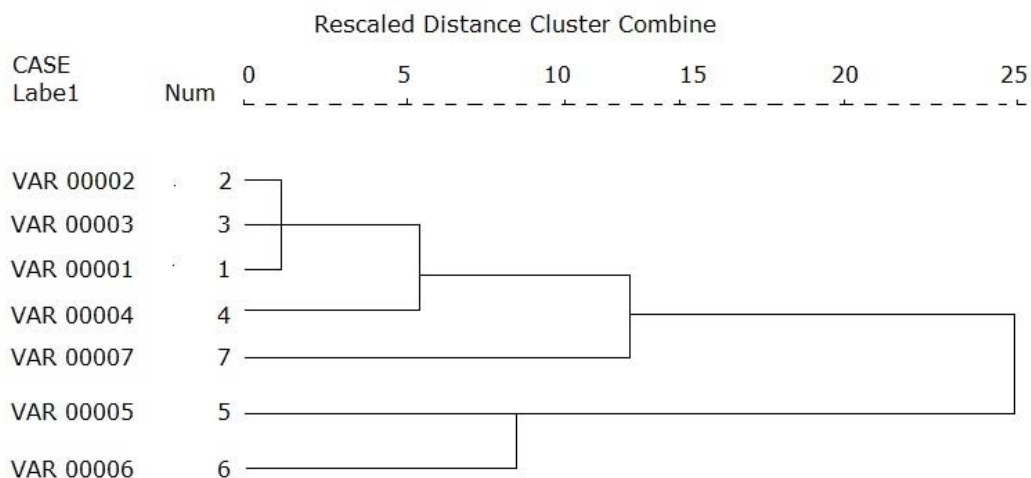


Figure 1: Phylogenetic relationship of Seven species of *Russula*

CONCLUSION

RAPD markers can be used identify and diversify the ectomycorrhizal species as indicated by the results. As species of *Russula* genus are known to have various antimicrobial and antioxidant activities so it is important to identify the species of this genus. These results may serve as reference information for researchers.

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CONFLICT OF INTEREST : Nothing