Pertanika J. Trop. Agric. Sci. 48 (4): 1109 - 1117 (2025)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Short Communication

Extraction of Useful DNA from Different Parts of the Durian (*Durio zibethinus*) Fruit

Wei Lun Ng[1,6], Xue Jing Wong[2], Shiamala Devi Ramaiya[3], Suzana Sabran[4], Bui Manh Hung[5] and Shiou Yih Lee[2,7]*

[1]*China-ASEAN College of Marine Sciences, Xiamen University Malaysia, 43900 Sepang, Selangor, Malaysia*
[2]*Faculty of Health and Life Sciences, INTI International University, 71800 Nilai, Negeri Sembilan, Malaysia*
[3]*Department of Crop Science, Faculty of Agriculture and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, 97000 Bintulu, Sarawak, Malaysia*
[4]*Sabah Forestry Department, Forest Research Centre, 90715 Sandakan, Sabah, Malaysia*
[5]*Department of Forest Inventory and Planning, Faculty of Forestry, Vietnam National University of Forestry, Chuong My, QL 21 Hanoi, Vietnam*
[6]*College of Ocean and Earth Sciences, Xiamen University, 361102 Xiamen, Fujian, China*
[7]*Centre of Health, Well-Being, and Environmental Sustainability, INTI International University, 71800 Nilai, Negeri Sembilan, Malaysia*

ABSTRACT

Successful deoxyribonucleic acid (DNA) extraction from the durian fruit allows effective molecular identification and genotyping of durian cultivars for quality assurance. While most genetic identification of durian samples used the leaf as the DNA source, studies exploring durian fruit as a source of DNA for molecular identification and genotyping are limited. In this study, four potential sources of genetic material: the peduncle, spine, carpel, and aril from the durian fruit were evaluated for DNA yield (in terms of concentration), quality (in terms of ratio of A_{260}/A_{280}), and suitability in PCR amplification (using genetic markers that target the chloroplast, nuclear, and microsatellite DNA regions). Results of this study show that the spine is a good potential source of genetic material alongside the peduncle.

Keywords: Cultivar genotyping, DNA extraction, fruit DNA, genetic resources

ARTICLE INFO

Article history:
Received: 18 July 2024
Accepted: 09 September 2024
Published: 30 May 2025

DOI: https://doi.org/10.47836/pjtas.48.4.03

E-mail addresses:
weilun.ng@xmu.edu.my (Wei Lun Ng)
i20018869@student.newinti.edu.my (Xue Jing Wong)
shiamala@upm.edu.my (Shiamala Devi Ramaiya)
suzana.sabran13@gmail.com (Suzana Sabran)
hungbm@vnuf.edu.vn (Bui Manh Hung)
shiouyih.lee@newinti.edu.my (Shiou Yih Lee)
*Corresponding author

INTRODUCTION

The fruit of the durian (*Durio zibethinus* L.; Malvaceae) is known for its unique smell, intense taste, and significant nutritional

value (Ali et al., 2020). Native to Southeast Asia, durian has a crucial role in the cultural and economic aspects of countries, including Malaysia, Thailand, and Indonesia (Thorogood et al., 2022). It is commonly referred to as the "King of Fruits" due to its extensive appeal and significant market value despite its divisive smell. In 2023, Thailand was the primary global durian supplier, with Vietnam and Malaysia following closely behind. On the other hand, China is the world's leading importer of durian, accounting for over 95% of all imports (Food and Agriculture Organization of the United Nations [FAO], 2023).

Consumer taste plays a pivotal role in determining the demand for durian, with preference for different cultivars changing through time as new cultivars emerge in the market; Malaysia, for example, currently has 129 durian cultivars registered with the National Crop List (http://pvpbkkt.doa.gov.my/NationalList/Search.php). Since different durian cultivars present different aromas, tastes, textures, and nutritional levels, the identification of the cultivar from which a durian fruit is derived has to be accurate and consistent (May et al., 2023) for quality assurance. Ensuring the authenticity and quality of exported durian fruit and its products is crucial for maintaining market confidence towards durian-producing countries.

So far, genotyping of DNA markers has shown potential in differentiating the various durian cultivars (Siew et al., 2018; Mursyidin et al., 2022). It has significant applications in the quality control and assurance of durian exports, given the international demand and high market price, by reducing cases of false cultivar claims or adulterated products, which can damage the reputation of the producing countries. Due to its size, durian fruits sold on the market do not have other tissues from the mother tree, such as leaves or branches attached. It means that if any DNA analyses were to be done on the fruit, the DNA would have to be extracted from the fruit itself. The DNA extraction and polymerase chain reaction (PCR) amplification steps are essential processes that ensure the success of a genetic analysis. However, obtaining high-quality DNA from plant tissues, especially from the fruit, poses distinct challenges due to high concentrations of polysaccharides, secondary metabolites, and other substances that complicate the extraction process and affect the quality and quantity of the DNA obtained (Japelaghi et al., 2011). Determining suitable parts of the high-value durian fruit as sources of quality genetic material would thus aid in efforts that enable molecular identification and genotyping of its different cultivars.

This study seeks to address the challenges in DNA extraction from the durian fruit by conducting a comparative analysis of the efficacy of DNA extraction from various parts of the durian fruit and using the extracted DNA for PCR amplification. By examining the quality and quantity of extracted DNA from the durian fruit peduncle, spine, carpel, and aril, as well as determining the suitability of each sample type for PCR amplification of chloroplast and nuclear DNA fragments and microsatellite DNA, this study provides vital information on the approach for extracting useful DNA from the durian fruit. We envision

that the findings in this study would enhance the efficiency of genetic investigations for the authentication of durian fruits at border controls or international trade platforms.

MATERIALS AND METHODS

Fruit Material and DNA Extraction

Three Musang King variety durian fruits were purchased from a local fruit seller. Prior to excising pieces ("sample") of the different parts of the fruit—the peduncle, spine, carpel, and aril (Figure 1)—the surfaces of each sample type were gently wiped and sterilised with a C-fold hand towel sprayed with 70% ethanol. After surface sterilisation, 100 mg of each sample type was obtained for DNA extraction.

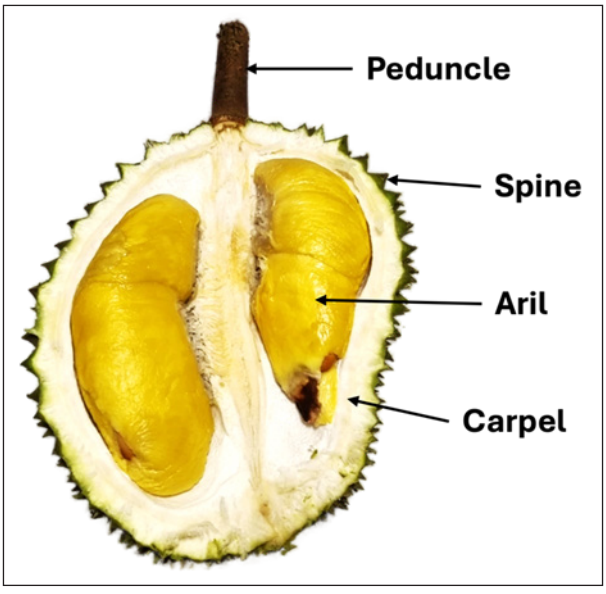

Figure 1. An opened durian fruit showing the different parts of the fruit sampled for DNA extraction: the peduncle, spine, carpel, and aril

Total genomic DNA was extracted using the FavorPrep Plant Genomic DNA Extraction Mini Kit (Favorgen Biotech, China) with minor modifications. Briefly, for the samples of the peduncle, spine, and carpel, the sample was cut into small cubes of 1mm×1mm×1mm or smaller using a sterile scalpel before being added into a 2 mL microcentrifuge tube containing 1 mL of lysis buffer (FAPG1 Buffer). In contrast, the sample of the aril was directly added to the lysis buffer. A sterile mini pestle was then used to grind the sample in the lysis buffer to ensure mixing and maximum contact of cells with the buffer. After adding 16 µL of RNase A solution (50 mg/mL), the mixture was vortexed vigorously and incubated at 65 ˚C for 2 h in a water bath. The mixture was let to cool down prior to

adding 280 µL FAPG2 Buffer and then incubated at -20 ˚C for 30 min. Subsequent steps to bind, wash, and elute the DNA were per the manufacturer's protocol. DNA extraction was conducted for each sample in duplicates as technical replicates. The quantity and quality of the extracted DNA were then determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

PCR Amplification and Verification of DNA Quality

Six primer pairs specific to the chloroplast and nuclear DNA regions were used to test for PCR amplification using the total genomic DNA extracted in this study. The marker loci were: (1) *mat*K (1R_KIM: 5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3' and 3F_KIM: 5'-CGTACAGTACTTTTGTGTTTACGAG-3') by Kim (unpublished), (2) *trn*L-*trn*F intergenic spacer (trnL-e: 5'-GGTTCAAGTCCCTCTATCCC-3' and trnF-f: 5'-ATTTGAACTGGTGACACGAG-3') by Taberlet et al. (1991), (3) ITS (ITS-p5: 5'-CCTTATCAYTTAGAGGAAGGAG-3' and ITS-S3R: 5'-GACGCTTCTCCAGACTACAAT-3') by Chen et al. (2010) and Cheng et al. (2016), (4) ITS2 (ITS-p3: 5'-GCCRAGATATCCGTTGCCGAG-3' and ITS-S3R: 5'-GACGCTTCTCCAGACTACAAT-3') by Chen et al. (2010) and Cheng et al. (2016), and microsatellite markers (5) DZ05 (DZ05_F2: 5'-ACACATACACAACTCACCTC-3' and DZ05_R: 5'-ATGCCCGATGAAATTGTAAC-3') and DZ07 (DZ07_F: 5'-ACACACCATCTTCCCTTTG-3' and DZ07_R: 5'-TGCACATGTTGTTTGTATATATG-3') by Siew et al. (2018).

Based on literature, the chloroplast partial gene sequence *mat*K and intergenic spacer region *trn*L-*trn*F would produce amplifications of about 850 bp and 500 bp, respectively. In comparison, the nuclear ribosomal DNA internal transcribed spacer (ITS) and ITS2 would produce amplifications of about 900 bp and 500 bp, respectively. The nuclear microsatellite markers DZ05 and DZ07 were expected to produce amplifications of about 200 bp and 440 bp, respectively. PCR amplifications were carried out in a 25 µL total reaction volume containing Taq Plus Master Mix (Dye Plus; Vazyme, China), 0.4 µM of each primer, and 15 ng of genomic DNA as a template. A negative control for each marker was included, using sterilised distilled water in place of the DNA template. PCR amplifications were conducted on a SelectCycler II thermal cycler (Select-Bioproducts, USA), with initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 15 s, annealing at 50 °C for 15 s, and extension at 72°C for 60 s, and a final extension at 72°C for 5 min. PCR amplification was carried out twice for each marker and sample (equivalent to two technical replicates). The amplified products were separated on 1.0% agarose gels containing the FloroSafe DNA Stain (1st BASE, Malaysia) and visualised under ultraviolet (UV) light.

RESULTS

DNA Quality and Yield

Using the selected DNA extraction method, we found that all four types of samples from the durian fruit exhibited different levels of total genomic DNA purity and concentration (Table 1). The ratio of absorbance at 260 nm to absorbance at 280 nm (A_{260}/A_{280}) of between 1.5 and 2.0 is typically the "acceptable" purity of a DNA extract for downstream applications (Lucena-Aguilar et al., 2016). For the peduncle sample, at least one technical replicate from Durian 1 (D1) and Durian 3 (D3) returned good DNA purity, with DNA concentrations of 35.5 ng/µL and 12.5 ng/µL, respectively. The DNA purity extracted from the spine sample was only acceptable in D1 but not in Durian 2 (D2) or D3. The DNA concentrations for the spine samples from D1 were 25–31.3 ng/µL. Of the six carpel samples, only one displayed an acceptable purity of 1.64 from D1. When using the aril as the tissue sample, four tissue samples from the three durian samples returned with at least one technical replicate with an absorbance ratio between 1.5 and 2.0, with DNA concentrations ranging from 36.6 to 149.9 ng/µL.

Table 1
The purity and concentration of DNA extracts of four sample types from the durian fruit

		Durian 1		Durian 2		Durian 3	
		Technical replicate 1	**Technical replicate 2**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 1**	**Technical replicate 2**
Peduncle	A_{260}/A_{280}	2.09	1.62	1.17	1.13	2.02	1.93
	Concentration (ng/µL)	7.40	35.50	34.10	148.70	12.40	12.50
Spine	A_{260}/A_{280}	1.89	1.79	2.47	1.47	2.26	2.72
	Concentration (ng/µL)	25.00	31.30	12.10	13.20	12.70	12.80
Carpel	A_{260}/A_{280}	1.64	1.20	1.37	2.22	2.08	1.29
	Concentration (ng/µL)	27.60	32.50	31.90	18.00	15.90	25.50
Aril	A_{260}/A_{280}	1.50	1.34	1.77	1.55	1.53	1.25
	Concentration (ng/µL)	36.60	71.60	149.90	75.50	68.40	104.20

PCR Amplification Using Different Genetic Markers

From the negative controls, all tested genetic markers showed no false signals from contamination (Figure 2). The success of the amplification varied among the samples and genetic markers. At the *mat*K locus, only the spine, carpel, and aril samples from D2 and D3 presented amplified DNA (including both technical replicates). For the peduncle

samples, both technical replicates from D2 showed positive amplifications for *mat*K, while D1 and D3 only showed one successful amplification in one of their technical replicates. All sample types (including both technical replicates) produced amplifications for the *trn*L-*trn*F and ITS2 loci. However, only one technical replicate for the peduncle sample of D1 and the carpel sample of D3 produced the amplicon of interest. DNA extracts from the peduncle of D1 and the carpels of all three durian samples did not produce amplicons for the ITS locus. Only one of the two technical replicates produced amplification for the peduncle sample of D2 and the spine sample of D3. At the microsatellite marker loci DZ05 and DZ07, PCR amplification was successful for both technical replicates of the peduncle sample of D3, the spine sample of D1 and D2, and the aril sample of D2. Only one replicate of the peduncle and aril samples from D2 and D3 produced amplification at the microsatellite loci. DNA extracts derived from the peduncle sample of D1, the spine sample of D3, the aril sample of D1, and all the carpel samples showed no amplification.

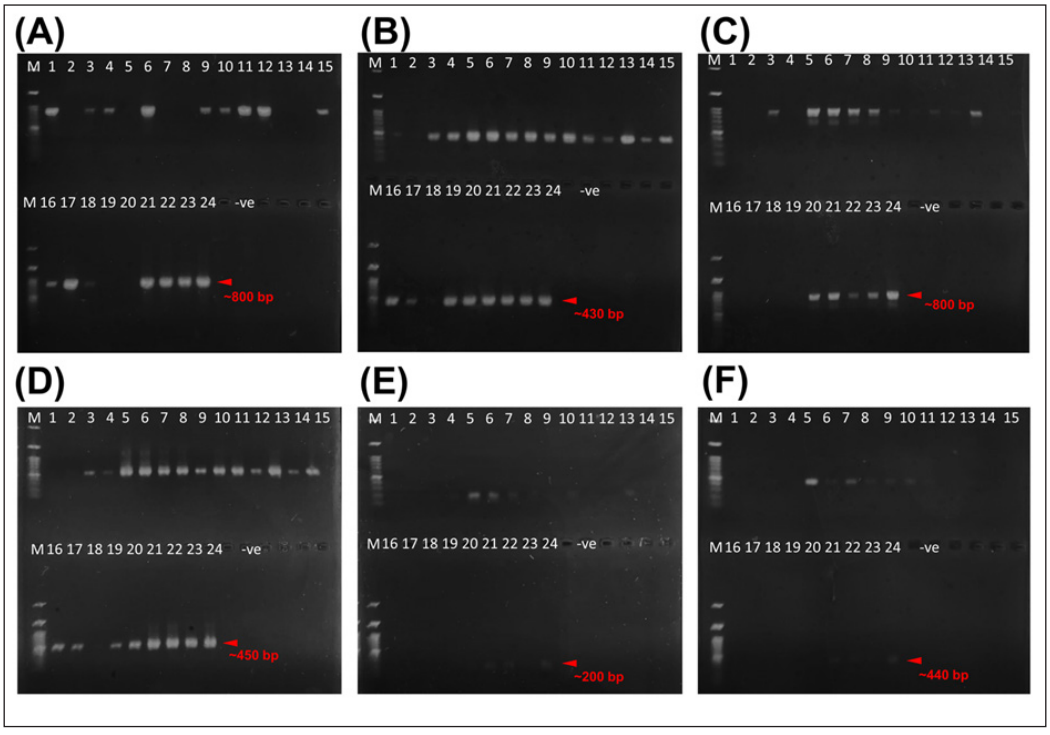

Figure 2. PCR amplification results for the genetic marker loci (A) *mat*K, (B) *trn*L-*trn*F intergenic spacer, (C) ITS, (D) ITS2, (E) DZ05, and (F) DZ07. Lane 1–2: D1 peduncle, 3–4: D1 spine, 5–6: D1 carpel, 7-8: D1 aril, 9–10: D2 peduncle, 11–12: D2 spine, 13–14: D2 carpel, 15–16: D2 aril, 17–18: D3 peduncle, 19–20: D3 spine, 21–22: D3 carpel, 23–24: D3 aril. Two technical replicates were produced for each sample. M:100 bp DNA ladder

DISCUSSION

To the best of current knowledge, this is the first study to report the evaluation of DNA yield and quality of the total genomic DNA extracted from the durian fruit. The results indicated that most sample types from the durian fruit did not achieve an acceptable absorbance ratio, whereas the DNA quality of the aril sample appeared promising. Generally, an A_{260}/A_{280} ratio of <1.5 could mean that there are proteins, phenols, or other contaminants that absorb strongly at or near 280 nm (Desjardins et al., 2010), while a ratio of >2.0 indicates the presence of RNA contamination that may inhibit amplification (Yuen et al., 2001).

As with many other plant species, genetic studies on durian usually involve using its leaf as the source of genetic material (Cheon et al., 2017; Nawae et al., 2023) due to its availability and ease of sample processing. However, as the fruits are marketed or exported, unlike some fruits, the durian leaves do not come with the fruit. Nonetheless, genetic studies using the durian fruit as a source of genetic material have been attempted in the past: Teh et al. (2017) used the peduncle as a source of genetic material for whole-genome sequencing of the durian, while Jantan et al. (2024) and Teh et al. (2017) also used the aril as the genetic material for transcriptomic studies on the durian fruit. The findings confirm that the peduncle and aril contain sufficient genetic material for genomic analysis. While the woody peduncle may seem to be lacking in chloroplasts, prior research has demonstrated that woody tissue can serve as a suitable source for obtaining extranuclear genetic material (Dumolin-Lapegue et al., 1999; Asif & Cannon, 2005).

Based on the findings in this study, the spine is proposed as a potential alternative source of genetic material for durian cultivar genotyping for its high success rate in PCR amplification of commonly used genetic markers. While the peduncle also produced good amplification results, not all durian fruit sold in the market come with a peduncle attached. On the other hand, the carpel and aril are impractical as sources of genetic material for quality assurance at checkpoints, as one has to open the fruit to obtain the samples. Thus, the spine (and, if available, the peduncle) would be an effortless and straightforward source of genetic material for total genomic DNA extraction.

Nonetheless, it is acknowledged that outcomes could vary when different DNA extraction protocols are used. As this study aimed to find a suitable source of sample from the durian fruit for quick and inexpensive DNA extraction, this work also did not consider an additional DNA purification step, which could improve the quality of the extracted DNA and, subsequently, the amplification success. Variations in the biochemical composition of fruit samples across different durian cultivars may also necessitate adjustments to the DNA extraction protocol to achieve optimal DNA yields.

CONCLUSION

This study evaluated the DNA yield and quality of total genomic DNA extracted from the durian fruit. While most sample types did not consistently achieve acceptable and/or consistent A_{260}/A_{280} ratios, DNA extracted from the durian aril showed promising DNA quality and concentration. Eventually, all sample types successfully amplified both the chloroplast *trn*L-*trn*F and nuclear ITS2 loci, demonstrating their suitability for downstream molecular applications.

ACKNOWLEDGEMENTS

This work is supported by the Ministry of Education, Malaysia, through the Fundamental Research Grant Scheme (FRGS) Fund, under Grants FRGS/1/2023/STG01/INTI/01/1 and FRGS/1/2020/STG03/UPM/02/10, and the INTI International University Research Seeding Scheme, under Grant INTI-FHLS-02-16-2023.

REFERENCES

Ali, M. M., Hashim, N., Abd Aziz, S., & Lasekan, O. (2020). Exploring the chemical composition, emerging applications, potential uses, and health benefits of durian: A review. *Food Control*, *113*, 107189. https://doi.org/10.1016/j.foodcont.2020.107189

Asif, M. J., & Cannon, C. H. (2005). DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Molecular Biology Reporter*, *23*, 185-192. https://doi.org/10.1007/BF02772709

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PloS One*, *5*(1), e8613. https://doi.org/10.1371/journal.pone.0008613

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom Plantae: New PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources*, *16*(1), 138-149. https://doi.org/10.1111/1755-0998.12438

Cheon, S.-H., Jo, S., Kim, H.-W., Kim, Y.-K., Sohn, J.-Y., & Kim, K.-J. (2017). The complete plastome sequence of Durian, *Durio zibethinus* L. (Malvaceae). *Mitochondrial DNA B Resources*, *2*(2), 763-764. https://doi.org/10.1080%2F23802359.2017.1398615

Desjardins, P., & Conklin, D. (2010). NanoDrop microvolume quantitation of nucleic acids. *Journal of Visualized Experiments*, *45*, e2565. https://doi.org/10.3791%2F2565

Dumolin-Lapègue, S., Pemonge, M. H., Gielly, L., Taberlet, P., & Petit, R. J. (1999). Amplification of oak DNA from ancient and modern wood. *Molecular Ecology*, *8*(12), 2137-2140. https://doi.org/10.1046/j.1365-294x.1999.00788.x

Food and Agriculture Organization of the United Nations. (2023). *Durian global trade overview 2023*. https://openknowledge.fao.org/server/api/core/bitstreams/84bbf2cb-d119-48c2-b4f1-f22bf802a7af/content

Jantan, S. Z., Poh, K. B., & Ginibun, F. C. (2024). Comparative transcriptomic study of matured fruit and post-fruit developmental stages in Malaysian durian varieties. *Plant Gene*, *39*, 100463. https://doi.org/10.1016/j.plgene.2024.100463

Japelaghi, R. H., Haddad, R., & Garoosi, G. A. (2011). Rapid and efficient isolation of high-quality nucleic acids from plant tissues rich in polyphenols and polysaccharides. *Molecular Biotechnology*, *49*, 129-137. https://doi.org/10.1007/s12033-011-9384-8

Lucena-Aguilar, G., Sánchez-López, A. M., Barberán-Aceituno, C., Carrillo-Avila, J. A., López-Guerrero, J. A., & Aguilar-Quesada, R. (2016). DNA source selection for downstream applications is based on DNA quality indicator analysis. *Biopreservation and Biobanking*, *14*(4), 264-270. https://doi.org/10.1089%2Fbio.2015.0064

May, O. S., Ismail, W. N. A. B. W., & Jun, C. (2023). The king of fruits dilemma in Malaysia: Discovering durian export challenges to China. *Journal of Engineering Research and Education*, *15*, 61-75. https://doi.org/10.58915/jere.v15.2023.652

Mursyidin, D. H., Makruf, M. I., & Noor, A. (2022). Molecular diversity of exotic durian (*Durio* spp.) germplasm: A case study of Kalimantan, Indonesia. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 39. https://doi.org/10.1186/s43141-022-00321-8

Nawae, W., Naktang, C., Charoensri, S., U-thoomporn, S., Narong, N., Chusri, O., Tangphatsornruang, S., & Pootakham, W. (2023). Resequencing of durian genomes reveals large genetic variations among different cultivars. *Frontiers in Plant Science*, *14*, 1137077. https://doi.org/10.3389/fpls.2023.1137077

Siew, G. Y., Ng, W. L., Salleh, M. F., Tan, S. W., Ky, H., Alitheen, N. B. M., Tan, S. G., & Yeap, S. K. (2018). Assessment of the genetic variation of Malaysian durian varieties using inter-simple sequence repeat markers and chloroplast DNA Sequences. *Pertanika Journal of Tropical Agricultural Science*, *41*(1), 321-332.

Siew, G. Y., Ng, W. L., Tan, S. W., Alitheen, N. B., Tan, S. G., & Yeap, S. K. (2018). Genetic variation and DNA fingerprinting of durian types in Malaysia using simple sequence repeat (SSR) markers. *PeerJ*, *6*, e4266. https://doi.org/10.7717/peerj.4266

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*, 1105-1109. https://doi.org/10.1007/bf00037152

Thorogood, C. J., Ghazalli, M. N., Siti-Munirah, M. Y., Nikong, D., Kusuma, Y. W. C., Sudarmono, S., & Witono, J. R. (2022). The king of fruits. *Plants, People, Planet*, *4*(6), 538-547. https://doi.org/10.1002/ppp3.10288

Yuen, P. S., Brooks, K. M., & Li, Y. (2001). RNA: a method to specifically inhibit PCR amplification of known members of a multigene family by degenerate primers. *Nucleic Acids Research*, *29*(6), e31-e31. https://doi.org/10.1093/nar/29.6.e31