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Genetic divergence study of wild edible mushroom in East Siang district of Arunachal Pradesh using simple sequence repeat markers

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ABSTRACT

The state of Arunachal Pradesh located in the eastern Himalayan region is part of the Himalaya biodiversity hotspots. It is the largest mountainous state of India and recognized as a globally important eco-region (out of 200). In the state, people collect and consume wild-growing mushrooms exclusively based on the indigenous knowledge of the morphology of fruiting bodies. Information on the level of genetic diversity among these varieties is scarce. The genetic diversity of 10 wild edible mushrooms (WEM) represent almost all the common mushrooms sold and eaten by local people of East Siang, Arunachal Pradesh was assessed using 21 simple sequence repeat (SSR) markers. These markers revealed polymorphism among the strains of WEM. The average number of polymorphic alleles (3.07) and polymorphic information content (PIC = 0.40) per locus were detected among the strains. The genetic similarity among varieties using the Jaccard's similarity coefficient ranged from 0.37 to 0.83. Unweighted pair group method using arithmetic mean (UPGMA) clustering showed two major clusters and three singletons. This study represents the first evidence of the presence of genetic diversity in WEM from East Siang, Arunachal Pradesh. Divergent varieties with complementing traits could be crossed to develop productive hybrid mushroom varieties.

Key words: *Genetic similarity, Indigenous people, Macro-fungi, Morphological variation, Wild mushrooms.*

Introduction

An enormous rise in the consumption of mushrooms has been attributed to growing public awareness of the health advantages of eating them as well as the food's functional properties (Li *et al*., 2021). Therefore, it's crucial for the development of new varieties to make the best breeding material selection through the assessment and administration of accession collections (An *et al*., 2021). There is a huge variety of macro-fungi on earth and new species from all over the world are constantly being added. There are over 27,000 species of mushrooms (Hawksworth, 2001 and Cannon, 2018) 10% of which are thought to be edible (Li *et al*., 2021). However, some areas remain unknown or are only partially investigated (Azeem *et al*., 2020). More fieldwork is required to investigate and catalogue the diversity of macrofungi, especially in the tropics, using morphological and molecular identification methods (Lopez Quintero *et al*., 2012; Ambrosio *et al*., 2015 and Iqbal *et al*., 2016). The diversity of wild

mushrooms is abundant in the North Eastern area of India, which is a part of the Indo Burma biodiversity hotspot (Verma *et al*., 1995). Indigenous people who live in hilly locations and ethnic tribes who live in the plains both commonly consume mushrooms that they gather from wild habitats as part of their daily diets and as a source of traditional medicines (Khaund and Joshi, 2013 and Ao *et al*., 2020). Only a few studies revealing the diversity of wild mushrooms from various regions of Northeast India used morphological characteristics to identify them (Kumar *et al*., 2013; Gogoi and Parkash, 2015; Kalita *et al*., 2016 and Singh *et al*., 2017). The taxonomy of numerous fungal groupings is constantly being reorganized as a result of divergences over the classification of fungi based on a certain set of morphological features (Yang, 2011). Because morphological markers are perplexed by environmental, pleiotropic and epistatic effects, morphological variation does not accurately reflect genuine genetic variation that exists at the DNA level. The diversity of wild mushrooms in Northeast India has very little molecular information accessible (Kakoti *et al*., 2021).

Materials and Methods

The present study used simple sequence repeat (SSR) markers to generate information on the level of genetic diversity and relationship and identify divergent parents among 10 wild edible mushroom strains (Fig.1) collected from different locations (Table 1) of East Siang district in Arunachal Pradesh (Table 1 and Fig.1) and represent almost all the common mushrooms sold and eaten by local people during 2011–2020. Genomic DNA (gDNA) was extracted from 400 mg mycelium aliquots of ten single pure cultures with the commercial DNeasy Plant Mini DNA extraction kit (Qiagen, Hilden, Germany), following manufacturer's instructions. Concentration of the gDNA was determined using UV-absorption spectrophotometer (Thermo Scientific UV1 model) as per the procedure described by (Sambrook, 2001). Samples were stored -80 °C for further analysis. Amplification of DNA was carried out using SSR primers reported by (Li *et al*., 2019). The amplification was carried out in 25??l of the reaction mixture containing 2.5 ?l of 10X PCR buffer, 2.0 ?l dNTPs (25 mM), 2.0 ?l MgCl2 (25 mM), 1.0 ?l forward primer (10 pm/??l), 1.0 ?l reverse primer (10 pm/??l), 5.0 5l DNA template, 0.5 ?l *Taq* Polymerase (2 U/?l), and 11.0 ?l nuclease free water. A cycle at 94 °C for three minutes was followed by 35 cycles at that temperature for 30 seconds, an adequate annealing temperature of 52 to 56 °C for 30 seconds, 72 °C for one minute, and a final extension at 72 °C for seven minutes.

Fig. 1. Local mushroom strainsused for the study

The bands obtained after electrophoresis were scored for presence and absence of band and subjected to analysis using NTSYS-pc version 2.10. Dendrograms was constructed using the genetic similarity coefficients using Unweighted Pair Group Method for Arithmetic Average (UPGMA) follow-

ing the Sequential Agglomerative Hierrarchial Network (SAHN) algorithm.

Results and Discussion

In the present investigation of genetic divergence study of wild edible mushroom in East Siang District of Arunachal Pradesh using simple sequence repeat markers results shows that:

SSR's are one of the most reliable marker-based diversity evaluator system. In the present investigation, 21 primer pairs (Li *et al*., 2019) were selected for diversity analysis of 10 mushroom strains. Out of 21 SSR, 15 exhibited polymorphic bands on gel electrophoresis. The gel electrophoresis pattern obtained using primers SSR1, SSR2, SSR4, SSR5, SSR7 and SSR15 were illustrated in (Fig. 2). The 15 polymorphic loci identified between 2 and 4 alleles with an average of 3.07 alleles per locus in 10 mushroom strains. The 15 SSR loci amplified a total of 46 alleles that were used for genetic analysis. The total number of alleles and size of SSR markers varied from the ones originally reported, indicating that the source of the material tested differed from that of the previously characterized collections. Moreover, six tested SSR (SSR22, SSR25, SSR46, SSR50, SSR53 and SSR62) had PIC values greater than 0.40, which is consistent with the results found for some of these loci. The SSR25 and SSR50 marker was highly polymorphic and had a high PIC value of 0.48 (Table 2). The estimates of mean RP were found to be the highest for the primer SSR22, SSR25 and SRR50 (0.80), followed by SSR62 (0.70) and was lowest for the

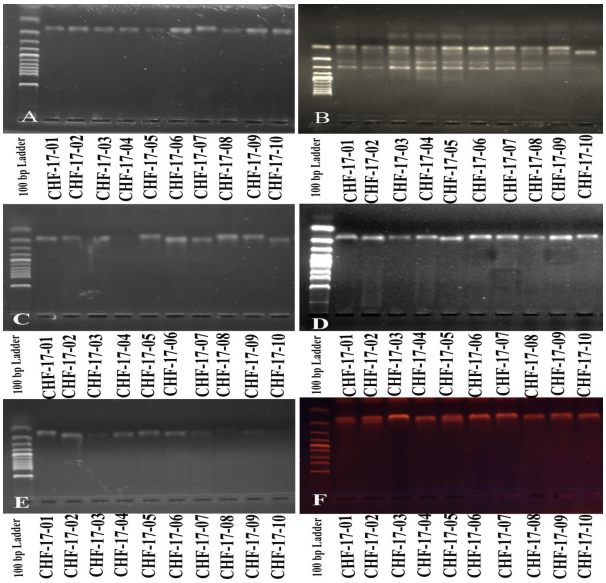

Fig. 2. A representative gel image showing SSR profiles of the six strains using SSR22 (A), SSR22 (B), SSR46 (C) SSR50 (D) SSR53 (E) and SSR62 (F) marker

Table 1. Ten local mushroom strains along with their origin and GPS

Sample No.	Habitat	GPS Data	Identification
CHF-17-01	Wooden stumps	Longitude N 28^0 04′44.79"Latitude E 095^{0}19′23.00"Altitude 155m	Cap and Stipe *(A)
CHF-17-02	Wooden (Dry mango tree)	Longitude N 28^0 03′44.23"Latitude E 095^{0}18′58p.31"Altitude 120m	Cap (A) Stipe *(P)
CHF-17-03	Soil	Longitude N 28^0 03′44.32"Latitude E 095^{0}18′58.33"Altitude 192m	Cap (A) Stipe (P)
CHF-17-04	Wooden stumps or trees	Longitude N 28^0 08′15.43"Latitude E 095^{0}20′09.08"Altitude 321m	Cap (A) Stipe (P)
CHF-17-05	Trees	Longitude N 28^0 08′15,35"Latitude E 095^{0}20′09.12"Altitude 319m	Cap (A) Stipe (P)
CHF-17-06	Saw dust	Longitude N 28^0 08′15.48"Latitude E 095^{0}20′09.28"Altitude 316m	Cap (A) Stipe (P)
CHF-17-07	Wooden stumps or trees	Longitude N 28^0 08′15.93"Latitude E 095^{0}20′08.90"Altitude 313m	Cap and Stipe (A)
CHF-17-08	Wooden stumps or trees	Longitude N 28^0 08′15.96"Latitude E 095^{0}20′08.95"Altitude 312m	Cap and Stipe (A)
CHF-17-09	Wooden stumps or trees	Longitude N 28^0 08′16.24"Latitude E 095^{0}20′08.09"Altitude 310m	Cap (A) Stipe (P)
CHF-17-10	Wooden stumps or trees	Longitude N 28^0 08′16.59"Latitude E 095^{0}20′07.76"Altitude 309m	Cap and Stipe (A)

* (A= Absent; P= Present)

primer SSR6 (0.50). The maximum MI was observed for the primer SSR57 (53.92) followed by the SSR50 (49.77) and SSR60 (49.08). The minimum MI was observed for the primer SSR62 (31.11). The Diversity Index (DI) and were ranged from 0.63 to 0.93 with average 0.84.

The genetic coefficients calculated from the molecular data on 15 polymorphic SSR markers revealed a wide array of genetic distances between the studied mushroom genotypes. The Jaccard similarity coefficient ranged from 0.370 to 0.826 owing to diversification in morphology and pedigree between the mushroom strains. The strainspairs CHF-17-01 and CHF-17-02 revealed the highest similarity of 0.826, followed by CHF-17-02 and CHF-17-06 (0.717) and CHF-17-09 and CHF-17-10 (0.717). However, the strainspairs CHF-17-07 and CHF-17-09 showed the lowest genetic similarity of 0.391, followed by CHF-17-01 and CHF-17-05 (0.370) (Table 3).

A dendrogram of ten mushroom strains was constructed based on similarity of SSR alleles among the genotypes by cluster analysis with the NTSYS-pc software. The ten mushroom strains were grouped into 2 major clusters ranging from 0.40 to 0.83 coefficient scale (Fig. 2A and Table 3). Clusters I contain six mushroom strains as CHF-17-01, CHF-17-02, CHF-17-06, CHF-17-03, CHF-17-07 and CHF-17-08. In this cluster, the mushroom strains CHF-17-01, CHF-17-02 and CHF-17-06 formed another sub cluster. The cluster II contains four mushroom strains *viz*., CHF-17-04, CHF-17-09, CHF-17-05 and CHF-

Table 2. Information on band polymorphism by the microsatellite markers

Sl.No.	Primer	NPB	PIC	DI	RP	MI
1	SSR4	3	0.39	0.86	0.67	40.10
2	SSR6	4	0.35	0.93	0.50	48.39
3	SSR8	3	0.36	0.85	0.53	37.33
4	SSR18	3	0.36	0.85	0.53	37.33
5	SSR22	2	0.46	0.63	0.80	31.80
6	SSR25	2	0.48	0.74	0.80	33.18
7	SSR38	4	0.37	0.89	0.60	51.16
8	SSR46	3	0.43	0.88	0.67	44.24
9	SSR47	3	0.37	0.85	0.53	38.71
10	SSR50	3	0.48	0.77	0.80	49.77
11	SSR53	3	0.43	0.85	0.73	44.93
12	SSR57	4	0.39	0.90	0.60	53.92
13	SSR60	4	0.36	0.90	0.55	49.08
14	SSR61	3	0.39	0.86	0.67	40.10
15	SSR62	2	0.45	0.88	0.70	31.11
Grand Mean	3.07	0.40	0.84	0.65	42.08	
Total	46.0	6.1	12.6	9.7	631.2	

[NPB: Number of Polymorphic Bands; PIC: Polymorphic information content; DI: Diversity Index; MI: Marker index; RP: Resolving power]

Table 3. Jaccard's similarity coefficient of the mushroomstrains based on SSR markers

Strain CHF-17-10	CHF-17-01	CHF-17-02	CHF-17-03	CHF-17-04	CHF-17-05	CHF-17-06	CHF-17-07	CHF-17-08	CHF-17-09	
CHF-17-01	1.000									
CHF-17-02	0.826	1.000								
CHF-17-03	0.609	0.609	1.000							
CHF-17-04	0.609	0.522	0.609	1.000						
CHF-17-05	0.370	0.413	0.413	0.674	1.000					
CHF-17-06	0.630	0.717	0.544	0.413	0.478	1.000				
CHF-17-07	0.630	0.587	0.544	0.457	0.522	0.522	1.000			
CHF-17-08	0.500	0.500	0.630	0.630	0.565	0.522	0.652	1.000		
CHF-17-09	0.587	0.544	0.544	0.761	0.696	0.522	0.391	0.565	1.000	
CHF-17-10	0.565	0.478	0.435	0.609	0.587	0.457	0.500	0.500	0.717	1.000

17-10 (Fig. 2A). The mushroom strain CHF-17-10 and CHF- 17-03 were unique as they have shown distant relation to the other members of their cluster (Fig. 2A). Dendrogram summarized the existing genetic similarity/ dissimilarity among genotypes within the cluster based on molecular marker (SSR) characteristics. The knowledge of genetic variation is useful to identified new strains.The principal co-ordinate analysis provides an alternative view of the genetic distances among mushroom strains compared to the UPGMA dendrogram (Fig. 2B). The first four eigenvectors accounted for 43.15% of the variation. The PCo-A graph corresponded very well to the UPGMA cluster analysis. The similarity between these two analyses supports the genetic diversity results observed in this study.

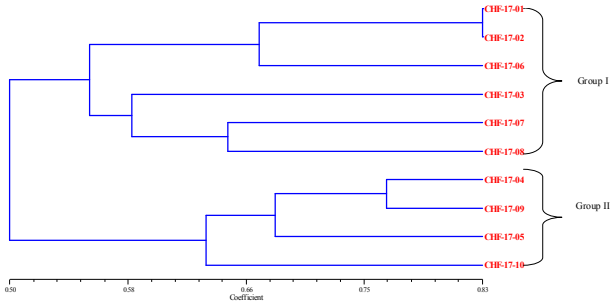

Fig. 2.(A) Cluster analysis of local Mushroom strains based on UPGMA and SAHN algorithm

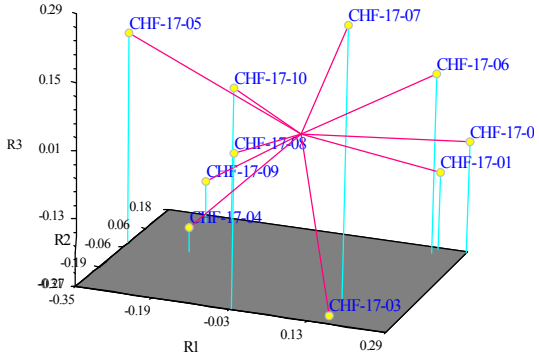

Fig. 2(B) Principal Co-ordinate Analysis diagram of relationship among local Mushroom strains

Cluster analysis based on genetic similarity values provided a clear resolution of relationships among all the 10 mushroom strains. UPGMA analysis of SSR marker variation corresponded well to the breeding histories for the cultivars evaluated with a few exceptions. These results indicate that SSR markers were effective in distinguishing mushroom strains. Additionally, SSR markers were useful in differentiating between closely related strains sources and could be used to supplement morphological data used for strains protection. In both the UPGMA dendrogram and the Principal Co-ordinate analysis of 10 mushroom strains showed a high similarity between CHF-17-01 and CHF-17-02 two of the strains belonging to cluster I and strain CHF-17-10 and CHF- 17-03 showed distant relation to the other members of their cluster.

Conclusion

The findings from the study conclude that the genetic diversity in mushroom was revealed at molecular level. The markers utilized in the study can serve as efficient, stable and reliable marker system. Of the 21 markers, a set of 15 markers were identified that was able to identify all 10 WEM. These SSR markers will be useful for analysis of WEM diversity and population structures. The materials under study may be a source of genes for widespread adaptability in the current climate change scenario that jeopardises the mushroom production.

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Conflict of Interest- None

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