DNA barcode for rambutan diversity in Thailand using chloroplast genome regions

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Abstract

Rambutan is one of the best-known fruits in Asia. Thailand is one of the largest producers of rambutan apart of Indonesia and Malaysia. The commercial rambutan, *Nephelium lappaceum* L., is considered closer to other *Nepheliums*, which are very difficult to distinguish from each other. Therefore, this study is to clarify the DNA barcode for diversity of rambutan using chloroplast genome regions (*psbA, trnL* and *rpoC*). Seventeen samples of rambutan were collected from the field at Chanthaburi Horticultural Research Center for analysis. Among these, 14 samples are classified as *Nephelium lappaceum* L. Six of the commercial cultivars and 8 hybrid cultivars including Rongrien, Seechompoo, Seethong, Bangyeekhan, Namtankraud, Jaemong and Pliew 1-8 were analyzed. The other 3 samples were Pulasan (*Nephelium ramboutan-ake* (Labill.) Leenh.), *Nephelium sp.* No. 1 and *Nephelium sp.* No. 2. Extracted DNA samples were evaluated with 3 universal primers of *psbA, trnL* and *rpoC*. The result showed that using psbA primer separated the group of Seethong and Pliew 2 (Seethong x Jaemong) and group of Nephelium sp. No.2 from the others. Whereas *trnL, rpoC* and combination of three primers could not explicitly explain the diversity within rambutan. Our study will further examine more specific primers to confirm the relationships of the Thai rambutans. The genetic diversity of cultivated rambutan from this study will be used as a genetic database for development of a future breeding program.

Keyword : rambutan genetics, phylogenetic relationships, barcode sequencing

Introduction

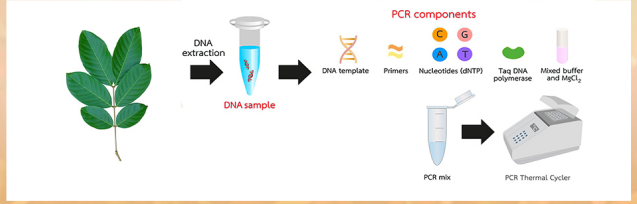
N. lappaceum is known as rambutan, which is commonly cultivated in orchards and home gardens in Thailand, Malaysia, and Indonesia. Another species such as pulasan (*N. ramboutan-ake*) is a close relative and is rarely cultivated. Thailand has a high diversity of rambutan clones, including Rongrian, Seechompoo, Seethong, and Bangyeekhan. However, the diversity of the *Nephelium* species has been not fully exploited.

DNA barcode is a technique used for identifying species using short orthologous DNA sequences. It is gradually being tested in many areas as a cost-effective tool for identifying and regulating pests, invasive and disease-carrying species, trade and sale of endangered species, and for many other species (Onuminya and Ogundipe, 2016). Hence, the aim of this research is to explore the diversity of rambutan in Thailand with particular emphasis on identification of the plant samples using DNA barcode sequences which can be shared publicly.

Materials & Methods

Rambutan (*N. lappaceum*) leaves were collected from Chanthaburi Horticultural Research Center, Tapon Sub-District, Khlung District, Chanthaburi province in Thailand. Leaf samples were collected from 17 species including Rongrien, Seechompoo, Seethong, Namtankraud, Bangyeekhan, Jaemong, Ngorkonson, Pliew 1 (Seechompoo x Rongrien), Pliew 2 (Seethong x Jaemong), Pliew 3 (Seechompoo x Seethong), Pliew 4 (Seechompoo x Rongrien), Pliew 5 (Seechompoo x Rongrien), Pliew 6 (Namtankraud x Rongrien), Pliew 7 (Seechompoo x Seethong), Pliew 8 (Seechompoo x Seethong), Pulasan and unknown (*Nephelium sp.* No.1 and No. 20) for this study.

DNA extrction and PCR amplification



Result & Discussion

The samples of rambutan were collected from the field at Chanthaburi Horticultural Research Center. From the sequences generatedthe phylogenetic relationship of all 17 samples are shown on Figures 1 to 4. DNA barcodes for *psbA, trnL* and *rpoC* have been used in several studies.Using the *psbA* primer, the phylogenetic analyses revealed the *Nephelium sp.* No. 2 was shown to be in the outer group. However, Seethong and Pliew 2 were separated within the same group. Accordingly, Pliew 2 is designated as a hybrid of Seethong x Jaemong. Furthermore, the distribution of the other groups did not show any relationships. Additionally, the primers of *trnL, rpoc* and combination of three primers, could not explicitly explain the diversity of rambutan. Additionally, using DNA barcoding in the context of quality control is both a well and poorly regulated supply system. Standardization of protocols for DNA barcoding and DNA sequence-based identification are necessary before DNA-based biological methods can be implemented as routine analytical approach. These must be approved by the competent authorities for use in regulated procedures (Raclariu *et al.*, 2017).

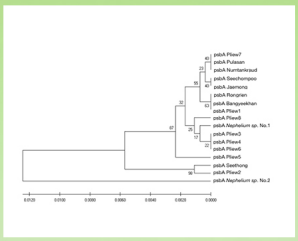


Figure 1 Phylogenetic relationships of 17 rambutans based on *psbA* barcode sequence data.

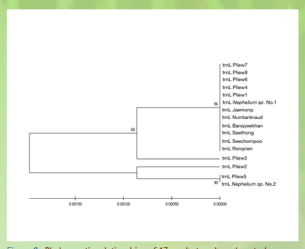


Figure 2 Phylogenetic relationships of 17 rambutans based on *trnL* barcode sequence data.

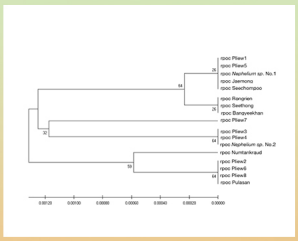


Figure 3 Phylogenetic relationships of 17 rambutans based on *rpoC* barcode sequence data.

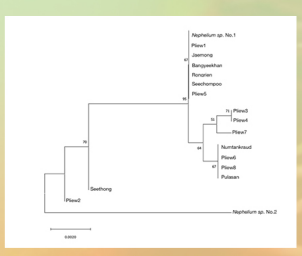


Figure 4 Phylogenetic relationships of 17 rambutans based on *psbA, trnL* and *rpoC* barcode sequence data.

Conclusion

The explanation of rambutan diversity using chloroplast genome regions (*psbA, trnL* and *rpoC*) is not clear. This study confirmed the need for further examination of different specific primers or other techniques such as GBS to confirm the relationships of Thai rambutan. The genetic diversity of cultivated rambutan shown by this study will be used as genetic database for a future breeding program.

References

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