Identification of dragon fruit (Selenicereus) species in Mekong Delta based on DNA barcode sequences

TRAN GIA HUY1, TRAN THANH MEN2, NGUYEN PHAM ANH THI1, DO TAN KHANG1,*

1Department of Molecular Biotechnology, Biotechnology Research and Development Institute, Can Tho University, 3/2 Street, Ninh Kieu District, Can Tho City, Viet Nam. Tel./fax. +84-919-813035, *email: dtkhang@ctu.edu.vn
2Department of Biology, School of Natural Sciences, Can Tho University. 3/2 Street, Ninh Kieu District, Can Tho City, Viet Nam

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Abstract. Huy TG, Men TT, Thi NPA, Khang DT. 2021. Identification of dragon fruit (Selenicereus) species in Mekong Delta based on DNA barcode sequences. Biodiversitas 22: 4216–4222. Selenicereus species is one of the valuable fruiting plants in Vietnam due to its properties, e.g., rich nutrition and medicine activity. Eight DNA barcodes applied to the discrimination power of dragon fruit species in the Mekong delta. Total DNA extracted from fresh roots and the loci of interest were amplified and sequenced. DNA sequences were aligned and determined variable regions. The findings revealed that four loci, including matK, rbcL, rpoC1, and atpF-H reached high PCR yield and specificity compared to those in ycf1b, psbK-L, and ITS. The atpF-H was the most variable region due to the number of single nucleotide polymorphisms (SNPs) and indel mutations, whereas rpoC1 was the least one. Based on sequence characteristics, each locus only discriminated some of the Selenicereus monacanthus from Southern Horticultural Research Institute identified by combining three loci, atpF-H, matK, and rbcL. The results elucidated the close genetic relationship between Mekong delta dragon fruits and National Center for Biotechnology Information (NCBI) database. Furthermore, this finding generated a DNA barcode database of ten dragon fruit accessions and suggested that multiple loci in the chloroplast genome should be a reliable solution for identifying this highly commercial fruiting plant.

Keywords: atpF-H, matK, plant authentication, rbcL, Selenicereus

INTRODUCTION

Selenicereus (A. Berger) Britton & Rose, pitahaya or dragon fruit, belongs to the family Cactaceae, is distributed from South America regions. Individuals of this genus are described as vine cacti with unique traits such as climbing, aerial roots, three angled stems, and glabrous large-scaled berry (Montoya-Arroyo et al. 2014). It is grown as ornamental plants in gardens and indoors for its size, aromatic, and abnormal time blooming flowers. At present, dragon fruits are being exported globally and have a high economic value as exotic fruit crops in harsh regions where water is limited. The Crassulacean acid metabolism pathway is utilized for carbon dioxide fixation and is highly tolerant to water stress (Ibrahim et al. 2018). The fruits have played a remarkable role in medicine, food, and ornamentally. Dragon fruit is rich in essential nutrients such as vitamins, minerals, complex carbohydrates, dietary fibers, and antioxidants (Wichienchot et al. 2010; Tenore et al. 2012). Dragon fruit can withstand prolonged drought. Therefore, it considers as a high potential fruit for horticultural development, especially in areas where drought is a limiting factor for other fruits. There are 14 species of dragon fruit in the world (Cisneros and Tel-Zur 2012). White flesh dragon fruit (Selenicereus undatus) and red flesh dragon fruit (Selenicereus monacanthus) cultivate in Vietnam. To the best of our knowledge, dragon fruits were classified into the Selenicereus genus base on DNA sequences in both nuclear and plastid (Korotkova et al. 2017). Thus, the accepted scientific name of dragon fruit is Selenicereus instead of Hylocereus.

DNA barcodes are short DNA fragments, around 400-800 bp, found in all plant species (DeSalle and Goldstein 2019). DNA is a specialized chain of “letters” that distinguish between organisms and/or individuals despite very similar morphological features between them. By integrating the advances of molecular biology, sequencing technologies, and bioinformatics, DNA barcodes provide a quick and accurate means to recognize previously known, described, and classified species and construct a DNA database for them (Kress 2017). As a result of the low nucleotide substitution rate in land plants, it is a challenge to determine a standard DNA barcode (Fazekas et al. 2012). Furthermore, several loci from the plastid genome analyze for species discrimination power. Two genes of matK and rbcL and their combination proposed as a standard DNA barcode for land plants (DeSalle and Goldstein 2019).

In Selenicereus species, some studies focused on the morphology and Inter-simple sequence repeat (ISSR marker) for genetic diversity and species identification (Tao et al. 2014; Aribami et al. 2021). However, morphological traits influenced by environmental factors and the band pattern may not be reliable because ISSR is a dominant marker. Based on nucleotide sequences, which is environment independence, DNA barcodes proposed a potential solution for plant authentication. This current study was to disclose the ability of DNA barcodes to identify Selenicereus species in The Mekong delta.
MATERIALS AND METHODS

Plant sampling
Ten samples of Selenicereus species were collected from Southern Horticultural Research Institute (SOFRI) and fruit gardens in The Mekong delta (Table 1). The S. monacanthus DF1 was the high yield variety cultivated ubiquitously in The Mekong delta. Such samples were verified by SOFRI staff.

DNA extraction and amplification
Fresh roots sterilized with 70% ethanol and then cut into tiny pieces. Samples were incubated at liquid nitrogen for 5 minutes and grind into powder. Total DNA was extracted using the CTAB-based protocol (Roger and Bendich 1988) with appropriate modifications. The quantity and purity of DNA were measured by Nanodrop spectrophotometer 2000C (Thermo Scientific, USA). DNA integrity was examined by 1% agarose electrophoresis.

Eight loci, including four genes matK, rpoC1, rbcL, ycf1b, and four noncoding spacers psbA-trnH, atpF-H, psbK-1, ITS were amplified by 30 µL of volume reaction. The reagents consist of 15 µL of master mix 2X (Bioline, United Kingdom), 0.4 µM of each forward and reverse primer, and DNA template. Primer sequences are listed in Table 2. The amplification process was performed in C1000 thermocycler (Bio-rad, USA) (Table 2). PCR products were separated by 2% agarose electrophoresis for 45 minutes at 50V, and the bands were visualized by Run-Safe stain (Cleaver Scientific, United Kingdom). Amplicons with clear bands and no nonspecific products were submitted to Nextgen Biotechnology corporation for sequencing (3500 Genetic Analyzer, Applied Biosystem).

Table 1. Main characteristics of ten dragon fruit samples in this study

| Code   | Species          | Shape          | Source   | Code   | Species   | Shape          | Source   |
|--------|------------------|----------------|----------|--------|-----------|----------------|----------|
| DF1    | S. monacanthus   | Red flesh, oval fruit | SOFRI, cultivated | DF6    | S. undatus | White flesh, oval fruit | Ben Tre, cultivated |
| DF2    | S. monacanthus   | Red flesh, oval fruit | Ben Tre, cultivated | DF7    | S. undatus | White flesh, oval fruit | Ca Mau, cultivated |
| DF3    | S. monacanthus   | Red flesh, oval fruit | Ca Mau, cultivated | DF8    | S. megalanthes | Yellow skin, white flesh, oval fruit | Dong Thap, cultivated |
| DF4    | Selenicereus sp. | Red flesh, round fruit | Ca Mau, cultivated | DF9    | S. megalanthes | Yellow skin, white flesh, oval fruit | Tien Giang, cultivated |
| DF5    | Selenicereus sp. | Purple flesh, oval fruit | SOFRI, cultivated | DF10   | Selenicereus sp. | Not available | An Giang, wild |

Note: DF1: S. monacanthus SOFRI; DF2: S. monacanthus BT; DF3: S. monacanthus CM; DF4: Selenicereus sp.; DF5: Selenicereus sp. SOFRI; DF6: S. undatus BT; DF7: S. undatus CM; DF8: S. megalanthes DT; DF9: S. megalanthes TG; DF10: Selenicereus sp. AG.

Table 2. Nucleotide sequences of primer pairs for amplification of DNA barcode candidates (Primer database from boldsystems.org)

| Locus   | Sequence (5'-3')      | Thermal cycle (35 cycles)          |
|---------|-----------------------|------------------------------------|
| matK    | F: CGATCTATTCATCTATATTC  
          | R: TCTAGCACAAGAAAGTCGAAGT        | 94°C-1 min; 94°C- 30 sec, 50°C-40 sec, 72°C-40 sec; 72°C-5 min |
| rbcL    | F: ATGTCAACCAAAACAGAGACTAAAGC  
          | R: GATAAATCAAGTCCACCCRCG         | 94°C-4 min; 94°C- 30 sec, 55°C-30 sec, 72°C-1 min; 72°C-10 min |
| psbA-trnH | F: GTATGCAATGAACTGAAATGCTC  
             | R: CGGCCATGTTGATTCACATCC         | 94°C-4 min; 94°C- 30 sec, 58°C-30 sec, 72°C-1 min; 72°C-10 min |
| rpoC1   | F: GCCAAGAGGGGAAGATTTCCG       | 94°C-4 min; 94°C- 30 sec, 51°C-40 sec, 72°C-40 sec; 72°C-5 min |
| atpF-atpH | F: ACTGCGACACACTCCCTTCCC     |
| psbK-psbI | F: TTAGCCTTTGTGGGCAAG  
             | R: TTAGCCTCTTGGGCAAG             | 95°C-5 min; 95°C- 30 sec, 57°C-30 sec, 72°C-1 min; 72°C-5 min |
| ITS     | F: TCCGGGAACCTGCAGG  
          | R: TCTCCTCCTTGTGATGC            | 95°C-5 min; 95°C- 30 sec, 57°C-30 sec, 72°C-1 min; 72°C-5 min |
| ycf1b   | F: TACTGACGAAAATCAGATTTGGAAT  
          | R: ATACATGCAAGTGTGGAAGA         | 95°C-5 min; 95°C- 30 sec, 51°C-40 sec, 72°C-1 min; 72°C-10 min |
Table 3. Accession numbers for DNA barcode sequences in this study

| Samples | atpF-H | rbcL | rpoC1 | matK |
|---------|--------|------|-------|------|
| DF1     | OK094559 | OK094539 | OK094549 | OK094529 |
| DF2     | OK094560 | OK094540 | OK094550 | OK094530 |
| DF3     | OK094561 | OK094541 | OK094551 | OK094531 |
| DF4     | OK094562 | OK094542 | OK094552 | OK094532 |
| DF5     | OK094563 | OK094543 | OK094553 | OK094533 |
| DF6     | OK094564 | OK094544 | OK094554 | OK094534 |
| DF7     | OK094565 | OK094545 | OK094555 | OK094535 |
| DF8     | OK094566 | OK094546 | OK094556 | OK094536 |
| DF9     | OK094567 | OK094547 | OK094557 | OK094537 |
| DF10    | OK094568 | OK094548 | OK094558 | OK094538 |

Note: DF1: S. monacanthus SORFI; DF2: S. monacanthus BT; DF3: S. monacanthus CM; DF4: Selenicereus sp.; DF5: Selenicereus sp. SORFI; DF6: S. undatus BT; DF7: S. undatus CM; DF8: S. megalanthus DT; DF9: S. megalanthus TG; DF10: Selenicereus sp. AG

Data analysis

Raw sequences were interpreted by Bioedit software version 7.2.1. The quality value checks to confirm the accuracy of the sequencing procedure, and unidentified nucleotides were verified based on the chromatogram. These sequences were submitted to Genbank for accession number registration (Table 3). Multiple sequence alignment was conducted by following the ClustalW algorithm. Conservative and variable regions determine by MEGA X software (Kumar et al. 2018). Homologous sequences belong to the *Selenicereus* genus on Nation Center Biotechnology Information (NCBI) were collected by Basic Local Alignment Search Tool (BLAST).

RESULTS AND DISCUSSION

Amplification of DNA barcode sequences

Eight DNA barcode sequences, including four protein-encoding genes (ycf1b, rbcL, rpoC1, and matK) and four non-coding sequences (atpF-H, psbA-trnH, psbK-I, and ITS), were amplified and visualized on the gel (Figure 1). From the gel patterns, amplicon size ranged from 400 to 900 bp for eight loci. It indicated that three genes rpoC1, rbcL, matK, and two spacers atpF-H, psbA-trnH showed unique bands, reflecting the PCR specificity. No band has appeared in the negative control sample, indicating that external contamination under-controlled. Therefore, such five loci were suitable to sequence and analyze species identification. On the other hand, the presence of nonspecific bands from the ycf1b gene, ITS, and psbK-I spacer made such loci unsuitable for sequencing.

Sequence analysis for DNA barcode candidates

Based on the chromatogram, the quality value of DNA sequences was verified. The psbA-trnH spacer indicated multiple unidentified characters while atpF-H, matK, rpoC1, and rbcL expressed clear nucleotide signals. Conservative and variable sites of such loci were detected based on multiple sequence alignment (Table 4). Based on the alignment result, all sequences showed a high amount of conserved sites. As a result, *Selenicereus* species expressed a close genetic relationship. However, the occurrence of SNPs and indel-mutations revealed the nucleotide diversity of such dragon fruit species. The atpF-H spacer was the highest variable locus with six variable sites. By contrast, three genes consist of matK, rpoC1, and rbcL were highly similar. In terms of indel-mutations, nine mutations were detected from the atpF-H sequence, while the frequency of this mutation was quite low in three other loci. Thus, it could suggest that the noncoding sequence was more variable than coding sequences and proposed as a potential barcode sequence for species discrimination.

A serial insertion of 9 nucleotides ATTAGGTAC was found on *Selenicereus* sp. DF4 (red flesh and round fruit) (Table 5). Because of this mutation, DF4 distinguish from other red flesh dragon fruits. This result confirmed that atpF-H spacer was highly polymorphic and able to identify this dragon fruit variety. Santos and Pereira (2018) analyzed more than 44,000 sequences belonging to 206 different plant families. Their findings suggested that the atpF-H spacer was a suitable region for SPInDel (Species Identification by Insertions/Deletions) concept. Furthermore, five substitution mutations were detected and classified into three SNPs as T/C, G/A, and G/A.

Sequence analysis from the rbcL gene reflected that this plastid gene was valuable for species delimitation (Table 6). The *S. monacanthus* DF1 (red flesh) and *Selenicereus* sp. DF5 (purple flesh) from SORFI were identified successfully from the other samples by four valuable SNPs, including G/C, A/C, G/T, and T/C, and two consecutive insertions with A and G. The rbcL gene was also evaluated as a hypervariable region, which is the promising DNA barcode to identify *S. monacanthus* and three species *S. anthonyanus, S. grandiflorus* and *S. validus* (Qin et al. 2021).

The rpoC1 gene, a coding sequence in the plastid genome, revealed the incorrect identification of dragon fruits (Table 7). For instance, samples DF1, DF2, and DF3 belonged to *S. monacanthus*, but their sequence was variable. Furthermore, four individuals DF1, DF5, DF6, and DF10 were different species, and their nucleotide sequences were identical. Although rpoC1 was one of 10 informative markers for phylogeny in Cactaceae (Köhler et al. 2020), this plastid gene was not a suitable barcode for *Selenicereus* species.
Figure 1. Amplicons of eight DNA barcode locus on 2% agarose gel. Note: M: 50 bp hyperladder (Bioline, England); DF1: S. monacanthus SOFRI; DF2: S. monacanthus BT; DF3: S. monacanthus CM; DF4: Selenicereus sp.; DF5: Selenicereus sp. SOFRI; DF6: S. undatus BT; DF7: S. undatus CM; DF8: S. megalanthus DT; DF9: S. megalanthus TG; DF10: Selenicereus sp. AG; NC: Negative control.

| Locus | Aligned length (bp) | Conserved sites | Variable sites | Parsimony informative sites | Indel mutations |
|-------|---------------------|----------------|----------------|-----------------------------|----------------|
|       |                     |                | Singleton sites |                              |                |
| atpF-H| 585                 | 571            | 5 (0.85%)      | 1 (0.17%)                   | 9 (1.54%)      |
| matK  | 782                 | 778            | 1 (0.13%)      | 3 (0.38%)                   | 0 (0%)         |
| rbcL  | 506                 | 502            | 1 (0.20%)      | 3 (0.59%)                   | 2 (0.40%)      |
| rpoC1 | 452                 | 450            | 1 (0.22%)      | 1 (0.22%)                   | 2 (0.44%)      |
Table 5. Single nucleotide polymorphisms (SNPs) and indel mutations of atpF-H spacer

| Accession number | Samples | Position |
|------------------|---------|----------|
| OK0945459        | DF1     | 1 1 1 1 1 2 2 2 2 2 2 2 2 4 5 9 |
| OK094560         | DF2     | C T T C T G T G T G T G T G T G T |
| OK094561         | DF3     | C A A A A A A A A A A A A A A A A |
| OK094562         | DF4     | C A A A A A A A A A A A A A A A C |
| OK094563         | DF5     | C A A A A A A A A A A A A A A A |
| OK094564         | DF6     | C A A A A A A A A A A A A A A A |
| OK094565         | DF7     | C A A A A A A A A A A A A A A A |
| OK094566         | DF8     | C A A A A A A A A A A A A A A A |
| OK094567         | DF9     | C A A A A A A A A A A A A A A A |
| OK094568         | DF10    | C A A A A A A A A A A A A A A A |

Note: DF1: S. monacanthus SORFI; DF2: S. monacanthus BT; DF3: S. monacanthus CM; DF4: Selenicereus sp.; DF5: Selenicereus sp. SORFI; DF6: S. undatus BT; DF7: S. undatus CM; DF8: S. megalanthus DT; DF9: S. megalanthus TG; DF10: Selenicereus sp. AG.

Table 6. Single nucleotide polymorphisms (SNPs) and indel mutations of rbcL gene

| Accession number | Sample | Position |
|------------------|--------|----------|
| OK094339         | DF1    | G A A G G T |
| OK094540         | DF2    | C - - - - - |
| OK094541         | DF3    | C - - - - - |
| OK094542         | DF4    | C - - - - - |
| OK094543         | DF5    | C - - - - - |
| OK094544         | DF6    | C - - - - - |
| OK094545         | DF7    | C - - - - - |
| OK094546         | DF8    | C - - - - - |
| OK094547         | DF9    | C - - - - - |
| OK094548         | DF10   | C - - - - - |

Note: DF1: S. monacanthus SORFI; DF2: S. monacanthus BT; DF3: S. monacanthus CM; DF4: Selenicereus sp.; DF5: Selenicereus sp. SORFI; DF6: S. undatus BT; DF7: S. undatus CM; DF8: S. megalanthus DT; DF9: S. megalanthus TG; DF10: Selenicereus sp. AG.

Table 7. Single nucleotide polymorphisms (SNPs) and indel mutations of rpoC1 gene

| Accession number | Sample | Position |
|------------------|--------|----------|
| OK094549         | DF1    | A T A A |
| OK094550         | DF2    | C - - - |
| OK094551         | DF3    | C - - - |
| OK094552         | DF4    | C - - - |
| OK094553         | DF5    | C - - - |
| OK094554         | DF6    | C - - - |
| OK094555         | DF7    | C G - - |
| OK094556         | DF8    | C - - - |
| OK094557         | DF9    | C - - - |
| OK094558         | DF10   | C - - - |

Note: DF1: S. monacanthus SORFI; DF2: S. monacanthus BT; DF3: S. monacanthus CM; DF4: Selenicereus sp.; DF5: Selenicereus sp. SORFI; DF6: S. undatus BT; DF7: S. undatus CM; DF8: S. megalanthus DT; DF9: S. megalanthus TG; DF10: Selenicereus sp. AG.

It illustrated that the matK sequence showed nucleotide polymorphism; however, this gene was non-informative to identify dragon fruits species (Table 8). Data from six dragon fruits species from Northern Vietnam (Huong et al. 2021) also showed that the matK gene contained variable sites, but these nucleotides could not discriminate against a particular species. On the other hand, the deletion of adenine in Selenicereus sp. DF5 compared to S. monacanthus DF1 SOFRI (position 817) made these two varieties distinguishable.

In comparison with dragon fruit sequences from the NCBI database, it showed that five species, S. undatus (NC_053698.1), S. tricca (LT745724.1), S. ocamponis (LT745687.1) (Korotkova et al. 2017), S. costaricensis (JQ590992.1) and S. peruvianus (AY015310.1) showed the identity percent ranging from 97-100% compared with the S. monacanthus SOFRI (Table 9). Selenicereus costaricensis is the purple flesh dragon fruit variety in Costa Rica (Viñas and Jiménez 2016), this species contained a high amount of bioactive compounds with no cytotoxic effects (Paško et al. 2021). Selenicereus tricca are the wild dragon fruit variety in Belize (Gómez-Hinostrosa et al. 2014) and S. ocamponis is the red flesh dragon fruit variety in Mexico (Ibrahim et al. 2018). The high identity percentage indicated a close genetic relationship among these accesses with S. monacanthus SOFRI. Based on sequence similarity, three loci, including matK, atpF-H, and rbcL could discriminate S. monacanthus SOFRI from dragon fruits in other countries.

The maturase matK is one of the fastest evolving genes in the chloroplast genome. It was widely applied for phylogenetic studies at the species level since it played a crucial role in the RNA splicing process (Schmitz-Linneweber et al. 2015). The utility of matK region tested for species-level identification on 528 species of Cactaceae, including approximately 75% of Mexican species. Yesson et al. (2011) find that the DNA barcode by matK could identify exactly 77% of collected species. However, the expectation value is more than that percentage, and the change of nucleotides in primer regions is the main...
drawback for PCR specificity of such gene (Yesson et al.
2011). Bell et al. (2017) generated the rbcrL database for the
accurate identification of plant mixture. This study succeeded in
accurate species-level identification for eight angiosperm
species: *Populus tremuloides* (Salicaceae), *Populus
deltaoides*, *Broussonetia papyrifera* (Moraceae), *Carya
 illinoinsensis* (Juglandaceae), *Bassia scoparia* (Amaranthaceae), *Ambrosia
artemisiifolia* (Asteraceae), *Artemisia tridentata* (Asteraceae), *Poa
pratensis* (Poaceae) and a family-level identification of *Zea mays* (Poaceae).
Thus, the rbcrL gene should be an improvement on ITS, a
cellular sequence has been utilized widely for plant
identification. The rbcrL (1, 5-ribulose bisphosphate
carboxylase/oxygenase large subunit) gene encodes a large
subunit of rubisco protein, which is an important enzyme
for photosynthesis (Fangru et al. 2020). Thus, *matK* and
rbcrL were responsible for key metabolism processes in
cells, so the changes of their sequences might be powerful
markers for species identification.

Frequent plant hybridization via mechanisms such as
polyplody and various breeding systems have given rise to
new hybrids formation (Fazekas et al. 2012). Several
breeding programs were submitted to increase the yield and
quality of dragon fruits lead to high intra- and interspecific
hybridization. This phenomenon generated taxonomical
confusion, leading to the complexity of dragon fruit species
and varieties (Jian et al. 2021). Therefore, a single locus
possesses insufficient discrimination power to identify
these species. In this study, the *S. monacanthus* SOFRI
variety was authenticated by three loci, *atpF-H + rbcrL +
matK*. Several studies contributed that species resolution
was improved significantly by multiple loci. Data from 12
plant genera showed that chloroplast genome sequences are
highly variable, and such regions should be the priority
when seeking the suitable loci to resolve closely related
plant species or varieties and for DNA barcoding (Dong et
al. 2012). The utilization of multiple loci considers as a
potential solution for accurate identification (Fazekas et al.
2012; Wu et al. 2017). A single DNA barcode only
identifies some of the 18 species in the Melilotus genus, a
herbal plant in North Africa. According to these findings,
the combination of five loci, *matK + rbcrL + trnL-F + trnH-
psba + ITS* showed the greatest species resolution while
the single rbcrL was the least. Khan et al. (2017) reported
that four coding regions *matK, rbcrL, rpoB*, and *rpoC1*
were highly conservative among the taxa. On the other
hand, the sequences of two intergenic spacers *psbK-psbI*
and *atpF-atpH* were variable, specifically identifying the
medicinal plant *Rhazya stricta* (Khan et al. 2017). Gogoi
and Bhau (2018) indicated that single-locus ITS or
combined with plastid *matK* expressed the better species
authentication of the genus Nepenthes based on barcoding
gaps. Vu et al. (2020) also suggested that the combination of
ITS + *matK* was the most potential DNA barcode for
Vietnamese *Paphiopedilum* species. Therefore, It was
reasonable to conclude that reliable identification was
strongly supported by more than one DNA barcode locus.
Last but not least, an effective locus for this species may
have some limitations for another species.

![Table 8](image1)

**Table 8. Single nucleotide polymorphisms (SNPs) and indel mutations of *matK* gene**

| Accession number | Sample | Positions |
|------------------|--------|----------|
|                  |        | 738 784 787 799 800 801 809 817 826 |
| OK094529         | DF1    | A A A   A T _ A A |
| OK094530         | DF2    | _ _ _   G _ _ _ |
| OK094531         | DF3    | _ C _   A T G G |
| OK094532         | DF4    | _ _ _   _ _ _ |
| OK094533         | DF5    | _ _ _   _ _ _ |
| OK094534         | DF6    | _ _ _   _ _ _ |
| OK094535         | DF7    | _ _ _   _ _ A |
| OK094536         | DF8    | _ _ _   _ _ _ |
| OK094537         | DF9    | _ _ _   _ _ _ |
| OK094538         | DF10   | _ _ _   _ _ _ |

Note: DF1: *S. monacanthus* SOFRI; DF2: *S. monacanthus* BT, DF3: *S. monacanthus* CM, DF4: *Selenicereus* sp.; DF5: *Selenicereus* sp.
SOFRI; DF6: *S. undatus* BT; DF7: *S. undatus* CM; DF8: *S. megalanthus* DT; DF9: *S. megalanthus* TG, DF10: *Selenicereus* sp. AG

![Table 9](image2)

**Table 9. Nucleotide polymorphism between *Selenicereus monacanthus* SOFRI and other *Selenicereus* species on NCBI database**

| Locus | Length (nt) | Coverage (%) | Identities (%) | Number of gaps | Accessions |
|-------|-------------|--------------|----------------|----------------|------------|
| atpF-H| 576         | 100          | 97.61          | 9              | *S. undatus* (NC_053698.1) |
| matK  | 782         | 100          | 99.74          | 0              | *S. tricca* (LT745724.1) |
| rbcrL | 506         | 100          | 99.41          | 2              | *S. undatus* (NC_053698.1) |
| rpoC1 | 452         | 100          | 100            | 0              | *S. costaricensis* (IQ590992.1) |
|       |             |              |                |                | *S. undatus* (NC_053698.1) |
This study successfully constructed DNA barcodes data for common *Selenicereus* species in the Mekong delta, consisting of four chloroplast loci: *rbcL*, *rpoC1*, *matK* and *atpF-H*. Such sequences showed high PCR yield and specificity as well as sequence quality. The *rpoC1* region was not a suitable barcode for this plant because of the highly conservative sequence. Based on the appearance of SNPs and indel mutations, three loci: *rbcL*, *matK* and *atpF-H* were able to distinguish some species. The integration of these three loci enhances the discrimination power and the successful identification of *S. monacanthus* DF1 varieties.

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