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Research Article

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Morphological characteristics and Genetic Diversity of some Red Dragon Fruit Samples in Field Trials and MatK Marker Sequencing

Nguyen Thi Thu Huong[1], Nguyen Quoc Hung[1], Doan Van Lu[2], Tran Dang Khanh[3]*

[1]Fruit and Vegetable Research Institute, Hanoi, Vietnam
[2]Vietnam National University of Agriculture, Hanoi, Vietnam
[3]Agricultural Genetics Institute, Hanoi, Vietnam

Abstract Dragon fruit (*Hylocereus undatus*) is an important economic fruit species and widely grown in many areas in Vietnam due to its rich nutrient contents and strong drought tolerance. The objectives of this study were to evaluate the main morphological characteristics of 6 dragon fruits collected in the different provinces and analyzed their genetic diversity by *MatK* marker sequencing. The results showed that the samples have relatively different morphological characteristics in terms of cladode, buds, flowers and fruits. By using *MatK* sequencing, the genetic similarity coefficient among the samples ranged from 86.8% to 98.6%; the nearest genetic distance is 0.01, while the farthest is 0.07. The attained results of this study have provided useful information on morphological characteristics and genetic diversity of dragon fruits for breeding programs.

Keywords Dragon fruit, morphological characteristic, MatK marker

Introduction

Dragon fruit (*Hylocereus undatus*) is a tropical and subtropical fruit that belongs to the climbing cactus vine (Cactaceae) family. In Vietnam, dragon fruit is the most popular fruit and is widely grown in all provinces in this country, especially in the southern regions. In 2017, Vietnam has been a leading exporter of dragon fruit in the world with over 40.000 ha devoted to dragon fruit and produce 1 million metric tons, which is equivalent to 896 million US dollars [1]. In recent years, the Fruit and Vegetable Research Institute (FVRI) has selected the red-flesh dragon fruit variety TL4 which is suitable for growing in the Northern provinces and additionally collected various dragon fruit varieties from domestic and foreign countries for dragon breeding programs. The dragon fruit samples have been initially evaluated for their ability to grow and develop in the ecological condition in Gia Lam- Hanoi. In order to obtain a complete set of data on agronomic characteristics of the samples, it needs to fully research their morphological characteristics and genetic diversity at the molecular level to make selection strategies and breeding approaches to generate the red dragon fruit with high quality. Therefore, the objective of this study was to evaluate the agronomic traits and genetic diversity of 6 red dragon fruits by *MatK* marker sequencing.

Materials and Methods

Materials collection

A total of 6 red flesh dragon fruit samples were collected from different provinces. Their names and origins and collection information were listed in Table 1.

Table 1: The red dragon fruit samples were used in this study

No.	Name of samples/varieties	Origins/collected provinces
1	Sample of red flesh dragon fruit QN1	Taiwan, collected in Quang Ninh province
2	Sample of red flesh dragon fruit QN2	
3	Sample of red flesh dragon fruit VP2	Taiwan, collected in Vinh Phuc province
4	Sample of red flesh dragon fruit TL4	Official variety awarded by MARD in 2017
5	Sample of red flesh dragon fruit TL5	Official trial variety awarded by MARD in 2017
6	Sample of red flesh dragon fruit LĐ1	Official variety generated by Fruits and Vegetable Institute, 2010

Evaluating agronomic traits of the samples in the field condition

The experiments were performed following the conventional method. Specifically, the planting method was evaluated based on the group of 10 posts/sample variety without replication. The samples were grown with the same density of 1000 posts/ha. Each supporting post was planted by 4 samples on each side of the post cylinder. The fertilizer and watering were applied following the conventional methods developed by FVRI. The morphological and agronomic characteristics of the samples including stems, branches, buds, flowers were conducted following to the DUS, national technical regulation for dragon fruit [2].

Total DNA extraction and amplification of *MatK*

The fresh samples were collected and kept samples dry at less than 60% humidity for DNA extraction. In this study, DNA extract was done following the CTAB method of Doyle and Doyle [3] with some minor modifications. The yielded DNA products were then confirmed and determined by the use of Spectrophotometer [4]. The forward primer *MatK* -390F was 5’-CGATCTATTCATTCAATATTTC-3’ and the reverse primer *MatK* -1326R was 5’-TCTAGCACACGAAAGAAGT-3’, respectively.

Amplification was made in a polymerase chain reaction (PCR) in the tuber 0.2 ml with total volume (15 µl), of which contained 9.0 µl deionized distilled water, buffer Mg+ 25 Mm (1.5 µl), dNTPs 10 Mm (0.3 µl), Taq ADN polymerase 5 U/µl (0.2 µl), the primer F 10 µM (1.5 µl), Primer R 10 µM (1.5 µl) and DNA 50 ng/µl (1.0 µl), respectively. PCR program was performed as the method described by Trung et al [5]. Briefly, denaturation was made for 94^{o}C, 56^{o}C for annealing, and 72^{o}C for extension and 72^{o}C for the final extension. Gel removal Qiagen Kit was applied for PCR products.

Sequencing and phylogenetic analysis

The *MatK* PCR products were sent to Macrogen (Korea) to sequence by applying ABI PRISM 3700 DNA Analyzer (Applied Biotech). The attained data were used to analyze by MEGA v 5.1 software to generate a phylogenetic tree with neighbor-joining (NJ) methods. BLAST tool was used to compared the obtained sequences to National Center for Biotechnology Information databases (NCBI).

Statistical Analyses

All raw data were statistically analyzed by Excel version 2016, ClustalW, Mega 6.0 and CLC 8.0 software.

Results and Discussion

Evaluating agronomic traits of the sample varieties

Unlike some other fruits, dragon fruit is belonging to the family of Cactus, the samples have stems with no leaves, on the other ways, the leaves are deformed into spines [6]. Therefore, the branch characteristics of the samples are shown by the number of spines per areolas, color of young buds and cladode color as shown in Table 1.

The results showed that the spiny areola spacing in dragon fruit among each sample variety is different with densities of thorns, flower buds that have appeared in each areola. Typically, the spikes were sparsely spaced for the red flesh dragon fruit TL5 (7.1 cm), followed by the sample LĐ1 (6.3 cm) and QN1 (6.0 cm), respectively. The sample VP2 has the distance between adjacent spikes at 4.3 cm in thickness. The dragon cladode usually has 3 flat edges, each edge forming a branch edges with the gray-brown spine. The number of spines appearing on the areolas mainly depend on the variety. Of which, there were about 2.2 to 2.5 main spines/areolas in the studied samples. The TL5 variety was the highest (3.3 spines).

The color of young buds of the samples QN1, QN2, TL5 and LĐ1 are all red, while the bud of samples VP2 and TL4 are green. The cladode color of TL4, TL5 and VP2 are dark green, and others QN1, QN2 and LĐ1 are green. Noteworthily that the cladodes of the studies sample varieties are very diverse, only the cladodes of the sample VP2 and TL4 were the same with a straight, thick edge and smooth surface. The surface of cladodes of dragon fruits has chalk (QN1 and QN2). Among the studied sample varieties, the wavy cladodes were found in QN1, QN2 and LĐ1, while the cladodes of TL5 have a slightly wavy edge (Table 2).

Table 2: Morphological characteristics of cladodes of the studies sample varieties

Samples	**Young buds**	**Cladode (cm)**		**Main spines/areola (thorn)**	**Distance of spines areolas (cm)**	**Cladode characteristics**
		Length	**Diameter**			
QN1	Red	134.2	6.4	2.3	6.0	Yellow-green, edges with little brown border, wavy, smooth chalk surface
QN2	Red	130.8	6.3	2.2	5.2	Yellow-green, brown and wavy edges, chalk smooth surface
VP2	Green	143.5	6.2	2.5	4.3	Dark green, straight edge, thick, smooth surface
TL4	Green	145.7	6.3	2.5	5.2	Dark green, straight edge, thick, smooth surface
TL5	Red	150.0	7.8	3.3	7.1	Dark green, thick edge with little brown border, slightly wavy, smooth surface
LĐ1	Red	148.6	7.3	2.3	6.3	Yellow-green, with brown edges, wavy edges, smooth surface

The length and diameter of the cladode of the red flesh dragon fruit are quite different, of which the TL5 and LĐ1 have the largest cladode length and diameter (148.6-150.0 cm and 7.3. -7.8 cm). Samples of QN1 and QN2 have a low cladode length and diameter (130.8-134.2 cm and 6.3-6.4 cm). Dragon fruit varieties TL4 and VP2 had a medium length of cladode, but low diameter than the others.

The bud shape is very different among the studied dragon fruit varieties, according to the instructions in QCVN 01- 129: 2013 / BNNPTNT on regulations on testing for distinctiveness, uniformity and stability (DUS) of the guide dragon varieties with 4 shapes. buds are different: buds are ovoid, elliptical, round and spherical. Among the experimental dragon fruit varieties, the sample varieties QN1, QN2 and TL4 showed elliptical flower buds, round buds. The varieties VP2 and LĐ1 have separate ovary buds. The sample TL5 has round flower buds, respectively.

Dragon fruit flowers of the studied varieties have large size, flower length ranges from 26.5 cm in the sample QN2 to 29.7 cm in sample TL5. The diameter of the flower stalk is the part that formed the fruit with the largest diameter in the same sample TL5 (reaching 4.2 cm). Most of the experimental dragon fruit-like samples had cream petals and shorter anther than the stigma; Particularly, TL5 has pale yellow petals and has pistil-like anther (Table 2). Regarding the fruit characteristics such as the length of fruit, color of apical bracts, pods, the ratio of fruit height/diameter and color of fruit flesh, the results showed that among the dragon fruit varieties which are available in Vietnam, have white flesh dragon fruit with the largest length of the apex, green color and stiffness, followed by the red flesh LĐ1.

Table 2: Some flower characteristics of the experimental dragon fruit varieties

Sample	Bud characteristics	Flower length (cm)	Flower stalk diameter (cm)	Flower characteristics
QN1	Wide, round and elliptical shape	28.4 ± 1.1	3.8 ± 0.3	Petals are cream-colored, anther shorter than pistil, red bract
QN2	Long, round, and elliptical shape	26.5 ± 1.6	3.7 ± 0.4	Petals are cream-colored, anther shorter than pistil, red bract
VP2	Long, pointed top pud, ovoid	27.3 ± 1.5	3.5 ± 0.2	Petals are cream-colored, anther shorter than pistil, bracts light red
TL4	Long, narrow, round, elliptical	28.5± 1.5	3.9 ± 0.2	The petals are cream-colored, the anther is shorter than the pistil, and the bracts are medium red.
TL5	Wide, pointed top bud, round	29.7± 1.3	4.2 ± 0.1	The petals are light yellow, covered with pistil, and red bract
LĐ1	Long, narrow, pointed top bud, avoid	28.6± 1.8	3.3 ± 0.1	The petals are cream-colored, the anther is shorter than the pistil, and the bracts are crimson

However, the sample varieties QN1 and QN2 were red flesh but apical bracts are reddish greenish and softer than the white flesh dragon fruits. Sample varieties VP2 and TL4 had an average length of bracts of 4.7 to 4.8 cm, respectively. The color of the peel and flesh of the fruit were shown different among the experimental varieties, of which the samples QN1 and QN2 had red, brown, and red skin; The samples VP2 and TL4 with red skin, purple-red flesh, TL5 sample with red skin, and the flesh of the fruit are all crimson red and like LĐ1, the pods are bright red and the flesh red. The ratio of fruit height/diameter of fruit forms the fruit shape, this ratio reaches 1.4 to 1.6 cm, making the elongated fruit (QN1, QN2 and LĐ1). The remaining varieties of dragon fruit have a medium tapered shape, the ratio of height/diameter of fruit reaches 1.2 cm (Table 3).

Table 3: Fruit characteristics of the studies dragon fruit samples

Samples	Length of apical bract (cm)	Peel color	Ratio of fruit height/diameter	Flesh color	Fruit characteristics
QN1	6.3	Red with brown veins	1.4	Bright red	Elongated, long bracts, soft and red
QN2	6.5	Red with brown veins	1.4	Dark red	Elongated, long bracts, soft and red
VP2	4.7	Red	1.2	Red-purple	Medium tapered and apical bracts, stiff, red with green border
TL4	4.8	Red	1.2	Red-purple	Medium tapered and apical bracts, stiff, red with green border
TL5	4.2	Dark red	1.2	Dark red	Medium tapered and apical bracts, stiff, red with green border
LĐ1	6.3	Bright red	1.6	Red	Elongated, long bracts, soft and red

Genetic diversity among the studied samples by *MatK* sequencing

In this study, total DNA extraction was perform using the CTAB of Doyle and Doyle [3] with minor modifications. The results of the total DNA extraction of 06 dragon fruit samples were checked by electrophoresis on 1% agarose gel. The DNA bands of samples were quite compact and uniform proved that the DNA quality of the samples was quite good and enough sufficient requirements to conduct further experiments (Figure 1).

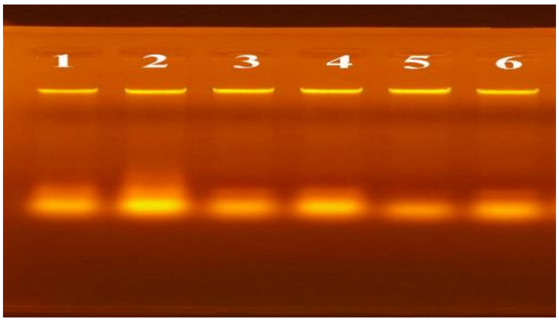


Figure 1: Total DNA extraction of 6 dragon fruit samples

The PCR amplification reaction with the primers pair *MatK* -390F/*MatK* -1326F, the attained results showed that the bands of 06 dragon samples were monomorphic bands with the size of approximately 900 bp (Figure 2)

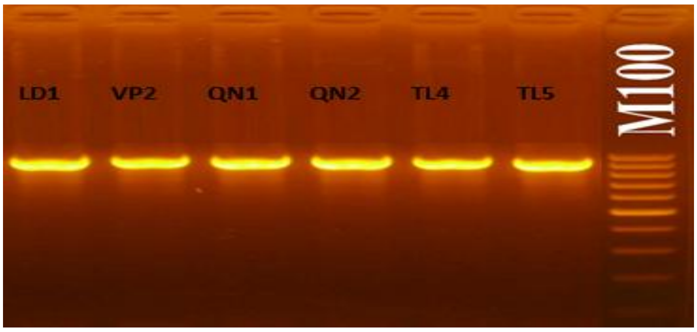


Figure 2: PCR amplification with the primer pair MatK -390F/MatK -1326F of the samples

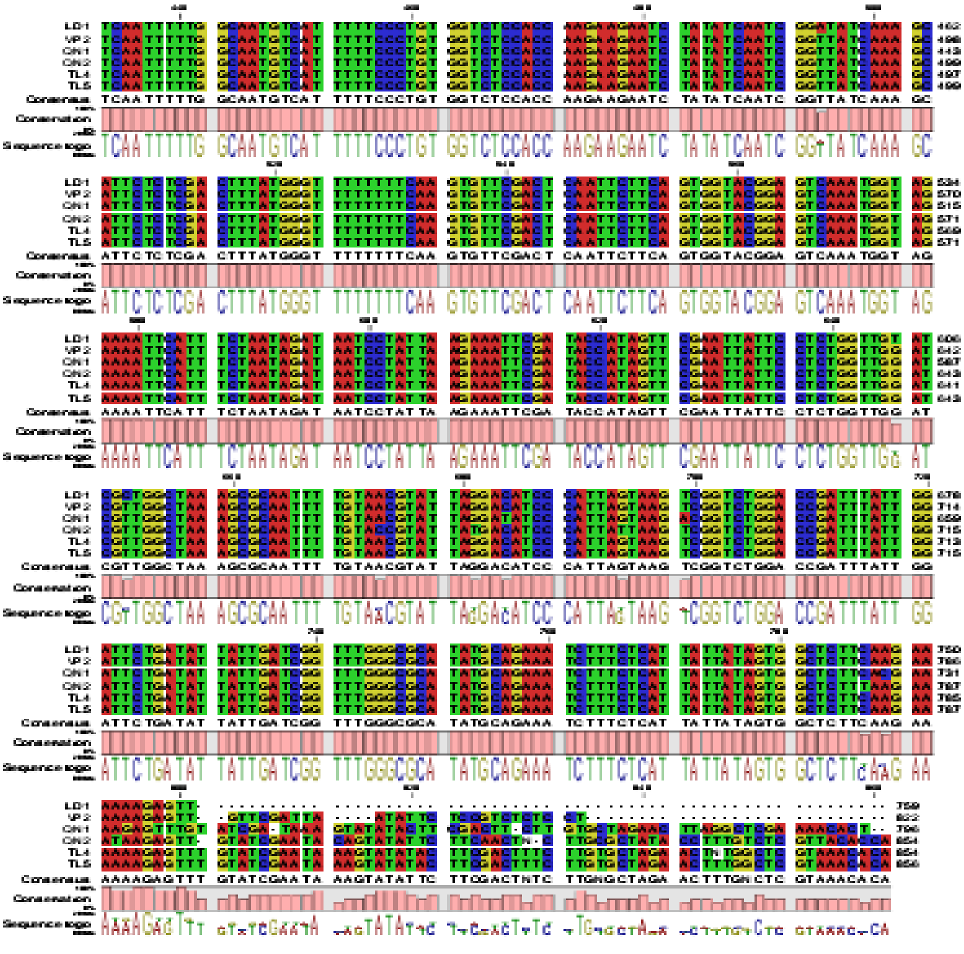


Figure 3: Comparison of nucleotide sequences of the studied samples

As above mentioned, the PCR amplification with the *MatK* -390F/*MatK* -1326F was sequenced directly by ABI PRISM 3,700 DNA Analyzer (Applied Biotech) Macrogen Company (Korea) and MEGA v5 software. The sequencing results of the *MatK* regions of the study samples were compared with each other by the ClustalW radical sequence tool of Mega 6.0 and CLC 8.0 software, the results are shown in Figure 3.

It showed that the difference between the sequences is single polymorphic sites (SNPs), where 1 nucleotide is replaced by another nucleotide in the first and last region sequences of the dragon fruit samples. We have found that there have many differences in *MatK* region sequence, of which the variation of sequence between the sample is most evident at the first 60 nucleotides and the last is 60 nucleotides, respectively.

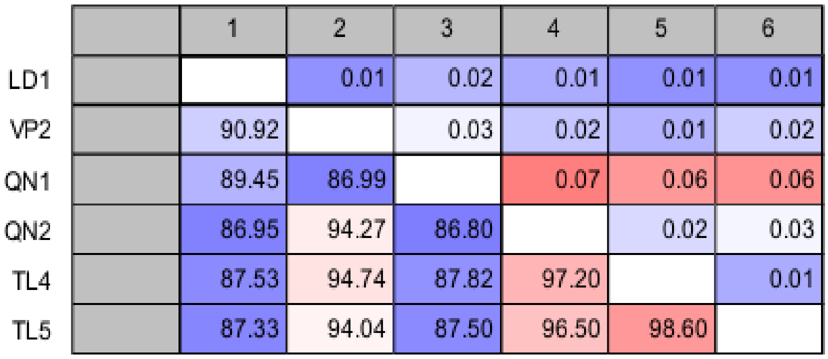
Based on the results of comparing the nucleotide sequences in Figure 3, it is possible to distinguish the dragon fruit samples. To further investigate the nucleotide composition of *MatK* sequences, the results showed in Table 3. The results presented in Table 3 revealed that based on the *MatK* regions, the composition of G (Guanin), C (Cytosine), A (Adenine) and T (Thymine) in the samples were different. Generally, the ratio of G and C were lower than A and T rations, on the other hand, the %GC was lower than %AT, respectively. Specifically, the samples of groups LĐ1 and LĐ1 and BK1 had the highest component (A + T) (66.0%) and the lowest component (G + C) (34.0%). The average percentage of components % (G + C) in all 06 studied varieties was 34.1% and % (A + T) was 65.9%, respectively (Table 4).

Table 4: The sequence length of the studied samples

No	Samples	T(U)	C	A	G	%GC	%AT
1	LĐ1	37.7	17.9	28.3	16.1	34.0	66.0
2	VP2	38.2	17.9	27.6	16.3	34.2	65.8
3	QN1	37.3	17.6	28.5	16.6	34.2	65.8
4	QN2	38.3	18.2	27.5	15.9	34.1	65.9
5	TL4	37.7	17.6	28.1	16.7	34.3	65.7
6	TL5	37.6	17.6	28.5	16.4	34.0	66.0
Average		37.8	17.8	28.1	16.3	34.1	65.9

The difference in the *MatK* region sequence between the samples was examined via the similarity coefficient of each pair of samples by calculated the genetic distance, measuring tool of CLC v8.02 as presented in Table 5.

Table 5: The similarity coefficient of each sample pair of the dragon fruit samples



		1	2	3	4	5	6
LD1			0.01	0.02	0.01	0.01	0.01
VP2		90.92		0.03	0.02	0.01	0.02
QN1		89.45	86.99		0.07	0.06	0.06
QN2		86.95	94.27	86.80		0.02	0.03
TL4		87.53	94.74	87.82	97.20		0.01
TL5		87.33	94.04	87.50	96.50	98.60	

Based on the *MatK* sequence, the results showed that the samples were originated from Taiwan, Tien Giang and Malaysia were less genetic diversity, and revealed high genetic similarity in 6 samples and coefficient was 98.6%, while the lowest coefficient was 86.8%; the nearest genetic distance is 0.01 and farthest 0.07. Thereby, it showed the genetic similarity and arising origin of the dragon fruit varieties among the studied dragon fruit samples. The genetic variations in the *MatK* region represent the diversity of the samples. Hence, it is possible to identify high-yield and high-quality genetic resources to serve the propagation, development and conservation of dragon fruit genetic resources of Vietnam. We have further generated the phylogenetic tree of the samples based on the nucleotide sequences of *MatK* region as shown in Figure 4. The results indicated that 6 samples were divided into 2 main groups: Group I: Including QN1 and LĐ1, and Group II: consisted of the samples TL5, TL4, QN2 and VP2, respectively.

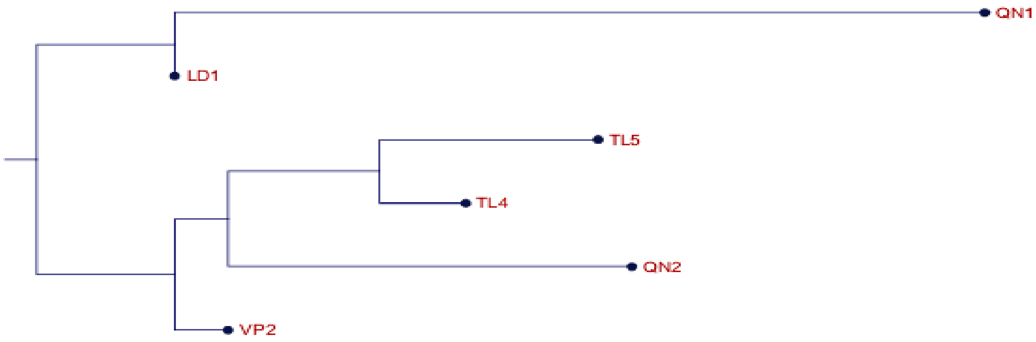


Figure 4: Phylogenetic tree of the samples

Conclusions

In summary, in this study, we have successfully evaluated the main agronomic traits of 6 dragon fruits collected in the different provinces in Vietnam. We have further assessed the genetic diversity of these samples by *MatK* sequencing. The results showed that the samples have relatively different agronomic characteristics in terms of branches, buds, flowers and fruits. By using *MatK* sequence, the genetic similarity coefficient among the samples ranged from 86.8% to 98.6%; the nearest genetic distance is 0.01, while the farthest is 0.07. The attained results of this study have provided useful information on morphological characteristics and genetic diversity of dragon fruits for the breeding program.

References

[1]. Australian Department of Agriculture and Water Resources. (2017). Final report for the review of biosecurity import requirements for fresh dragon fruit from Vietnam. Department of Agriculture and Water Resources, Canberra, Australia. http://www.agriculture.gov.au/biosecurity/riskanalysis/plant/dragon-fruit-from-vietnam/final-report.

[2]. DUS- Distinctness, Uniformity and Stability. (2013). National Technical Regulation on Testing for Distinctness, Uniformity and Stability of Dragon Fruit Varieties (MARD). QCVN 01-129:2013/BNNPTNT.

[3]. Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*, 19, 11-15.

[4]. Ha, T.T.T, Khanh, T.D., Trung, K.H. (2020). Evaluation of genetic diversity and identification of *Huperia* species collected in some different areas in Vietnam by molecular markers. *Inter Let Nat Sci*, 80: 13-23.

[5]. Trung, K.H., T.D. Khanh, L.H. Ham, T.D. Duong, N.T. Khoa. (2013). Molecular phylogeny of the endangered Vietnamese Paphiopedilum species based on the internal transcribed spacer of the nuclear ribosomal DNA. *Adv. Stud. Biol*, 5(7): 337-346.

[6]. Gunasena, H.P.M., Pushpakumara, D.K.N.G., Kariyawasam, M. (2007). Dragon fruit (*Hylocereus undatus* (Haw.) Britton and Rose. Chapter 4. In: Pushpakumara, D.K.N.G., Gunasena, H.P.M., Singh, V.P. (Eds.), Under- utilized fruit trees in Sri Lanka. World Agroforestry Centre, South Asia Office, New Delhi, pp.110-141.