The Secret Behind Blue Curcumin:

DNA PRIMERS in ACTION

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Synopsis

Ever heard of PCR? It is a laboratory technique that copies tiny DNA pieces in a fast manner, but it needs a 'primer' (like a GPS) to mark where to start. PCR method helps in studying Indonesia's blue curcumin (*Curcuma aeruginosa*). By picking the right primers, genetic tags for genes like ITS1, rbcL, or matK, can help scientists in copying key DNA segments, which is known as. DNA barcoding: using genes as a plant's 'ID card' for identification. Here we show how finding these primers will aid to the conservation and science-based herbal medicine. Small tools, big impact on biodiversity!

Introduction

Blue Curcumin: The "Blue" of the Zingiberaceae family

Ever seen a turmeric that's blue? Not the spice in your kitchen, but a rare turmeric colored wonder called Blue Curcumin (*Curcuma aeruginosa*). This exotic member of the ginger family isn't just pretty; it's packed with potential for modern medicine! Found in Indonesia, it's so special that India even protects it as a rare species.

But here's the problem: it's tricky to tell apart from its "cousin," Black Turmeric. They look similar, both have striped leaves but Blue Curcumin's rhizomes glow like ocean blue, while Black Turmeric's are deep indigo. Looks can be deceiving though! You can't rely on color or leaf shape alone because their DNA is incredibly similar. So how do scientists tell them apart?

DNA Barcoding: The "Supermarket Scanner" for Plants

Imagine scanning a plant's DNA like a barcode at the checkout! That's DNA Barcoding. A genius method using tiny pieces of DNA of a species like an ID tag. For plants like Blue Curcumin, scientists use special markers from the plant's chloroplast (matK, rbcL) or cell nucleus (ITS).

To read these tags, you need a "key." That's where primers come in! Primers are short, custom-made DNA strips that act like molecular bookmarks. In a process called PCR (Polymerase Chain Reaction),

Table 1 PCR primers for matK, rbcL, trnH-psbA, ITS1 & ITS2 markers.

Loci	Primer	Sequences
matK	F	5'- CGA TCT ATT CAT TCA ATA TTT C -3
matK	R	5'- TCT AGC ACA CGA AAG TCG AAG T -3'
rbcL	F	5'- ATG TCA CCA CAA ACA GAG ACT AAA GC -3'
rbcL	R	5'- GTA AAA TCA AGT CCA CCA CG -3'
psbA	F	5'- GTT ATG CAT GAA CGT AAT GCT C-3'
trnH	R	5'- GCA TGG TGG ATT CAC AAT CC -3'
ITS1	F	5'- GGT GAA CCT GCG GAA GGA TCA TTG -3'
ITS1	R	5'- CCG AGA TAT CCA TTG CCG AGA GTC-3'
ITS2	F	5'- CGC CTG CTT GGG CGT CAT GGC -3'
ITS2	R	5'- GGG CCT CGC CTG ACT TGG GGC C -3'

Notes: Forward (F) and reverse (R) primers with their 5'-3' sequences used for the amplification of plastid DNA sequences.
Source: Data obtained from laboratory analysis at SEAMEO BIOTROP.

they stick to the start and end of a target gene, signaling: "Copy this section!". Think of them as forward and reverse bookends that help make millions of DNA copies.

Get the primer right, and you can identify a species from a speck of leaf. Get it wrong? You get nothing or copy the wrong gene.

Why Finding the Perfect Primer Matters?

For researchers, choosing the right primer isn't just science, it's a critical mission. A mismatch primer means no results, wasted time, or misidentifying this rare plant. With Blue Curcumin's conservation and medical potential on the line, we can't afford guesswork.

That's why we tested 5 different primers (matK, trnH-psbA, rbcL, ITS1, ITS2) on Blue Curcumin DNA. Our goal is to find which primer reliably unlock the genetic code of Blue Curcumin, so that we can protect the species, study its healing powers, and secure its future.

Copying Nature's Code: Cracking the Genetic Barcode of Blue Curcumin

Imagine a tiny molecular photocopier that can make millions of DNA copies in just a few hours. That's PCR (Polymerase Chain Reaction), a powerful tool we use to reveal the unique genetic "barcode" of Blue Curcumin, a rare and medicinally valuable plant. First, we start by preparing a tiny mix of sample, just 50 µL, containing a special enzyme mix (MyTaq™), ultra-pure water, a speck of Blue Curcumin DNA, and two primers per gene. These primers act like molecular bookends, marking exactly where the DNA-copying machine should start and stop. Table 1 shows the primers used.

The PCR process run in three simple steps, repeated 35 times: First, heating to 95 °C to "unzip" the DNA strands (like splitting a zipper). Next, the cooling down step i.e., 52 - 53 °C for matK/trnH-psbA, 56 °C for rbcL, and 59 °C for ITS genes, to allow the primers to stick to their matching spots, like puzzle pieces locking in. Finally, we raised the temperature again to 72 °C, so the enzyme could build new DNA strands.

To see if the copying worked, we used DNA gel electrophoresis, a method that lets us watch DNA pieces "race" through a jelly-like gel under electric current. We inserted the samples into a gel like tray, zapped them with electricity, and watched as DNA

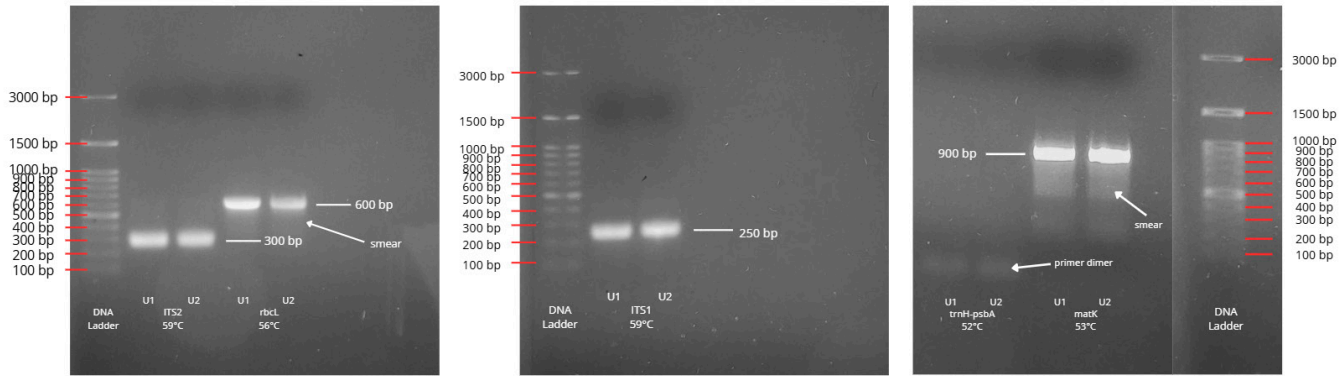

Figure 1 Electrophoresis results of Blue Curcumin DNA samples
Source: Laboratory experiment at SEAMEO BIOTROP (2025).

fragments raced toward a finish line (Fig. 1). We saw that smaller pieces moved faster. We used a blue dye (made the DNA glow under UV light) and a "DNA ruler" to measure the gene sizes.

The Primer Showdown: Which Keys Unlocked Blue Curcumin's DNA?

Out of five primer pairs tested, four worked perfectly. Only trnH-psbA failed, producing blurry smears. This likely happens because its forward and reverse primers stuck to each other instead of the DNA, a "primer dimer", like two keys locking together before reaching the door. This often occurs when DNA is scarce, or the primer isn't a perfect match.

The other genes delivered clear results: Chloroplast genes matK (900 bp) and rbcL (600 bp) are ideal for barcoding due to their stability across species. Nuclear genes ITS1 (~300 bp) and ITS2 (~250 bp) also performed well, creating short but powerful ID tags. ITS2 is especially precise in distinguishing even closely related species, like telling identical twins apart!

We've now stored Blue Curcumin's full genetic code in the NCBI GenBank, a global DNA library. This means, Conservationists can ID and protect this rare plant with 100% accuracy. Scientists can explore its medicinal secrets (like blue-curcumin's health potential). Future studies can mix-and-match primers: matK/rbcL for stability, ITS2 for precision! And that fastidious trnH-psbA primer? It's not hopeless; adjusting temperatures or redesigning the primer (like recutting a key) could make it work, as seen in Camellia plant studies.

Conclusion

So, what's the takeaway? Think of primers as specialized "keys" that unlock specific sections of DNA and after rigorous testing, we've found the perfect set for Blue Curcumin: matK (chloroplast); rbcL (chloroplast); ITS1 (nuclear); and ITS2 (nuclear). These markers didn't just work; they delivered crystal-clear genetic barcodes, making them ideal for sequencing Blue Curcumin's DNA. Most impressively, the ITS1 and ITS2 primers emerged as rockstars, amplifying DNA with pinpoint accuracy every time.

Future Directions/Call to Actions

This study is just the beginning. The next step is to sequence the DNA fragments that were successfully amplified using PCR. By determining the exact order of DNA building blocks (called nucleotides), scientists can compare them with other species in online databases like GenBank. This comparison will help confirm whether Blue Curcumin is genetically distinct from similar plants such as Black Turmeric (*Curcuma caesia*) or *Curcuma aeruginosa*, which share similar appearances.

In the long run, this will lead to the creation of a unique genetic barcode specifically for Blue Curcumin—a sort of biological ID card. This barcode will help researchers, farmers, and herbal medicine producers to distinguish the plant accurately and avoid misidentification. To deepen the study, researchers can also apply methods like DNA fingerprinting (e.g., ISSR) to explore genetic diversity within and between Blue Curcumin populations. This could provide insights into how the plant has evolved and how best to conserve its genetic richness.

Acknowledgment

The author would like to thank the team from the Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP) for the research facilities and experiences that have been provided as well as the internship supervisor and mentor Ms. Dewi Rahmawati, MSi for her guidance during the 3-month activities at SEAMEO BIOTROP.

Readings

Chandrasekara, C. B., Naranpanawa, D. N. U., Bandusekara, B. S., Pushpakumara, D., Wijesundera, D. S. A., & Bandaranayake, P. C. G. (2021). Universal barcoding regions, rbc L, mat K and trn H-psb A do not discriminate *Cinnamomum* species in Sri Lanka. PLoS One, 16(2), e0245592. DOI: 10.1371/journal.pone.0245592

Fadhilah, N., Idami, Z., & Dur, S. (2025). Analisis polimorfik genetik DNA ikan mas koki genus *Carrasius* menggunakan metode RAPD (Random Amplified Polymorphic DNA) [Genetic polymorphic analysis on DNA of goldfish, genus *Carrasius*, using RAPD method (Random Amplified Polymorphic DNA)]. Jurnal Biosense, 8(2), 157-71. DOI: 10.36526/biosense.v8i2.5137

Ho, V. T., Nguyen, T. H., Nguyen, L. B. N., Nguyen, T. T. N., & Nguyen, M. P. (2023). Comparison of rbcL and trnH-psbA DNA barcodes in diverse *Camellia* species collection in Vietnam. SABRAO J. Breed. Genet, 55(3), 877-85. DOI: 10.54910/sabrao2023.55.3.22

Ismail, M., Ahmad, A., Nadeem, M., Javed, M. A., Khan, S. H., Khawaish, I., Sthanadar, A. A., Qari, S. H., Alghanem, S. M., Khan, K. A., Khan, M. F., & Qamer, S. (2020). Development of DNA barcodes for selected Acacia species by using rbcL and matK DNA markers. Saudi Journal of Biological Sciences, 27(12), 3735-42. DOI: 10.1016/j.sjbs.2020.08.020

Jose, S., & Thomas, T. D. (2014). Comparative phytochemical and anti-bacterial studies of two indigenous medicinal plants *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. International Journal of Green Pharmacy (IJGP), 8(1). DOI: 10.22377/ijgp.v8i1.356

Khan, A. Z., Fernando, A. P., Pamungkas, E. A., Andhira, A., Ishlah, R. N., Resmi, D. D. C., Damayanti, F., & Rahmawati, D. (2025). Effectiveness of DNA barcoding primers in red algae (Rhodophyta) identification. BIODIVERS-BIOTROP Science Magazine, 4(1), 1-8. DOI: 10.56060/bdv.2025.4.1

Kumar, A. M., Vasundhara, M., Sathyanarayana, B. N., Shyamalamma, S., Doreswamy, C., & Anil, V. S. (2021). Authentication and DNA barcoding of *Curcuma caesia* Roxb. genotypes and other Zingiberaceae species using ITS-2 gene. Mysore Journal of Agricultural Sciences, 55(3), 171.

Prasetya, E., Lazuardi, L., Harahap, F., Rachmawati, Y., Yusuf, Y., Al Idrus, S. I., & Prastowo, P. (2023). Region of nuclear ribosomal DNA (ITS2) and chloroplast DNA (rbc L and trn LF) as a suitable DNA barcode for identification of Zingiber loerzingii Valeton from North Sumatera, Indonesia. Journal of Tropical Biodiversity and Biotechnology, 8(3), 76956. DOI: 10.22146/jtbb.76956

Rahmayani, L., Harahap, F., Manalu, K., Idami, Z., & Prasetya, E. (2021). DNA barcoding of Zingiber loerzingii Valeton using Ribulose-1, 5-biphosphate Carboxylase-Oxygenase Large subunit Gene (rbcL) gene locus. Jurnal Biosains Unimed, 7(3), 166-73. DOI: 10.24114/jbio.v7i3.2892

Saha, K., Dholakia, B. B., Sinha, R. K., & Sinha, S. (2020). DNA barcoding of selected Zingiberaceae species from North-East India. Journal of Plant Biochemistry and Biotechnology, 29(3), 494-502. DOI: 10.1007/s13562-020-00563-y

Vinitha, M. R., Kumar, U. S., Aishwarya, K., Sabu, M., & Thomas, G. (2014). Prospects for discriminating Zingiberaceae species in India using DNA barcodes. Journal of Integrative Plant Biology, 56(8), 760-73. DOI: 10.1111/jipb.12189

Wardi, E. S., Verawati, V., Juita, A. I., & Nova, B. (2023). Desain primer dan deteksi gen Chs (Chalcone Synthase) pada tanaman gambir (*Uncaria gambir* (Hunter) Roxb.) Tipe Udang. [Primers design and gene detection of Chs (Chalcone Synthase) in Gambier plant (*Uncaria gambir* (Hunter) Roxb.) shrimp type] FASKES: Jurnal Farmasi, Kesehatan, Dan Sains, 1(2), 150-63. DOI: 10.32665/faskes.v1i3.2407

Short Author's Biographies

Gracecielo Isaiah Poetra Andriyanto Gunawan is an active undergraduate student majoring in Biology with a strong interest in biodiversity conservation and molecular studies of wildlife. His academic focus spans from ecology, ornithology, to biotechnology. He currently serves as a practicum and research assistant in zoology major and is actively involved in DNA barcoding and bioinformatics research at the Biotechnology Laboratory of SEAMEO BIOTROP.

Short Co-author Biography

Dewi Rahmawati is a senior laboratory supervisor at the Biotechnology Laboratory of SEAMEO BIOTROP and a civil servant staff member at IPB University. With extensive experience in molecular biology, bioinformatics, plant tissue culture, and biotechnology, she plays an active role in supervising research and training programs. Her work focuses on applying molecular techniques to biodiversity studies and conservation.