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Genetic Diversity and Nutritional Values of *Dendrocalamus yunnanicus* Species in the Northern Mountainous Regions of Vietnam

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Abstract

This study was to assess the genetic diversity of 12 *D. yunnanicus* samples collected from 5 different provinces in Vietnam and analyze the nutritional value of their shoots. The genetic similarity among the samples ranged from 98.47 - 100%. The length of ITS1-5,8S-ITS2 region of the samples has a size of 653 nucleotides. The GC accounted for 60% and the (A+T) was 40%. Based on the differences of ITS1-5,8S-ITS2 sequences, the samples were divided into two main groups: group 1 (1T and 1A), group 2 clustered into 4 subgroups 2.1 (2A), 2.2 (2H), 2.3 (1T) and 2.4 (1V, 3H, 1P, 1H, 3A, 2V and 2P). In nutritional value analysis of *D.yunnanicus* shoots, it showed a high nutritional property and variation among the samples, of which the highest calcium content (35.59g/100g) in Bac Kan province.

Keywords: *Dendrocalamus yunnanicus*, genetic diversity, nutritional content

Introduction

Dendrocalamus yunnanicus Hsueh is called “giant bamboo” and belongs to the subfamily of Bambusoideae. Over 200 worldwide commercial edible bamboo

species (*Phyllostachy*, *Bambusa* and *Dendrocalamus*) have been used for food products [1]. *D. yunnanicus* is considered as hexaploid, the chromosome number is presumably 2n= 72±2 [2]. This species is a large bamboo, underground tuber-shaped body, sporadic bamboo stem, drooping tops, 15-25 m in height. The common diameter is about 12-15cm, and the body wall thickness is 1-3cm, and few branches. This species has great value for collecting bamboo shoots and stems as raw materials for the food, construction and paper industry [3]. *D. yunnanicus* was first discovered in Yunnan province, China in 1988 [4]. This species is widely distributed in Asian countries including Vietnam. Vietnam locates in the center of bamboo distribution and *D. yunnanicus* estimated over 40 species with both *Bambusa* and *Dendrocalamus* species [5]. Especially, *D. yunnanicus* is found in the northern provinces such as Phu Tho, Tuyen Quang, Bac Kan and Thai Nguyen, Hoa Binh provinces from 50 to 200m above sea level [6].

The advantages of molecular techniques can identify the diversity at the molecular level, provide the basis for the assessment of species conservation value, identification of plant varieties, selection of parents for breeding, and conservation of genetic resources such as AFLP, RAPD, SSR, ISSRs and ITS sequence. Those molecular markers have been widely applied for the identification of numerous plant species including bamboo species [7]. Amongst them, the internal transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA (ITS1, 5.8S and ITS2) has been broadly performed for plant systematic to represent the phylogenetic relationships at the different taxonomic levels [8]. ITS region is useful for low-level phylogenetic analysis, comprising infra-generic level, because of its relatively rapid rate of evolution [9]. On the other way, ITS is one of the most comprehensively utilized molecular markers for angiosperm phylogenetic inference and genetic involvement in plants. Typically, ITS sequences have given molecular evidence to assess the phylogeny of taxonomic groups in numerous plant species.

With a highly commercial and ecological value, *D. yunnanicus* is being overexploited and extremely reduced in nature, which caused soil erosion and a decline in biodiversity in this country. Currently, there is currently no research available to assess the genetic diversity or analyze the nutritional content of bamboo shoots of *D. yunnanicus* in this country. Therefore, it is an urgent need to give effective protection and conserve this germplasm resource.

Materials and Methods

Material collection

A total of 12 leaf samples of *D. yunnanicus* were collected in 5 different provinces in Vietnam. The detailed information of the samples as shown in Table 1 and Figure 1.

Table 1. List of the collected *D. yunnanicus* samples used in this study

No	Origin	Code	No	Origin	Code
1	Bac Kan	1A	7	Ha Giang	2H
2	Ha Giang	1H	8	Phu Tho	2P
3	Phu Tho	1P	9	Thai Nguyen	2V
4	Tuyen Quang	1T	10	Bac Kan	3A
5	Thai Nguyen	1V	11	Ha Giang	3H
6	Bac Kan	2A	12	Tuyen Quang	3T

Fig. 1. The map of the sample collection. Some typical *D. yunnanicus* samples collected in the 5 different provinces in the northern areas, Vietnam

DNA extraction, ITS amplification and sequencing and ITS sequencing and phylogenetic analysis

A total of DNA extraction was done following the CTAP method with some minor modifications. The yielded DNA products were checked on the agarose gel (1%). The nucleotide sequences of ITS1 primer: TCCGTAGGTGAACCTGCGG, while, ITS8 primer was TCCTCCGCTTATTGATATGC [10]. The amplification of the primers and PCR program was performed as the method of Trung et al [8]. The DNA of all samples was sent to Macrogen company (Korea) for sequencing.

The data were used to analyze by MEGA 5 software to generate a phylogenetic tree with neighbour-joining (NJ) methods.

Nutritional content analyses

Five fresh sample species of each *D. yunanicus* shoot were collected from the 5 different provinces as mentioned above, were then analyzed for their nutritional contents. Analyzing these criteria was focused on: protein, total sugar, lipid, glucid, cellulose, calcium, iron, vitamin C, B2, A, D, and E following the National Regulation Standards method.

Results and Discussion

Molecular markers application to analyze the *D. yunnanicus* samples based on the ITS region sequences

Therefore, we used the ITS sequence to evaluate the genetic diversity of some *D. yunnanicus* species. The results showed that all DNA bands were relatively compact, clear and uniform which has been enough DNA quality for further experiments. All products were single-shaped bands with a size of about 800 bp.

Table 2: Composition of four types of nucleotides of the samples

No	Sample	Percentage %					
		A	C	G	T	A+T	C+G
1	1A	25.6	30.6	29.1	14.7	40.3	59.7
2	1H	25.6	30.8	29.2	14.4	40.0	60.0
3	1P	25.6	30.8	29.2	14.4	40.0	60.0
4	1T	25.6	30.6	29.4	14.4	40.0	60.0
5	1V	25.4	30.6	29.4	14.5	40.0	60.0
6	2A	25.7	30.8	29.1	14.4	40.1	59.9
7	2H	25.6	30.6	29.4	14.4	40.0	60.0
8	2P	25.6	30.8	29.2	14.4	40.0	60.0
9	2V	25.6	30.8	29.2	14.4	40.0	60.0
10	3A	25.6	30.8	29.2	14.4	40.0	60.0
11	3H	25.4	30.8	29.4	14.4	39.8	60.2
12	3T	25.6	30.8	29.2	14.4	40.0	60.0
	Avg	25.6	30.7	29.3	14.4	40.0	60.0

A: Adenine, C: Cytosine, G: Guanin, T: Thymine

In the fact that young leaves of bamboos are generally challenging to extract the DNA due to their high fiber and RNA contents [11], we have successfully extracted DNA products of 12 samples. We have further analyzed the length of the region ITS1-5,8SrRNA-ITS2 and collated with the sequences of the same genus taxon on the Genbank. However, all the samples had a similar size and composition ratio (G+C) of ITS1-5.8SRRNA-ITS2 region. On the other way, the result revealed no differences with the samples representing the surveyed taxon. All samples

shared the size of 653 nucleotides. The nucleotide compositions were manifested in Table 2. Generally, the samples have a higher ratio of Cytosine (C) and Guanin (G) than the ratio of Adenine (A) and Thymine (T). In other words, they have had % CG content greater than the % AT component. The 3H sample had the highest (C+G) component (60.2%) and the lowest (A+T) component (39.8%). The average % (C+G) in all samples was 60% and the percentage (A+T) averaged 40%. Our results implied that the greater CG% and AT% contents indicate the higher gene complexity to compare with *MatK* and *psbA-H* [11].

Comparing the nucleotide sequence of ITS1-rRNA-ITS2 region among the samples

The sequence of ITS1-rRNA-ITS2 of the *D. yunnanicus* samples was compared using the ClustalW sequencing tool of Mega 6.0 software. The results disclosed that the differences among the sequences were mainly single polymorphic values (SNP), in which one nucleotide was replaced by another nucleotide (Figure 2).

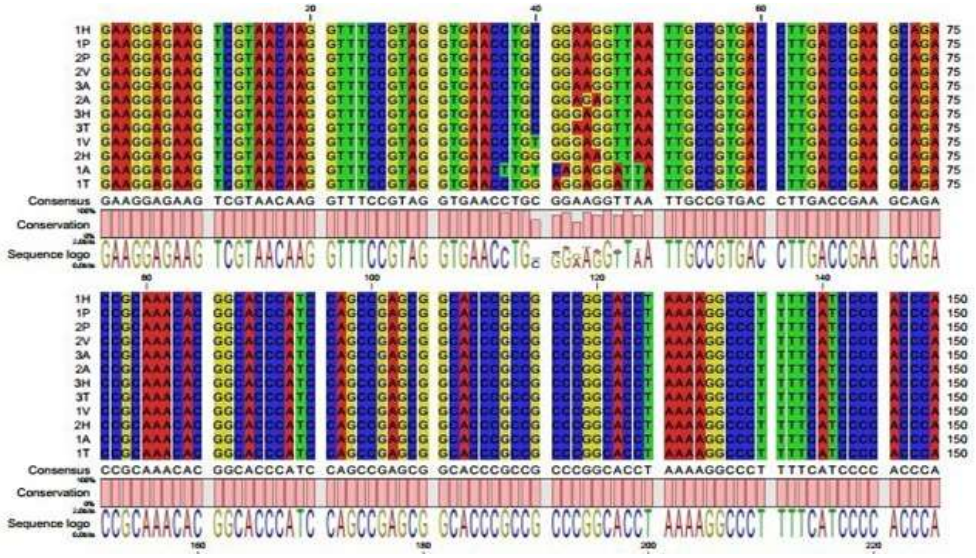

Fig.2. Comparison of nucleotide sequences among the samples

Besides, there are some gaps between those sequences. This may be resulted in the deletion and insertion of one or several nucleotides in the ITS1-rRNA-ITS2 region. The variation of sequences was most evident in the first 200 nucleotides and the last 200 nucleotides. These were empty areas without code from two sides of 5.8S rRNA gene. In other words, the variation spontaneously occurs in the ITS1 and ITS2 regions but less in the 5.8S rRNA gene region. The differences in the sequence of ITS1-rRNA-ITS2 region between the surveyed samples are expressed through the similarity coefficient of each pair of samples, determined by the genetic distance measuring tool of CLC v8.02. The highest homologous coefficient was 100 %, the lowest was 98,47% (Figure 3).

Constructing the genetic relationship tree among the *D. yunnanicus* samples based on the nucleotide sequence of ITS1-rRNA-ITS2 region

After determining the nucleotide sequence of ITS1-rRNA-ITS2 region, the construction of the relation tree was generated by Mega 6.0 software under the Maximum likelihood method (Figure 5). Based on the classification tree which was expressed by the sequence of ITS1-rRNA-ITS2 region, 12 *D. yunnanicus* samples were divided into 2 main groups. Group 1 consisted of only 2 taxons: 1T and 1A. While, group 2 included 10 taxa and was clustered into 4 subgroups as follows: Subgroup 2.1 was 1 taxon (2A). Subgroup 2.2 comprised of 1 taxon (2H). Subgroup 2.3 included 01 taxon (3T). Subgroup 2.4 consisted of 07 taxons (1V, 3H, 1P, 1H, 3A, 2V and 2P) (Figure 4). Currently, much worldwide effort has been focused on the genetic variation of bamboos, including intra and interspecies. However, few successes were obtained due to a lack of floral characters.

		1	2	3	4	5	6	7	8	9	10	11	12
1H	1		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
1P	2	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
2P	3	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
2V	4	100.00	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
3A	5	100.00	100.00	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.01	0.01
2A	6	99.54	99.54	99.54	99.54	99.54		0.01	0.01	0.01	0.01	0.02	0.01
3H	7	99.85	99.85	99.85	99.85	99.85	99.39		0.00	0.00	0.00	0.01	0.01
3T	8	99.69	99.69	99.69	99.69	99.69	99.23	99.54		0.01	0.01	0.01	0.01
1V	9	99.69	99.69	99.69	99.69	99.69	99.23	99.85	99.39		0.00	0.01	0.01
2H	10	99.54	99.54	99.54	99.54	99.54	99.39	99.69	99.23	99.69		0.01	0.01
1A	11	98.93	98.93	98.93	98.93	98.93	98.47	99.08	98.62	99.23	98.93		0.01
1T	12	99.23	99.23	99.23	99.23	99.23	98.77	99.39	98.93	99.39	99.39	99.39	

Fig. 3. Genetic distance between *D. yunnanicus* samples based on the sequence of ITS1-rRNA-ITS2 region

In the current study, we showed that ITS sequence had had good potential for the identification of bamboo specimens at the species level and phylogenetic relationships. Also, ITS sequence is not only simply amplify but also discloses accuracy for over 95% [11] and should be applied as the standard level.

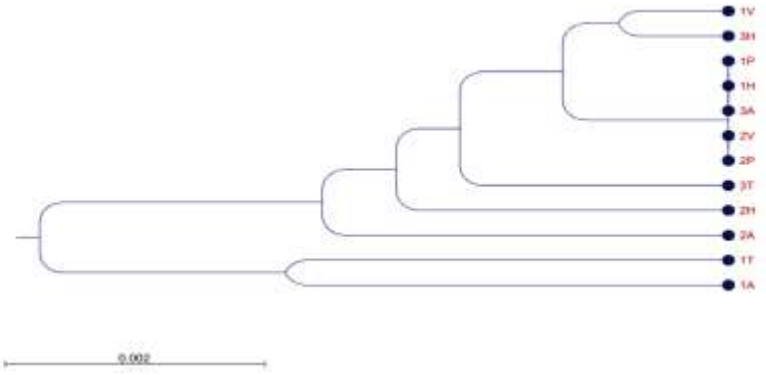

Fig 4: A relation tree generated among the samples

Analysis of nutritional compositions of *D. yunnanicus* shoot samples in the different origins

The analysis of nutritional contents of *D. yunnanicus* shoots was presented in Table 3. The highest calcium Bac Kan sample (35.59mg/100g), followed by Thai Nguyen (6.96mg/100g), the last was in Phu Tho, Tuyen Quang and Ha Giang provinces (1.57; 1.48g/100g), respectively. For carbohydrate (Glucid) contents among the samples, the highest was in Ha Giang sample (5.06g/100g), then followed by the Phu Tho, Bac Kan and Thai Nguyen provinces (4.83; 4.47 and 4.01g/100g), respectively. Whereas, the lowest content was detected in Tuyen Quang (3.18g/100g) (Table 3). Regarding the total sugar index among the provinces, there was not much difference. The greatest sugar content in Phu Tho province samples was 4.00 g/100g, next to the samples in Ha Giang, Bac Kan and Thai Nguyen provinces were 3.73; 3.57; 3.44g/100mg, respectively. The lowest was found in Tuyen Quang province samples was 3.73g/100mg. For the protein content, the highest protein was 2.24g/100g in Tuyen Quang province sample, followed by the Thai Nguyen sample was 2.08g/100g.

Table 3. Average of the main nutritional content of *D. yunnanicus* shoot samples collected in the different origins

Contents (mg/100g)	Provinces				
	Bac Kan	Ha Giang	Phu Tho	Tuyen Quang	Thai Nguyen
Total fiber	0.56	1.02	0.63	0.75	0.58
Sugar	3.57	3.73	4.00	2.17	3.44
Carbohydrates	4.47	5.06	4.83	3.18	4.01
Lipid	0.19	0.07	0.20	0.16	0.23
Protein	1.89	0.90	1.63	2.24	2.08
Calcium	35.59	1.48	1.74	1.57	6.96
Average (%)	7.70	2.0	2.17	1.67	2.88

The lowest was in Ha Giang sample was 0.9g/100g. Similarly, the lipid content was the highest in Thai Nguyen sample (0.23g/100g), then other samples showed low lipid contents were ranged from 0.07 to 0.19mg/100g, respectively. For the total fiber content, the highest was recorded in Ha Giang (1.02g/100g), then followed by Tuyen Quang, Phu Tho, Thai Nguyen province samples were 0.75; 0.63; 0.58g/100g and the lowest was in Bac Kan 0.56g/100g.

Generally, the nutritional contents of 5 bamboo shoots showed relatively high and varied in the different origins, especially the calcium content. For the total average nutritional contents of 5 samples were ranked as follows: the highest was Bac Kan (7.7%), followed by Thai Nguyen (2.88%), Phu Tho (2.17%), Ha Giang (2.0%) and Tuyen Quang (1.67%), respectively (Figure 5).

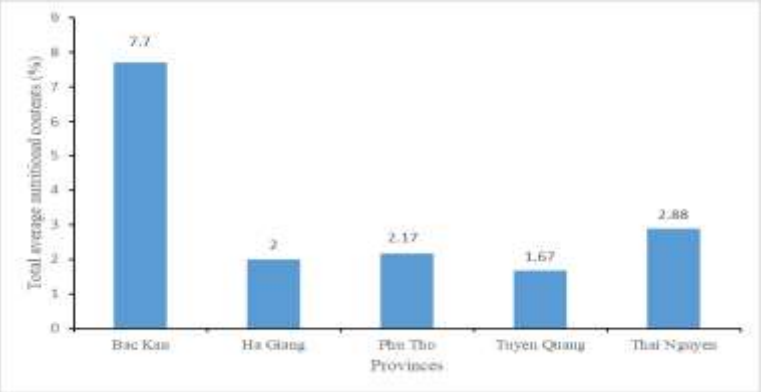

Fig. 5. Total average of the nutritional contents among the samples in the different origins

According to the report of Nguyen and Le [12], fresh *D. yunnanicus* shoot consists of these components: water content of 92.4%; protein 1.81%; sugar 2.14%; gluxite 2.71%; cellulose 0.51%; lipid 0.18%, respectively. Numerous reports showed that bamboo shoots have the main nutrients are proteins, carbohydrates, amino acids, minerals, sugar, fat, fiber, and inorganic salts. Protein contains 1.49 - 4.04 g (average 2.65g) per 100 g of fresh bamboo shoots. The protein in fresh *D. yunnanicus* shoots includes 17 amino acids, of which 8 amino acids are significantly essential for the human body [13]. The chemical composition of fresh bamboo shoots of *Bambusa vulgaris* and the nutritional content of commonly used bamboo shoots as fiber: 4.24 ± 0.01%; Carbohydrates: 6.51 ± 0.05%; Fat: 0.50 ± 0.01%; Protein: 3.64 ± 0.03% [14]. If comparing bamboo shoots of *Bambusa vulgaris* with *D. yunnanicus* shoots in Vietnam as above showed that the nutritional contents of *D. yunnanicus* shoot in Vietnam much higher.

Conclusions

The sequence analysis of ITS1-rRNA-ITS2 region of 12 *D. yunnanicus* has a size of 653 nucleotides. The average percentage of component (G+C) in all 12 samples was 60%, while (A+T) was 40% on average. The ITS genome sequence of *Densrocalamus* samples have a similarity rate of up to 98.47%. Based on the sequence of ITS1-rRNA-ITS2 region, the samples were divided into 2 main groups: group 1 (1T and 1A), group 2 clustered 4 subgroups 2.1 (2A), 2.2 (2H), 2.3 (1T) and 2.4 (1V, 3H, 1P, 1H, 3A, 2V and 2P). The analysis of the nutritional content of the shoots in five provinces affirmed that *D. yunnanicus* contains a high nutritional value.

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