

DNA Based Characterization of Various Morel Species

¹Naheed Fazal, ²Habib Ahmad, ¹Zubeda Chaudhry, ¹Shaista Bibi,

³Zafar Ali Yousafzai and ⁴Imtiaz Ahmed Khan

¹Department of Botany,

²Department of Genetics, Hazara University, Mansehra, Pakistan

³Hussain and Co, Mangora, Swat

⁴Department of Genetics, Karachi University, Pakistan

Abstract: Morels (*Morchella* species) are important edible mushrooms belonging to family Helveliaceae. Despite its economic and medicinal importance, not much scientific work (especially molecular work) has been done in Pakistan for improvement of morels. During present research, genomic DNA profiles of five morels species obtained from upper Swat valley, Khyber Pukhtoonkhawa province were studied using four Randomly Amplified Polymorphic (RAPD) primers GLE-05, -06, -07 and -09. Moderate amount of genetic diversity (average GD ranging from 27-62%) was observed among the material studied. The DNA fragments amplified using 4 RAPD primers ranged in size from approximately 100 to 1100 bp. Two, 3.0, 4.6 and 2.2 alleles per accession were amplified using GLE-05, -06, -07 and -09, RAPD primers, respectively. To elaborate phylogenetic relationship among the accessions, a dendrogram was constructed. It is recommended that similar kinds of research should be conducted on larger scale so that a more clear estimate of existing genetic diversity in Pakistani morels can be established which will help in formulating better breeding programs for the improvement of morels in Pakistan.

Key words: Genetic distance, morels, *Morchella* species, PCR, phylogeny, RAPD

INTRODUCTION

Morels (*Morchella* species e.g., *Morchella conica*, *Morchella esculenta*, *Morchella ultima*, *Morchella rotunda*, *Morchella semilibera*, *Morchella elata*, *Morchella crassipes*) are important mushrooms belong to family Helveliaceae Fungi. The fruit bodies of all the *Morchella* species of the genus are edible and are mainly used as flavoring in soups and gravies. Morels are cylindrical in shape. The stalk of morel is 1.0 to 4.0 cm long, 0.5 to 3.0 cm thick, hollow and of variable shapes. The upper part of a morel is called pileus and contains 70 to 80% of the total weight of the plant. It is generally brown, yellow, black or pale in color. About 90% of the total morel produce of Pakistan is collected from the Hindu Kush- Himalayan mountain ranges of Northern Pakistan. Morels are locally used as medicinal plants. In Swat District, annually 5000 tons morels are collected most of which is exported to western countries including France, Belgium, Switzerland, Austria and Germany where they are used for medicinal purposes (Anonymous, 1998). Their metabolites are also used as adaptogens and immunostimulants, and now are considered to be one of the most useful anti tumor agents for clinical use (Pankaj *et al.*, 2002). Prices of morels greatly depend upon the quality, processing and area of collection. Generally one kg of dried morel fetches Rs. 3000 to collector, Rs. 9000

to the wholesaler, Rs. 12000 in the National market and Rs. 20000 in the international markets (Chaudhary *et al.*, 2000; Hamayun *et al.*, 2003).

Despite enormous economic and medicinal value of the morels, not much scientific work has been done in Pakistan for conservation strategies, marketing and improvement of morels. During present research study, commonly found species of morels from Swat valley of Khyber Pukhtoonkhawa province were studied by documenting genetic structure of the species based on RAPD (Randomly Amplified Polymorphic DNA) markers.

MATERIALS AND METHODS

Five species of *Morchella* (viz; *M. esculenta*, *M. conica*, *M. delicosa*, *M. elata* and *M. crassipes*) commonly found in upper Swat valley were collected. Laboratory work was conducted at Department of Genetics, Karachi University during 2010. Total genomic DNA was isolated using modified small scale isolation procedure originally developed by Kobayashi *et al.*, (1998) and Weining and Langridge (1991). Polymerase Chain Reactions (PCR) were carried out using four 10 base pair RAPD primers (obtained from Gene Link, Inc. 1052 NY, USA) for estimation of genetic diversity (calculated as genetic distance). Only the score able

Table 1: Sequence information of RAPD primers used to estimate genetic diversity in morels species

S. No.	Oligo name	Sequence	Mol. wt (%)	GC content
1	GLE-05	TCAGGGAGGT	3108	60
2	GLE-06	AAGACCCCTC	2956	60
3	GLE-07	AGATGCAGCC	3037	60
4	GLE-09	CTTCACCCGA	2947	60

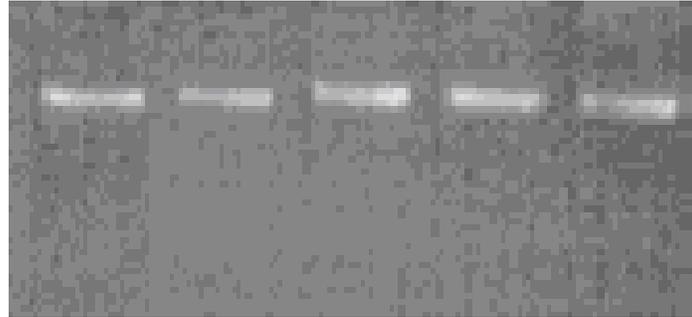


Fig. 1: Genomic DNA isolated from five species of *Morchella* collected from upper Swat valley, Pakistan. 1 = *M. esculenta*, 2 = *M. conica*, 3 = *M. delicosa*, 4 = *M. elata*, 5 = *M. crassipes*

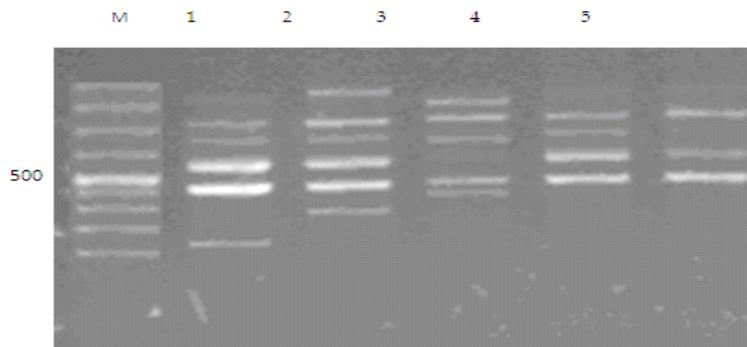


Fig. 2: PCR amplification profile of five species of *Morchella* using RAPD primer GLE-07. M= Molecular size maker (100 bp ladder). Size of the fragment (in base pairs) is presented on left. 1 = *M. esculenta*, 2 = *M. conica*, 3 = *M. delicosa*, 4 = *M. elata*, 5 = *M. crassipes*

Table 2: Average genetic distance estimates (G.D) among 5 accessions of morels using 4 RAPD primers viz; GLE-05, GLE-06, GLE-07 and GLE-09

	1	2	3	4
2	0.59			
3	0.48	0.49		
4	0.56	0.58	0.62	
5	0.27	0.46	0.50	0.40

1: *M. esculenta*; 2: *M. conica*; 3: *M. delicosa*; 4: *M. elata*; 5: *M. crassipes*

bands were included in the analysis. Every scoreable band was considered as single locus/allele. Presence or absence of each single DNA fragment was coded as “1” (present) or “0” (absent), respectively (Nei and Li, 1979). The following formula was used for the calculation of GD:

$$GD = 1 - \frac{d_{xy}}{d_x + d_y - d_{xy}}$$

where, GD = Genetic distance, d_{xy} = Total number of common bands in two genotypes, d_x = Total number of

bands in genotypes # 1 and d_y = Total number of bands in genotype #2. A dendrogram was constructed using computer program Popgene 32, ver. 2.3 (Yeh *et al.*, 1999)

RESULTS AND DISCUSSION

Quality of the DNA samples was checked on 1% agarose/TBE gel (Fig. 1). No detectable amount of RNA was present in the DNA samples and hence DNA samples were not treated with RNase. It is also evident from Fig. 1 that almost similar amount of DNA was present in the five samples. DNA was diluted to 1:4 using double distilled, autoclaved, deionized water and was used for PCR. In total 4 RAPD primers (obtained from GeneLink Inc, NY10528, USA) were used to amplify genomic DNA from five accessions of morels (Table 1). An example of amplification profile of 5 morel species is presented in Fig. 2.

Various kinds of amplification profiles were observed. Size of the DNA fragments was estimated using 100 bp DNA ladder (purchased from Gene Link, 1052,

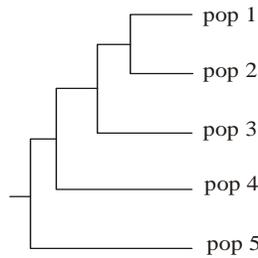


Fig. 3: Dendrogram constructed for 5 accessions of morels using 4 RAPD primers GLE-05, GLE-06, GLE-07 and GLE-09. 1 = *M. esculenta*, 2 = *M. conica*, 3 = *M. delicosa*, 4 = *M. elata*, 5 = *M. crassipes*

NY, USA). The DNA fragments amplified ranged in size from approximately 300 to 1100 bp, 800 to 1100, 100 to 900 and 300 to 1100 bp using RAPD primers GLE-05, GLE-06, GLE-07 and GLE-09, respectively. Total of 10, 15, 23 and 11 DNA fragments were amplified in 5 morel species using GLE-05, GLE-06, GLE-07 and GLE-09, respectively. Average of 2.0, 3.0, 4.6 and 2.2 alleles per accession were amplified using GLE-05, GLE-06, GLE-07 and GLE-09, respectively. Nei and Li (1979) procedure of Unweighted Pair Group of Arithmetic Mean (UPGMA) was used to estimate genetic diversity among the 5 species of morels based on data obtained from PCR using four RAPD primers. Average genetic distance estimates among the 5 morel accessions ranged from 27% to 62% (Table 2). One comparisons viz., *M. delicosa* - *M. elata* showed maximum genetic diversity (G.D = 62%) closely followed by *M. esculenta* - *M. conica* (59%) and *M. conica* - *M. elata* (58%). One comparisons *M. esculenta* - *M. crassipes* showed minimum genetic diversity (G.D = 27%) using RAPD primers GLE-05, -06, -07 and -09.

Bivariate 1-0 data matrix was also used for the construction of dendrogram. Computer program "Popgene ver. 3.2" was used to construct the dendrogram (Yeh *et al.*, 1999). Phylogenetically the five species of morels were genetically related in the order *M. esculenta*, *M. conica*, *M. delicosa*, *M. elata*, *M. crassipes* (Fig. 3).

CONCLUSION

Present research is the first documented attempt to utilize modern molecular biology techniques for the characterization and ultimate improvement of morels in Pakistan. During present work a small scale DNA isolation protocol was optimized. Polymerase Chain reactions using four Randomly Amplified Polymorphic DA (RAPD) primers were performed. Moderate estimates of

Genetic Distance (GD ranging from 27-62 and 0-63%) were observed during present study which indicate the potential of improving of morels in Pakistan using genetic means. It is recommended that similar kinds of research should be conducted on larger scale so that better picture regarding existing genetic diversity in Pakistani morels can be estimated which will help in formulating better strategy for the improvement of morels in Pakistan.

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