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Chloroplast Analysis of Genetic Diversity of *Dolichandrone spathacea* Collected in the Central Coastal Regions

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Abstract

In this research, we investigated the diversity of genetic diversity of (*Dolichandrone spathacea* (L. f.) K. Schum) collected from the Central Coastal regions in Vietnam through the use of chloroplast markers. The results indicated that most of our samples exhibited high homogeny with *D. spathacea* MW432177, with a similarity range from 96.6% to 98.99%, except for two initial samples (Q23 and Q27),which were more closely related to *D. spathacea* LC129130. Additionally, among the Quao samples, a high level of homogeneity was observed, ranging from 95.5% between Q2 and Q15 to 99.90% between Q39 and Q40. The samples were divided into four groups: Group 1 contained the respective samples (Q16, Q8, Q40, Q39, Q41, Q43, Q21, Q13, Q12, Q15, Q25, Q1, Q10, Q4, Q2, Q30 and Q22); group 2 included 31 samples that were further divided into three sub-groups; group 3 only contained the sample Q23; and group 4 only contains Q37. Our study could provide useful information for selection of dominant genes and investigation of individual plants in natural ecosystems.

Keywords: *Dolichandrone spathacea*, Genetic diversity, chloroplast marker

Introduction

In Vietnam, *Dolichandrone spathacea* (L. f.) K. Schum) is a plant species belonging to the Bignoniaceae family, which grows in the mangrove forests along the coastal regions ranging from Da Nang to An Giang provinces. It is called "Quao" in Vietnamese, it has been used for various traditional medicinal purposes, such as liver detoxification, antitumor, antiseptic, flatulence, and treatment of nervous diseases in Southeast Asian countries [1-2].

Quao is a valuable species for coastal protection, known for its unique ecological and economic importance in Vietnam. Despite its significance, the conservation of Quao is lacking and requires greater attention for protection and preservation, as indicated by the IUCN and the Red List of Threatened Species of Vietnam [3]. Unfortunately, there has been limited genetic research on *D. spathacea*, few reports were studied on the morphology, ecology, and medicinal composition of this species. To the best of our knowledge, the complete chloroplast genome showed that the whole genome length is 159.156 bp (Genbank accession no.MW173028), including 86.053 bp belonging to the larger single-copy (LSC) while 12.635 bp belonging to the small single-copy (SSC). These two segments are separated by the insert region (IR), which packs 30.234 bp. These three regions' respective figures are 36.18%, 33.30%, and 41.43%. The genome contains 134 genes, 80 of which are coding for protein, 8 for rRNA, and 36 for tRNA, with the GC content being 37.95%. Finally, according to the phylogenetic analysis by Ting Yua [4], *D. spathacea* established a close relationship with *S. campanulata*.

Therefore, the objective of this study was to evaluate the genetic diversity of 50 Quao samples collected from different coastal regions in Vietnam by using chloroplast markers.

Materials and methods

Material collection

Fifty samples of Quao leaves were collected in the Central Coast Region for this research. The information is illustrated in Table 1 as below.

Table 1: List of *D.spathacea* leaves used in this study

No.	Name of sample	Location	Sample symbol	No.	Name of sample	Location	Sample symbol
1	Doli.TTH1	Thuan Hoa	Q1	26	Doli.TTH26	Thuan Hoa	Q26
2	Doli.TTH2	Thuan Hoa	Q2	27	Doli.TTH27	Thuan Hoa	Q27
3	Doli.TTH3	Thuan Hoa	Q3	28	Doli.TTH28	Thuan Hoa	Q28
4	Doli.TTH4	Thuan Hoa	Q4	29	Doli.TTH29	Thuan Hoa	Q29
5	Doli.TTH5	Thuan Hoa	Q5	30	Doli.TTH30	Thuan Hoa	Q30
6	Doli.TTH6	Thuan Hoa	Q6	31	Doli.TTH31	Thuan Hoa	Q31
7	Doli.TTH7	Thuan Hoa	Q7	32	Doli.TTH32	Thuan Hoa	Q32

Table 1 (continued): List of *D.spathacea* leaves used in this study

8	Doli.TTH8	Thuan Hoa	Q8	33	Doli.TTH33	Thuan Hoa	Q33
9	Doli.TTH9	Thuan Hoa	Q9	34	Doli.TTH34	Dien Truong	Q34
10	Doli.TTH10	Thuan Hoa	Q10	35	Doli.TTH35	Dien Truong	Q35
11	Doli.TTH11	Thuan Hoa	Q11	36	Doli.TTH36	Dien Truong	Q36
12	Doli.TTH12	Thuan Hoa	Q12	37	Doli.TTH37	Dien Truong	Q37
13	Doli.TTH13	Thuan Hoa	Q13	38	Doli.TTH38	Dien Truong	Q38
14	Doli.TTH14	Thuan Hoa	Q14	39	Doli.TTH39	Dien Truong	Q38
15	Doli.TTH15	Thuan Hoa	Q15	40	Doli.TTH40	Dien Truong	Q40
16	Doli.TTH16	Thuan Hoa	Q16	41	Doli.TTH41	Ru Cha	Q41
17	Doli.TTH17	Thuan Hoa	Q17	42	Doli.TTH42	Ru Cha	Q42
18	Doli.TTH18	Thuan Hoa	Q18	43	Doli.TTH43	Ru Cha	Q43
19	Doli.TTH19	Thuan Hoa	Q19	44	Doli.TTH44	Ru Cha	Q44
20	Doli.TTH20	Thuan Hoa	Q20	45	Doli.TTH45	Ru Cha	Q45
21	Doli.TTH21	Thuan Hoa	Q21	46	Doli.TTH46	Ru Cha	Q46
22	Doli.TTH22	Thuan Hoa	Q22	47	Doli.TTH47	Ru Cha	Q47
23	Doli.TTH23	Thuan Hoa	Q23	48	Doli.TTH48	Ru Cha	Q48
24	Doli.TTH24	Thuan Hoa	Q24	49	Doli.TTH49	Ru Cha	Q49
25	Doli.TTH25	Thuan Hoa	Q25	50	Doli.TTH50	Ru Cha	Q50

The chloroplast primers [5].

*rbc*L-F: ATGTCACCACAAACAGAGACTAAAGC

*rbc*L-R: CTTCTGCTACAAATAAGAATCGATCTC

In this study, we used typical bio-molecular chemicals that Merck and Sigma provided, for example, CTAB, Tris base, Boric acid, NaCl, dNTPs, EDTA, 6X orange loading dye solution, Taq Polymerase, Ethanol, 2-propanol, Acetic acid glacial, Phenol, Chloroform, isoamyl alcohol and Agarose.

Total DNA extraction

The total DNA of each *D. spathacea* leaf was extracted following the CTAB protocol proposed by Obara and Kako [6] with some modifications.

PCR reaction

The PCR was performed with a final volume of 15 µl, containing 1.5 µl 10X PCR buffer; 0.3 µl dNTPs (10mM); 0.2 µl Taq DNA polymerase (5U/µl); 1.5 µl forward primer (5µM); 1.5 µl Reverse primer (5µM); 1µl DNA (30ng/µl); 9 µl deionized water. The reaction took place in a thermocycler. First, it began with the initiation at 94°C for 5 min. Then, it repeatedly executed the denaturation for 35 to 37 cycles in which 50s at 94°C, 45s at 59°C, and 50s at 72°C. Last but not least, the process was finished with a final cycle that lasted for 7 min at 72°C for a final extension.

Electrophoresis

The PCR products were checked by gel electrophoresis in agarose gel 1% and visualized under UV after staining by ethidium bromide. After undergoing the electrophoresis, the PCR products were purified by a gel purification kit (Qiagen) and sequenced by Apical Scientific Company (Malaysia).

Result and Discussion

Sequencing the *Rbc*L – rDNA region

The result compilation of the sample sequence is shown in Table 2. In detail, the shortest size belonged to sample Q15 with 625bp only. Then, the size of six

Table 2: The proportion of each nucleotide

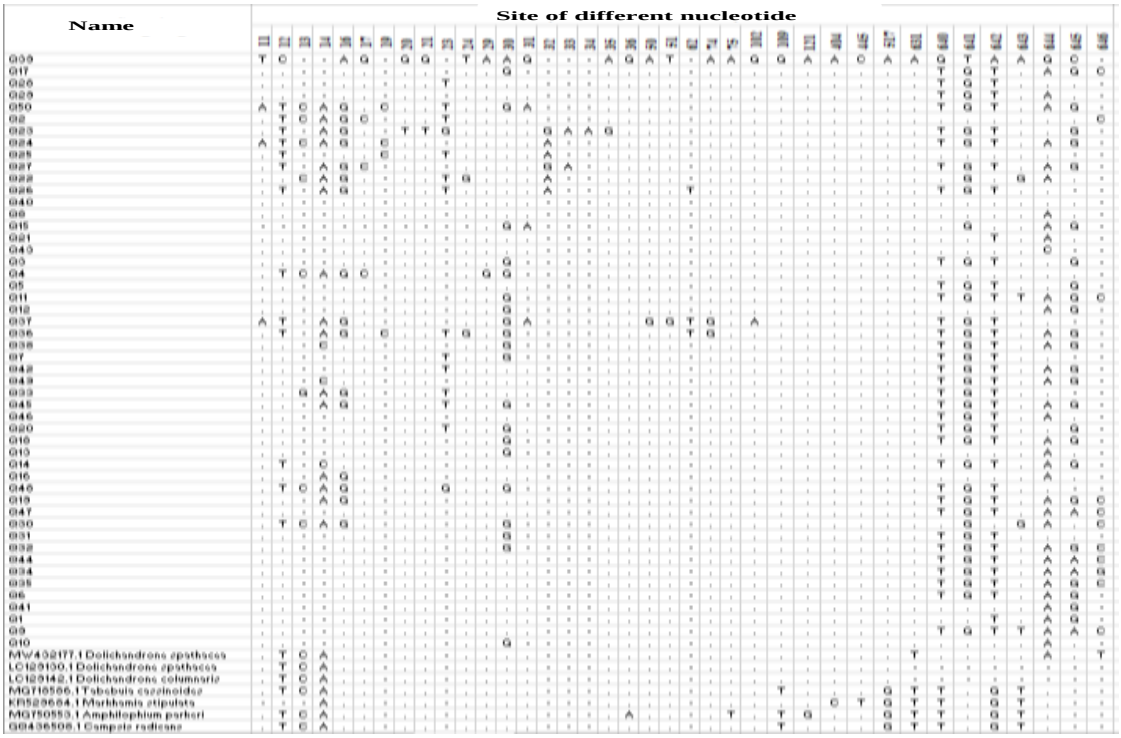
No.	Name	T(%)	C(%)	A(%)	G(%)	N	No.	Name	T(%)	C(%)	A(%)	G(%)	N
1	Q1	28.1	20.2	28.5	23.2	643.0	26	Q26	28.4	20.2	28.0	23.4	644.0
2	Q2	28.1	20.6	28.1	23.2	**647.0**	27	Q27	28.3	20.1	28.3	23.2	646.0
3	Q3	28.2	20.2	28.0	23.5	642.0	28	Q28	28.2	20.3	28.2	23.3	645.0
4	Q4	28.1	20.5	28.0	23.4	644.0	29	Q29	28.2	20.4	28.2	23.2	642.0
5	Q5	28.1	20.2	28.3	23.4	644.0	30	Q30	28.0	20.6	27.7	23.7	642.0
6	Q6	28.1	20.2	28.3	23.3	643.0	31	Q31	28.2	20.4	28.0	23.4	642.0
7	Q7	28.3	20.2	28.0	23.4	642.0	32	Q32	28.2	20.4	28.0	23.4	642.0
8	Q8	28.0	20.3	28.6	23.1	644.0	33	Q33	28.4	20.2	27.8	23.7	645.0
9	Q9	28.3	20.3	28.3	23.1	644.0	34	Q34	28.1	20.2	28.4	23.3	644.0
10	Q10	28.0	20.4	28.3	23.3	643.0	35	Q35	28.1	20.3	28.3	23.3	644.0
11	Q11	28.3	20.3	28.1	23.3	644.0	36	Q36	28.5	20.3	27.6	23.6	641.0
12	Q12	28.0	20.2	28.3	23.4	642.0	37	Q37	28.2	20.3	27.9	23.6	639.0
13	Q13	28.0	20.4	28.3	23.2	642.0	38	Q38	28.2	20.4	28.0	23.4	642.0
14	Q14	28.3	20.2	28.3	23.3	644.0	39	Q39	28.0	20.3	28.4	23.3	644.0
15	Q15	28.2	20.5	28.5	22.9	**625.0**	40	Q40	28.0	20.4	28.3	23.3	643.0
16	Q16	27.9	20.3	28.5	23.3	645.0	41	Q41	28.0	20.2	28.5	23.3	643.0
17	Q17	28.1	20.4	28.1	23.3	643.0	42	Q42	28.3	20.2	28.1	23.3	643.0
18	Q18	28.3	20.3	28.0	23.4	640.0	43	Q43	28.0	20.4	28.3	23.2	642.0
19	Q19	28.1	20.3	28.2	23.4	645.0	44	Q44	28.1	20.3	28.4	23.1	644.0
20	Q20	28.3	20.2	28.0	23.6	644.0	45	Q45	28.3	20.2	28.3	23.3	644.0
21	Q21	28.1	20.4	28.3	23.2	643.0	46	Q46	28.3	20.3	28.3	23.1	644.0
22	Q22	27.7	20.7	28.1	23.5	643.0	47	Q47	28.1	20.3	28.4	23.1	644.0
23	Q23	28.5	20.1	28.1	23.3	643.0	48	Q48	28.5	20.2	27.8	23.5	643.0
24	Q24	28.0	20.3	28.2	23.5	646.0	49	Q49	28.1	20.3	28.2	23.4	645.0
25	Q25	28.3	20.2	28.1	23.4	644.0	50	Q50	28.2	20.3	28.1	23.4	645.0
				Average					28.2	20.3	28.2	23.3	643.0

samples (Q16, Q119, Q28, Q33, Q49, Q50) and two other samples (Q24 and Q27) were next up, with the respective sizes being 645 and 646 bp. Finally, the longest one was sample Q2 (647 bp).

Analysis the diversity

The obtained sequence was first compared with the reference data given in NCBI (7 samples, including MW432177.1 *Dolichandrone spathacea*, LC129130.1 *D. spathacea*, LC129142.1 Dolichandrone columnaris, MG718586.1 T*abebuia cas sinoides*, KR529684.1 *Markhamia stipulata*, MG750553.1 *Amphilophium parkeri* and GQ436508.1 *Campsis radicans*, then fed to the MEGA v6.06 system to evaluate the phylogeny of the samples.

Table 3: Location of nucleotides between studied and reference sample



The data provided that the nucleotides of the *Rbc*L region had little difference among collected samples. Each of them held a unique share of the whole portion (Thymine – 28.2%, Adenine – 28.2%, Guanine – 23.3%, Cytosine – 20.3%). Comparing the nucleotides in the chloroplast region between experimented and reference samples using the *Rbc*L-F/*Rbc*L-R marker, we discovered that there were 38 sites containing nucleotide variation (Table 3). The CLC v8.0 software further investigated the difference in the chloroplast region by examining the homogenic values and the genetic distance. After running the software, the values established a range of 4.4% (from 95.5% between Q2 and Q15 to 99.90% between Q39 and Q40). Most of our samples showed a high homogeneity with MW432177 *D. spathacea* (96.06% to 98.99%) but not for samples Q23 and Q37 since they were more homogeneous to LC129130.1 *D. spathacea*.

The phylogeny of 50 Quao samples was built by combining the data from the software MEGA v6.06 and the maximum likelihood method. The result is shown in Figure 1.

Our samples could be divided into four main groups:

Group I: contained 17 samples: Q16, Q8, Q40, Q39, Q41, Q43, Q21, Q13, Q12, Q15, Q25, Q1, Q10, Q4, Q2, Q30 and Q22. The homogeneity witnessed a significant range from 95.5% between Q2 and Q15 to 99.9% between Q39 and Q40. Additionally, the highest homogenic values (99.2% to 99.8%) were Q39, Q40, Q41 and Q43. Conversely, the lowest value (98.6%) was held by both Q1 and Q10. Moreover, considering the similarity with MW432177 *D.spathacea* could be up to 98.9% (MW432177 and Q16, Q8, Q40, Q41, Q2).

Table 4: Homogeneity rates compared with references

Name	MW432177.1 *Dolichandrone spathacea*	LC129130.1 *Dolichandrone spathacea*	LC129142.1 *Dolichandrone columnaris*	MG718586.1 *Tabebuia cassinoides*	KR529684.1 *Markhamia stipulata*	MG750553.1 *Amphilophium parkeri*	GQ436508.1 *Campsis radicans*
Q16	98.99	96.90	96.12	84.98	84.98	91.90	90.06
Q8	98.99	96.90	96.12	84.98	84.98	91.90	90.06
Q40	98.99	97.06	96.43	85.12	85.12	92.06	90.21
Q39	98.84	97.06	96.28	84.98	84.98	92.06	90.21
Q41	98.99	96.90	96.27	85.12	85.12	91.90	90.06
Q43	98.83	97.05	96.42	85.25	85.25	92.05	90.21
Q21	98.68	96.75	95.97	84.83	84.83	91.75	89.90
Q13	98.53	96.44	95.66	84.52	84.52	91.44	89.59
Q12	98.68	96.59	95.96	84.81	84.81	91.59	89.75
Q15	96.06	96.01	94.12	82.35	82.35	89.12	87.27
Q25	98.38	96.76	96.28	84.85	84.85	91.76	89.91
Q1	98.68	96.75	96.12	85.12	85.12	91.75	89.90
Q10	98.68	96.45	95.81	84.67	84.67	91.45	89.60
Q4	98.68	96.75	95.66	84.81	84.81	91.75	89.90
Q2	98.99	96.91	95.83	85.01	85.01	91.91	90.07
Q30	98.68	96.45	95.96	84.83	84.83	91.45	89.60
Q22	97.92	95.99	96.11	84.26	84.26	90.99	89.14
Q26	97.76	96.60	96.43	84.70	84.70	91.60	89.76
Q24	98.22	96.60	95.98	84.85	84.85	91.60	89.76
Q46	98.68	96.91	96.12	85.14	85.14	91.91	90.06
Q49	98.37	96.90	96.12	84.98	84.98	91.90	90.06
Q33	98.06	96.90	96.28	84.96	84.96	91.90	90.06
Q45	97.60	96.14	95.81	84.23	84.23	91.14	89.29
Q50	97.91	96.30	95.81	84.54	84.54	91.30	89.45
Q36	97.30	95.69	95.95	83.95	83.95	90.69	88.84
Q48	98.22	97.06	96.12	85.12	85.12	92.06	90.21
Q27	98.07	96.60	95.82	84.72	84.72	91.60	89.76
Q44	98.52	96.75	96.12	84.98	84.98	91.75	89.91
Q47	98.52	96.75	96.12	84.98	84.98	91.75	89.91
Q34	98.52	96.75	96.12	84.98	84.98	91.75	89.91
Q29	98.52	96.75	96.42	84.96	84.96	91.75	89.90
Q35	98.52	96.75	96.12	84.98	84.98	91.75	89.91
Q42	98.22	96.60	96.27	84.83	84.83	91.60	89.75
Q6	98.52	96.90	96.27	85.12	85.12	91.90	90.06
Q19	98.52	96.75	96.12	84.98	84.98	91.75	89.91
Q14	98.68	97.06	96.27	85.27	85.27	92.06	90.21
Q9	98.37	96.91	96.28	84.98	84.98	91.91	90.06
Q5	98.22	97.06	96.28	84.98	84.98	92.06	90.21
Q28	98.22	96.91	96.13	84.85	84.85	91.91	90.06
Q7	97.60	96.59	96.12	84.52	84.52	91.59	89.75
Q3	97.75	96.59	95.81	84.52	84.52	91.59	89.75
Q20	97.76	96.60	96.12	84.54	84.54	91.60	89.75
Q31	97.91	96.59	95.81	84.52	84.52	91.59	89.75
Q32	98.06	96.29	95.66	84.52	84.52	91.29	89.45
Q18	98.06	96.59	96.27	84.65	84.65	91.59	89.75
Q38	98.06	96.44	96.11	84.65	84.65	91.44	89.59
Q17	98.21	96.45	95.81	84.67	84.67	91.45	89.60
Q11	97.91	96.60	95.82	84.54	84.54	91.60	89.75
Q23	95.55	**96.11**	95.38	83.41	83.41	90.39	88.55
Q37	95.68	**96.24**	95.82	83.59	83.59	90.82	88.98

Group II: comprised of 31 Quao samples. This group could be further divided into three subgroups:

Subgroup II.1: 5 samples (Q26, Q24, Q46, Q49 and Q33). This group has a moderate range since the value fluctuated from 97.8 to 99.2%. When comparing with the same reference sample as mentioned above, the highest rate was up to 98.7%.

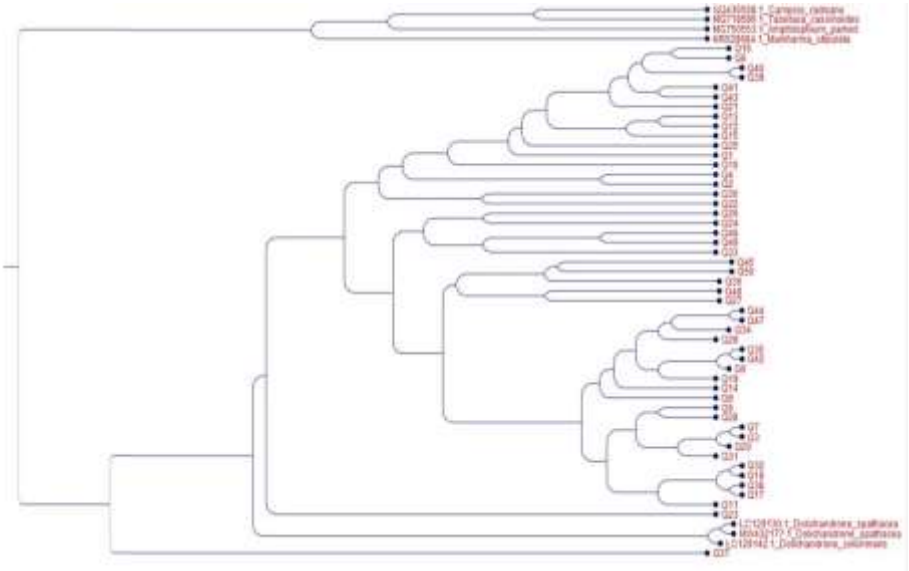


Figure 1: The phylogenetic tree of 50 Quao samples

Subgroup II.2: 5 samples (Q45, Q50, Q36, Q48 and Q27). Q45 and Q50 held this subgroup's highest homogeneity rate (99.2%). Compared with the reference, we derived the homogeneity rate from 97.3% to 98.2%.

Subgroup II.3: even though this was a subgroup, it included most of our samples (21 samples): Q44, Q47, Q34, Q29, Q35, Q42, Q6, Q19, Q14, Q9, Q5, Q28, Q7, Q3, Q20, Q31, Q32, Q18, Q38, Q17 and Q11. More than that, this group successfully occupied the most considerable homogenic rate, from 98.5% (Q7 and Q19) to 99.8% (Q44 and Q47). Compared with the reference, the returned values were between 97.6% and 98.7%.

Group III: this group was unique since it only had one element. The homogeneity of this strain with the others was from 95.9% (with Q10) to 97.8% (with Q27). Compared with the two reference samples MW432177 D.spathacea and LC129130.1 D. spathacea, this rate was 95.6% and 96.1%, respectively.

Group IV: this group had only one component (Q37) as well, with the homogenic rate compared with others being from 96.3% (with Q41) to 98.1% (with Q36). The respective figures, when compared with the identical references as group III, were 95.7% and 96.2%. Generally, 50 *D. spathacea* samples collected in numerous provinces of Vietnam's central and highland regions exhibited a high homogenetic rate in this study. The *D. spathacea* samples in this investigation exhibited low regional spread, although there was inter-location interference. The

result displayed on the phylogenetic tree of *D. spathacea* groups tended to congregate at some representative locations. For example, the group I had had 17 samples, but 13 came from Thuan Hoa. Similarly, group II contained three subgroups, each of which had the dominant characteristic mostly from specific regional properties. In detail, 3/5 of group II.1 were collected from Thuan Hoa, 3/5 of group II.2 were from Rucha, and the remaining group even had 15/21 samples taken in Thuan Hoa. This contribution might be attributed to the samples' origin or ecological location. Furthermore, these samples might indicate asexual propagation or seed movement caused by the wind. Our recent reports have successfully identified some precious plant species and genetic diversity and genetic relationships of *Dendrocalamus*, *Machilus*, *Disporopsis*, and *Huperzia* species by using molecular markers [7-10],

Our study had been the pioneer in the genetic research field of *D.spathacea* utilizing the chloroplast markers. Henceforth, it may be an excellent tool for distinguishing and selecting *D.spathacea* in future studies.

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