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Genetic diversity of Burmese grape (*Baccaurea ramiflora* Lour.) cultivars and Ha Chau cultivar identification based on DNA barcodes

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Abstract. *Khang DT, Huy TG, Thi NPA, Quyen DTH, Nhu NTB, Binh BN, Men TT, Dung TN. 2022. Genetic diversity of Burmese grape (*Baccaurea ramiflora *Lour.) cultivars and Ha Chau cultivar identification based on DNA barcodes. Biodiversitas 23: 3513-3520.* Burmese grape (*Baccaurea ramiflora* Lour.) is an underutilized fruit tree in the Mekong Delta and has the potential for food sources as well as landscape plants, especially Ha Chau cultivar. It has been considered an indigenous plant in Can Tho city, Vietnam with specific taste. However, their genetic diversity, utilization, and identification have not been dealt with in-depth. This study aimed to characterize genetic diversity among the five cultivars of Burmese grape in the Mekong Delta and to distinguish Ha Chau from other cultivars based on DNA barcodes, namely *matK*, *rbcL*, *ycf1b*, *rpoC1*, *psbK-I*, *atpF-H*, and ITS. DNA of twelve individuals belong to five Burmese grape cultivars was extracted prior to further amplification and sequencing. Sequences were analyzed to detect variable sites and the phylogenetic tree was constructed by the Maximum Likelihood method. Based on substitution sites and indel mutations, the plastid intergenic spacer *atpF-H* and *ycf1b* gene reflected the genetic diversity in five Burmese grape cultivars. Moreover, these sequences were valuable DNA barcodes for discrimination of Ha Chau cultivar. In combination of four markers (*rbcL*, *rpoC1*, *ycf1b*, and *psbK-I*) for phylogenetic construction, our finding revealed that Ha Chau cultivar is closely related to Red cultivar with highly supported bootstrap value of 89%. Such data could be applied for reliable identification of Ha Chau cultivar from other *B. ramiflora* cultivars in plant authentication.

Keywords: *atpF-H*, *Baccaurea ramiflora*, DNA barcoding, molecular identification, underutilized fruits, *ycf1b* gene

INTRODUCTION

Burmese grape (*Baccaurea ramiflora* Lour.) belongs to the family of Phyllanthaceae and is a fruit crop that originated in northeastern India to southern China and Peninsula Malaysia (POWO 2022). As a nutritional fruit, the species is not only rich in vitamin C and minerals, but also produces antioxidant compounds (Padayatty et al. 2003; Sundriyal and Sundriyal 2004). With a blend of sweet and sour, the pulp could be utilized in the food industry due to its economic efficiency and commercial importance. In the Mekong Delta, an indigenous variety of well-known Burmese grapes are Ha Chau, Green, Yellow, Red, and Xiem cultivars.

Ha Chau Burmese grape has light yellow color, thin skin, long fruit shapes, fruit has 3-4 segments, sweet and sour taste and specific aroma. Ha Chau Burmese grape was selected from indigenous cultivars by gardeners in Phong Dien district and developed in neighboring areas (Tran and Le 2012). The cultivar was recognized by the Intellectual Property Office of Vietnam as a specialty of Phong Dien district, Can Tho city in 2006 (Nguyen et al. 2018). Ha Chau Burmese grape has become a typical plant in Phong Dien district, Can Tho city, Vietnam and their plantation is the main income source for many horticulturists.

The propagation of Ha Chau Burmese grape is often through the intercrossing of selected plants with traits of interest. Nevertheless, long-term intraspecific hybridization could lead to inbreeding depression and thus, diminishing genetic background. On the other hand, cultivar identification with traditional techniques would be restricted due to the similarity in morphological features (Tran and Le 2012; Kodsara et al. 2021). Two popular molecular identification methods are AFLP (amplified fragment length polymorphism) and RAPD (random amplification of polymorphic DNA). However, each method has its limitations, such as the high cost of the AFLP technique and the accuracy of the RAPD technique (Kostamo et al. 2013). Therefore, the development of DNA based method for Burmese grape cultivar identification is of great potential.

Species-specific DNA barcodes have been an effective and reliable approach for genetic diversity evaluation as well as cultivar identification. This method provides a powerful tool for distinguishing various organisms, including animals, plants, and microbes at genus or species levels due to the comparison of a short and standardized genetic region (DeSalle and Goldstein 2019). Kodsara et al. (2021) reported that plastid loci and *rpoC1* could be utilized for discrimination of nine *Phyllanthus* species. The combination of barcoding and phylogenetic analysis indicated that *P. acidus* was the most genetically distinct from the rest of the analyzed species in the group. At the same time,

this study also confirmed the close relationship between two pairs of species *P. emblica* - *P. urinaria* and *P. emblica* - *P. reticulatus*. Some DNA barcodes commonly applied in plant taxonomy are known as *matK*, *psbA-trnH*, *rpoB*, *rpoC1*, *psbK-I*, *atpF-H*, *trnL-trnF* (Kress 2017). Therefore, this study aimed to characterize genetic diversity among the five cultivars of Burmese grape in the Mekong Delta and to distinguish Ha Chau to other cultivars based on DNA barcodes.

MATERIALS AND METHODS

Plant materials

Burmese grapes were cultivated from fruit gardens in Can Tho city, Hau Giang, and Ben Tre provinces in the Mekong Delta, Viet Nam. In this study, five common cultivars were utilized including Ha Chau, Green, Yellow, Red, and Xiem. Twelve individuals were selected based on fruit yield and identified by morphological characteristics (Table 1).

Procedures

DNA extraction

The procedure of DNA extraction was modified from the protocol of Rogers and Bendich (1988). One mL of extraction buffer and 50 µL of 10% SDS (w/v) were added to the powder of fresh leaves and incubated at 65°C for 30 minutes, followed by centrifugation at 12,000 rpm for 10 minutes. The supernatant was then transferred into a new tube containing 800 µL of isopropanol and placed at -20°C for at least 3 hours. Another centrifugation was carried out to collect the precipitate. RNA removal was obtained by the addition of RNase before the incubation with 2% CTAB at 65°C for 15 minutes. 800 µL of chloroform and isoamyl alcohol (24:1, v/v) was added. The upper phase was taken up by centrifugation and precipitated by absolute ethanol. The supernatant was then discarded while the pellet was washed with 70% ethanol twice to remove residual NaCl. DNA was dried for 10 minutes at 45°C in a vacuum centrifuge concentrator and stored in 100 µL of 0.1 X TE at -20°C prior to analysis. The quality and intact DNA were evaluated by agarose gel electrophoresis.

Amplification and sequencing

Seven DNA barcode loci were amplified using primer sequences as listed in Table 2. PCR mixtures were performed in a volume of 30 µL containing 2 µL DNA template (3 ng/µL), 0.5 µL of forward and reverse primers (0.4µM) respectively, 15 µL of MyTaq mix 2X (Bioline, England), and 12 µL of ddH_2O. The PCR thermal cycles to amplify seven DNA barcodes followed Fazekas et al. (2012) with modifications by gradient PCR (Table 3). PCR products were running in 2% agarose gel, then amplicons with clear and specific bands were sequenced by Sanger technology (ABI 3130) at Nextgen company (Ho Chi Minh city, Vietnam).

Data analysis

Target sequences of Burmese grapes amplified were analyzed with corresponding data in the GenBank database to investigate barcode sequences. Sequences were aligned by Clustal W algorithm in Bioedit program. The consensus sequence for each cultivar was created from individual sequences, and variable sites were enumerated. Phylogenetic tree was constructed by Maximum Likelihood method in MEGA X software (Kumar et al. 2018) with Kimura-2-Parameter (K2P) model and 1000 bootstrap replicates. Other options were followed as default setting.

Table 1. Burmese grape cultivars used in this study

Cultivar	Source (city/town, province)	Code
Green Burmese grape	Chau Thanh, Hau Giang	DXAHG
Red Burmese grape	Can Tho, Can Tho	DDVX
Xiem Burmese grape	Can Tho, Can Tho	DXVX
Ha Chau Burmese grape	Can Tho, Can Tho	DHCVX
Ha Chau Burmese grape	Can Tho, Can Tho	CHCVX
Green Burmese grape	Can Tho, Can Tho	DXAVX
Ha Chau Burmese grape	Can Tho, Can Tho	CHCOM
Xiem Burmese grape	Can Tho, Can Tho	DDXOM1
Xiem Burmese grape	Can Tho, Can Tho	DDXOM2
Green Burmese grape	Cho Lach, Ben Tre	DXABT
Ha Chau Burmese grape	Can Tho, Can Tho	CHC9H
Yellow Burmese grape	Can Tho, Can Tho	DV9H

Table 2. Primer sequences for seven DNA barcode loci

Primers	Nucleotide sequences (5'-3')	Product length (bp)	References
ITS	ITS1: TCCGTAGGTGAACCTGCGG ITS4: TCCTCCGCTTATTGATATGC	500-700	White et al. (1990)
matK	*matK*-390F: CGATCTATTCATTCAATATTTC *matK*-1326R: TCTAGCACACGAAAGTCGAAGT	100-900	Sun et al. (2001)
atpF-H	*atpF*: ACTCGCACACACTCCCTTTCC *atpH*: GCTTTTATGGAAGCTTTAACAAT	196-573	Vijayan and Tsou (2010)
psbK-I	*psbKF*: TTAGCCTTTGTTTGGCAAG *trnHF05R*: CGCGCATGGTGGATTCACAATCC	185-576	Fazekas et al. (2012)
ycf1b	*ycf1b*F: TCTCGACGAAAATCAGATTGTTGTGAAT *ycf1b*R: ATACATGTCAAGTGATGGAAAA	909-962	Dong et al. (2015)
rpoC1	*rpoC1*_2F: GGCAAAGAGGGAAGATTTCG *rpoC1*_4R: CCATAAGCATATCTTGAGTTGG	450-490	Fazekas et al. (2012)
rbcL	*rbcL*aF: ATGTCACCACAAACAGAGACTAAAGC *rbcL*aR: GTAAAATCAAGTCCACCRCG	550-600	Wang et al. (2011)

Table 3. Thermal cycles for amplification of seven DNA barcodes

Loci	Initial denaturation	30 cycles			Final extension	Storage
		Denaturation	Annealing	Extension		
ITS	95°C	95°C	55°C	72°C	72°C	4°C
	5 min	30 sec	30 sec	1 min	7 min	
matK	94°C	94°C	50°C	72°C	72°C	
	1 min	30 sec	40sec	40 sec	5 min	
rpoC1	95°C	95°C	52°C	72°C	72°C	
	2 min	30 sec	30 sec	1min	5 min	
atpF-H	94°C	94°C	51°C	72°C	72°C	
psbK-I	4 min	30 sec	40 sec	40 sec	5 min	
rbcL	94°C	94°C	55°C	72°C	72°C	
	4 min	30 sec	30 sec	1 min	10 min	
ycf1b	94°C	94°C	52°C	72°C	72°C	
	4 min	30 sec	40 sec	1 min	10 min	

RESULTS AND DISCUSSION

Amplification of DNA barcode candidates

Based on the gel pattern, the size of seven amplicons ranged from 500-900 bp (Figure 1). Five DNA barcode loci showed clear bands and high specificity, including *psbK-I*, *atpF-H*, *ycf1b*, *rbcL*, and *rpoC1*. Bands with similar lengths were also reviewed in other DNA barcode studies in plants (Kress 2017; Santos and Pereira 2018). No band was detected in non-template sample, reflecting the contamination was controlled. On the other hand, ITS region showed non-specific bands. In case of *matK* gene, no PCR products have appeared. Therefore, it should be considered for the variation of DNA template leading to the problem in primer binding.

Sequence characteristics

DNA sequence were characterized based on the number of variable sites and indel mutation (Table 4). two noncoding intergenic spacers, namely *atpF-H* and *psbK-I* had 11 and 12 variable sites, respectively. Such sites were much higher than those in three coding sequences, including *rpoC1*, *ycf1b*, and *rbcL*. Furthermore, *atpF-H* sequence also expressed a higher number of indel mutations compared to other loci.

atpF-H intergenic spacer

The sequence of *atpF-H* spacer in Ha Chau Burmese grape was approximately 740 bp in length. Red Burmese grape witnessed a loss of 492th-705th nucleotide segment while there was a replacement of thymine with adenine in nucleotides 699 and 701 of the Green ones. At nucleotide positions 716, 723, 736, 742, 743, 745, 747, 762, 767, and 768, consensus sequence of Ha Chau cultivar was also incompatible. There was also a difference in nucleotides 746, 758, and 771 in Ha Chau Burmese grape in comparison with two out of three control samples (Tables 5 and 6). There were 11 substitutions and 21 insertions between consensus sequences of Ha Chau Burmese grape and three other cultivars, with a similarity of 98.24%. Besides, the use of *atpF-H* sequence or the combination of *atpF-H* and *psbK-I* spacers have successfully identified medicinal plants or *R. stricta* (Techen et al. 2013). Based on the number of indel mutations, *atpF-H* was utilized to distinguish five cultivars of *Tabernaemontana divaricata* (Jena et al. 2019). Rehman et al. (2021) reported that a specific intron was detected in *atpF* sequence in only *B. ramiflora*, which was absent in all Phyllanthaceae species examined. This finding points to the usefulness of *atpF-H* sequence to discriminate Ha Chau Burmese grape from other cultivars.

psbK-I intergenic spacer

The sequence length of *psbK-I* intergenic spacer was about 470 bp. Currently, there is only *psbK-I* sequence belonging to *Baccaurea ramiflora* on the NCBI database, so it is impossible to compare the sequence variation within the genus *Baccaurea*. Rehman et al. (2021) reported that such sequences belonging to the Photosystem II group showed not many variable sites for species specific identification in Phyllanthaceae. There was an apparent difference in the consensus sequence between such plants and other cultivars, particularly at the nucleotides of 94, 95, 113, 121, and 215 (Table 7). By contrast, its target sequence was similar to that of Red Burmese grape at nucleotides 54 and 431 and to that of Yellow one at nucleotides 244, 249, 289, 291, and 301.

rpoC1 gene

The sequence of *rpoC1* gene in Burmese grape was 438 bp in length. We could not find any similar target sequence with that of Ha Chau cultivar on the GenBank database at the species level. Some studies focusing on higher levels revealed that PCR product sizes of such gene in *Balanops balansae* and *Mammea americana* were 2060 bp (Jin et al. 2020) and 2190 bp (Xi et al. 2012), respectively. This proves that there was not any published scientific research on *rpoC1* gene in Ha Chau cultivar.

A difference in consensus sequence of Ha Chau cultivar with that of others was shown due to the use of BioEdit software (Figure 2). In Ha Chau cultivar, a 2nd-7th nucleotide fragment in the target sequence was depleted. Only the Red cultivar did not lose a nucleotide at position 1, compared with the remaining. This was similar to the Green one, where nucleotide 8 remained. In addition, the target sequence in almost cultivars was deprived of nucleotide 17 and nucleotide 32, except for Yellow and Xiem cultivars, respectively.

Table 4. Variable sites and indel mutations of five DNA barcode sequences

Sequence	Variable sites	Indel mutations
atpF-H	11	21
psbK-I	12	0
rpoC1	0	10
ycf1b	5	11
rbcL	5	3

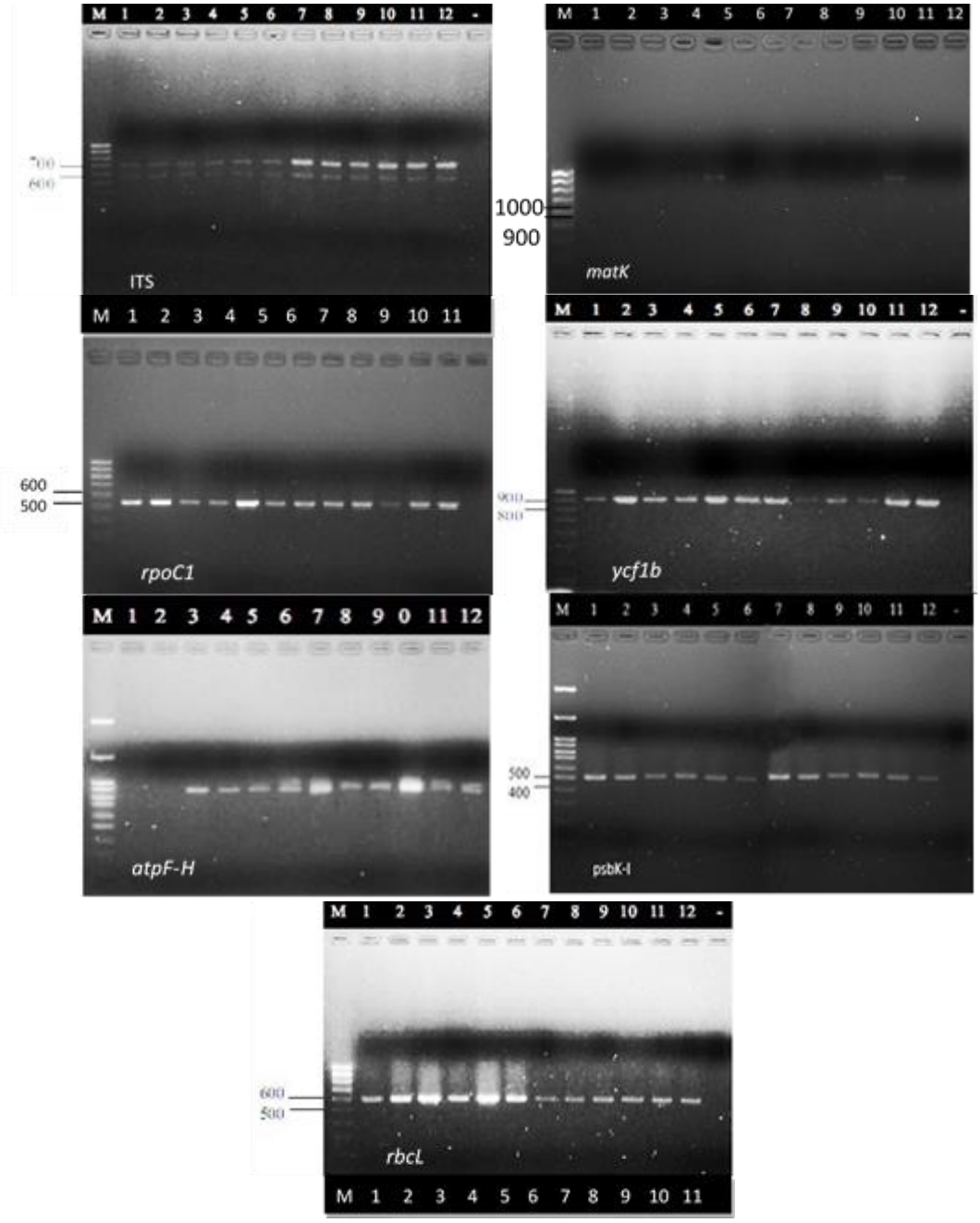

Figure 1. Amplicons of seven DNA barcode loci on 2% agarose gel. Note; M: 100 bp DNA ladder, 1: DXAHG, 2: DDVX, 3: DXVX, 4: DHCVX, 5: CHCVX, 6: DXAVX, 7: CHCOM, 8: DDXOM1, 9: DDXOM2, 10: DXABT, 11: CHC9H, 12: DV9H, (-): Negative control)

Table 5. Nucleotide variation of *atpF-H* spacer between Ha Chau Burmese grape and other cultivars

Cultivar	Nucleotide position															
	492	**493**	**494**	**495**	**496**	**497**	**498**	**499**	**698**	**699**	**700**	**701**	**702**	**703**	**704**	**705**
Consensus HC	C	C	T	T	T	G	T	T	T	T	T	T	T	T	T	T
Consensus DD	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Consensus DX	.	.	.	.	.	.	.	.	.	.	.	.	.	.	_	_
Consensus DXA	.	.	.	.	.	.	.	.	.	A	.	A	.	.	_	_

Table 6. Nucleotide variation of *atpF-H* spacer between Ha Chau Burmese grape and other cultivars (continuous)

Sample	Nucleotide position													
	706	**716**	**723**	**736**	**742**	**743**	**745**	**746**	**747**	**758**	**762**	**767**	**768**	**771**
Consensus HC	G	T	T	C	G	A	T	C	C	G	T	G	T	A
Consensus DD	A	A	_	G	A	T	C	_	G	T	_	_	C	_
Consensus DX	A	A	_	G	A	T	C	_	G	.	_	_	C	.
Consensus DXA	A	A	_	G	A	T	C	.	G	T	_	_	C	_

Note: The sign "." exhibits nucleotide similar to that of the first sample; the sign "_" exhibits the missing nucleotide position; HC: Ha Chau cultivar, DD: Red cultivar, DX: Xiem cultivar, DXA: Green cultivar

Table 7. Nucleotide variation of *psbK-I* sequence between Ha Chau Burmese grape and other cultivars

Sample	Nucleotide position											
	54	**94**	**95**	**113**	**121**	**215**	**244**	**249**	**289**	**291**	**301**	**431**
Consensus HC	A	C	C	C	A	A	T	T	G	T	T	A
Consensus DD	.	A	A	A	T	G	G	G	A	C	C	.
Consensus DV	C	G	A	A	C	G	.	.	.	.	.	T
Consensus DX	C	A	A	A	T	G	G	T	A	C	C	T
Consensus DXA	C	A	A	A	T	G	G	T	A	C	C	T

Note: The sign "." exhibits nucleotide similar to that of the first sample; The sign "_" exhibits the missing nucleotide position; HC: Ha Chau cultivar, DD: Red cultivar, DV: Yellow cultivar, DX: Xiem cultivar, DXA: Green cultivar

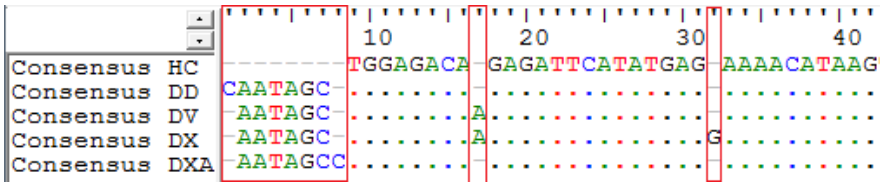

Figure 2. Nucleotide variation between Burmese grape cultivars based on *rpoC1* gene. Note: The sign "." exhibits nucleotide similar to that of the first sample; the sign "_" exhibits the missing nucleotide position; HC: Ha Chau cultivar, DD: Red cultivar, DV: Yellow cultivar, DX: Xiem cultivar, DXA: Green cultivar

In comparison with previous studies in other land plants, *rpoC1* gene showed highly conservative among closely related species in the genus *Selenicereus* (Huy et al. 2021). Although *rpoC1* gene exhibited 100% for amplification success, this plastid gene was not able to distinguish *Dendrobium* (Orchidaceae), indicated by the species resolution was only 38.89%, which was the lowest percentage among five tested markers including ITS, *matK*, *rbcL*, *rpoB*, and *rpoC1* (Singh et al. 2012). Studying the plastid genome of Phyllanthaceae, which contained the genus *Baccaurea*, *rpoC1* was not considered a polymorphic locus (Rehman et al. 2021). Thus, the results of this work were in complete agreement with other studies in the world, indicating that *rpoC1* gene was ineffectual for discriminating Ha Chau cultivar and other Burmese grape cultivars.

***ycf1b* gene**

There was a difference in positions in nucleotides 617 and 749 between the consensus sequence of Ha Chau cultivar and that of others, where T nucleotides were replaced by G and A nucleotides, respectively in Ha Chau cultivar. Depletions of nucleotides 747 and 748 and 707-715 nucleotide fragments in almost remaining cultivars were investigated, except for yellow cultivar. The sequence of yellow cultivar showed the discrepancy of nucleotides 707, 710, and 747 when compared with Ha Chau cultivar (Table 8). Particularly, from the position of nucleotide 592 onwards, its sequence greatly differed that of Ha Chau Burmese grape. The *ycf1b* gene was one of the potential plastid DNA barcode for the identification of several plant groups due to the high sequence variation in *Pinus*, *Calycanthaceae*, *Iris*, *Armeniaca*, *Paeonia*, *Quercus*, and *Panax* (Dong et al. 2015). Amar et al. (2020) reported that *ycf1* gene, including *ycf1a* and *ycf1b* inside was a valuable plastid coding gene for the identification of *Prunus persica*, which is close relationship with other *Prunus* species.

***rbcL* gene**

The *rbcL* gene length of Burmese grape cultivars ranged from 550 bp to 600 bp. These values were also presented by Heckenhauer et al. (2017) and Dean et al. (2018), where the amplicons of *rbcL* gene were 697 bp and 636 bp in length for *Baccaurea* sp. JH-2017 and *Baccaurea racemosa*, respectively. Seven sequences, including MF435503.1, MH332500.1, MH332478.1, MH332456.1, MH332445.1, MG838505.1, and MG838493.1 were found to have a similarity of 100% with consensus sequence of

Ha Chau Burmese grape using BLAST tool. Other six sequences also showed resemblance with that of Burmese grapes but with lower similarity (approximately 99.8%), i.e. MF435509.1, AY663570.1, AB925671.1, MH332455.1, MG838502.1, and MG838491.1.

After comparing the consensus sequence of Ha Chau Burmese grape with the sequence of four other cultivars, the data illustrated the occurrence of 6 single nucleotide polymorphisms (SNPs) and 2 indel mutations. At nucleotide positions 65, 101, 113, and 302 the consensus sequence of Ha Chau Burmese grape showed differences from the Red Burmese grape (Table 9). There were 8 different nucleotide positions between target sequences of Ha Chau Burmese grapes and other cultivars. This Burmese grape showed a similar position at nucleotide 35, but different positions at nucleotides 65, 101, 113, and 302 with the Red cultivar. In comparison with Yellow cultivar, consensus of Ha Chau cultivar was inserted by thymine at nucleotide 523. In addition, there were two single nucleotide depletions at nucleotides 511 and 526 in consensus sequences of Red and Yellow cultivars, revealing the difference with Ha Chau cultivar.

The *rbcL* gene showed limitation in low discrimination power when the accuracy rate of this gene was only 41.67% of *Dendrobium* species (Singh et al. 2012). Huang et al. (2015) reported that *rbcL* gene was recommended for the identification of tropical plants at a species level, but they did not provide adequate proof of this finding. Only a small number of plants among several plants surveyed were identified with low success rate. Thus, the *rbcL* gene was not enough variable to distinguish Ha Chau cultivar from others in this study.

Phylogenetic tree analysis

The phylogenetic analysis involved 4 nucleotide sequences, including three coding genes (*rbcL*, *rpoC1*, *ycf1b*) and the intergenic spacer *psbK-I*. The Yellow cultivar was missed in *atpF-H* sequence, so this spacer was ignored for phylogenetic tree construction. All positions containing gaps and missing data were eliminated (complete deletion option). In combination with five DNA barcode candidates for phylogenetic construction, such cultivars in this study were classified into two main groups (Figure 3).

Group A consists of two sub-branches, the first sub-branch includes Ha Chau and Red cultivars with the genetic distance between these two cultivars being 1.57%, and the reliability of the branch is reinforced with highly supported bootstrap value of 89%. The second sub-branch consisted of Xiem and Green cultivars with a genetic distance with highly supported bootstrap value of 99%. The Yellow cultivar has a relatively large genetic distance compared with other varieties. Thus, through genetic distance, it is possible to identify Ha Chau cultivar compared to other common Burmese cultivars in the Mekong delta. combination of more than two DNA barcodes increased the species resolution in various plant species. Wu et al. (2017) reported that a single locus only distinguishes some of the 18 species in *Melilotus*, a medicinal plant in North Africa.

Table 8. Nucleotide variation of *ycf1b* gene between Ha Chau Burmese grape and other cultivars

Sample	Nucleotide position												
	617	**707**	**708**	**709**	**710**	**711**	**712**	**713**	**714**	**715**	**747**	**748**	**749**
Consensus HC	G	G	G	G	A	G	G	A	G	A	A	A	A
Consensus DD	T	_	_	_	_	_	_	_	_	_	_	_	T
Consensus DV	T	C	.	.	G	.	.	.	.	.	G	.	T
Consensus DX	T	_	_	_	_	_	_	_	_	_	_	_	T
Consensus DXA	T	_	_	_	_	_	_	_	_	_	_	_	T

Note: The sign "." exhibits nucleotide similar to that of the first sample; The sign "_" exhibits the missing nucleotide position; HC: Ha Chau cultivar, DD: Red cultivar, DV: Yellow cultivar, DX: Xiem cultivar, DXA: Green cultivar

Table 9. Nucleotide variation of *rbcL* gene between Ha Chau Burmese grape and other cultivars

Code	Nucleotide position							
	35	**65**	**101**	**113**	**302**	**511**	**523**	**526**
Consensus HC	A	A	G	G	G	T	T	A
Consensus DD	.	C	C	C	A	_	.	_
Consensus DV	C	.	.	.	.	_	_	_
Consensus DX	C	.	.	.	.	.	.	.
Consensus DXA	C	.	.	.	.	.	.	.

Note: The sign "." exhibits nucleotide similar to that of the first sample; the sign "_" exhibits the missing nucleotide position; HC: Ha Chau cultivar, DD: Red cultivar, DV: Yellow cultivar, DX: Xiem cultivar, DXA: Green cultivar

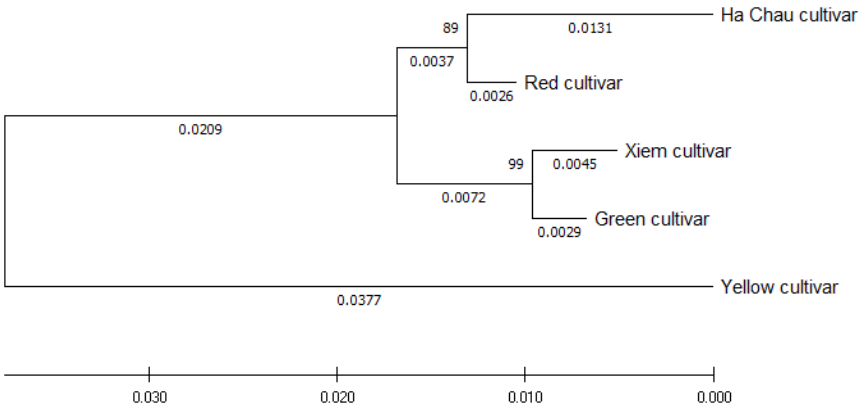

Figure 3. Phylogenetic relationship of Burmese grape cultivars based on four DNA barcode markers, namely *rcbL*, *rpoC1*, *ycf1b*, and *psbK-I*. scale bar indicates the percentage of difference in nucleotide variations

Data from this finding illustrated that the combination of five loci, namely *matK*, *rbcL*, *trnL-F*, *psbA-trnH*, and ITS indicated accurate species resolution while the single *rbcL* was less effective. Vu et al. (2020) also suggested that combination of plastid DNA barcodes increased the species identification in Vietnamese *Paphiopedilum* species. At the genome level, phylogenetic tree generated by 14 chloroplast genomes elucidated that *B. ramiflora* showed a closed genetic relationship with *Phyllanthus*, *Glochidion*, and *Flueggea* species (Hu et al. 2021).

In conclusion, the Burmese grape cultivars had genetic diversity based on the combination *rcbL*, *rpoC1*, *ycf1b*, and *psbK-I* sequences. This finding supports the idea that the sequence region at *atpF-H* could be used to distinguish Ha Chau Burmese grape from other cultivars in the Mekong Delta. Further studies on larger sample sizes and survey sites or the remaining regions should be carried out to investigate the appropriate sequence for identifying Ha Chau cultivar among others.

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