

DNA BARCODING-BASED MOLECULAR PROFILING

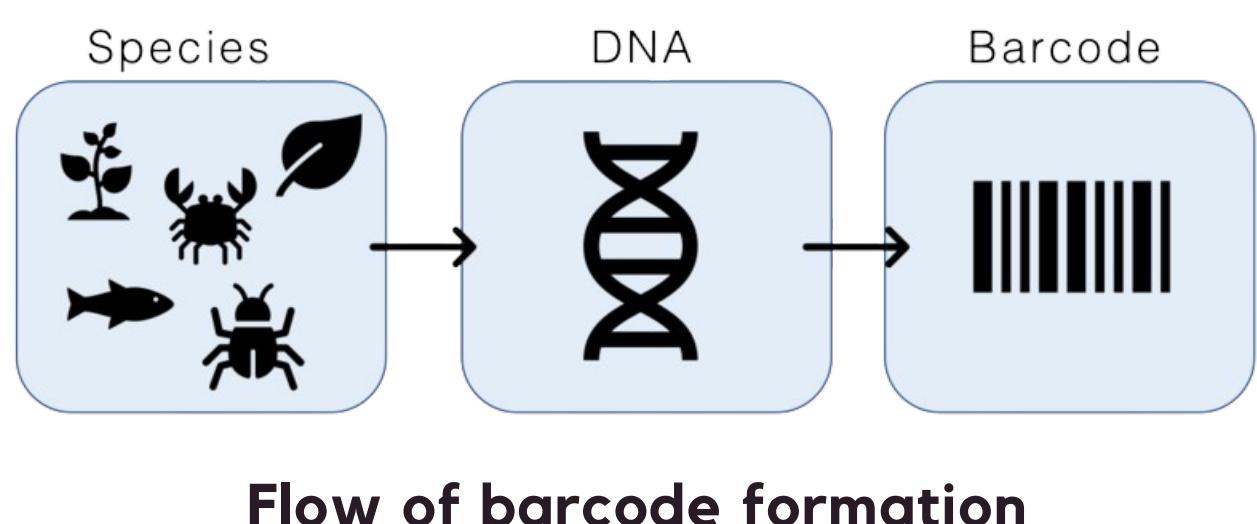
Bougainvillea, Dianthus, Plumeria using *matK* locus

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INTRODUCTION

- DNA barcoding uses short, standardized DNA segments to identify species, much like fingerprints for individuals.
- Each species has a **unique barcode**, which can be compared to a reference library for identification.

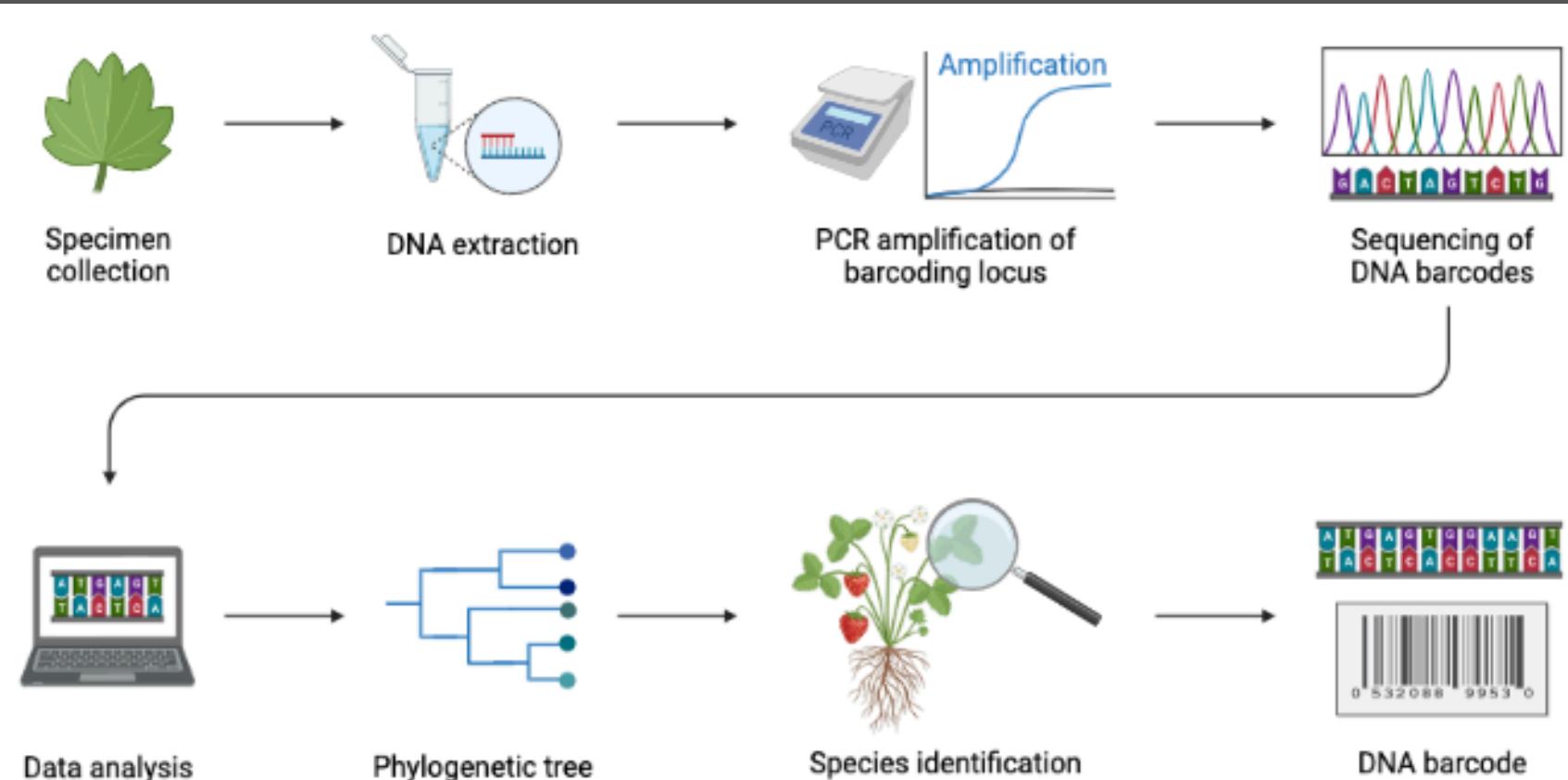


WHY DNA BARCODING WORKS?

- Barcode regions have enough genetic variation between species. For ex. *matK*, *rbcl*, *ITS*, *trnK-matK*, *psbA*
- We used **maturase K** (*matK*), a relatively conserved region within a species, allowing each species to be uniquely identified by its barcode.



METHODOLOGY



- We used leaf samples for DNA Extraction
- DNAeasy Extraction kit by Qiagen
- Designed Primer specific to species using Primer3

Made a database of **50+** species present on the campus on IITGN with more than **3 varieties** using **Pl@ntNet**



SCAN TO EXPLORE THE FLORA OF IITGN!!

How can we accurately differentiate and identify plant species?



RESULTS AND DISCUSSION

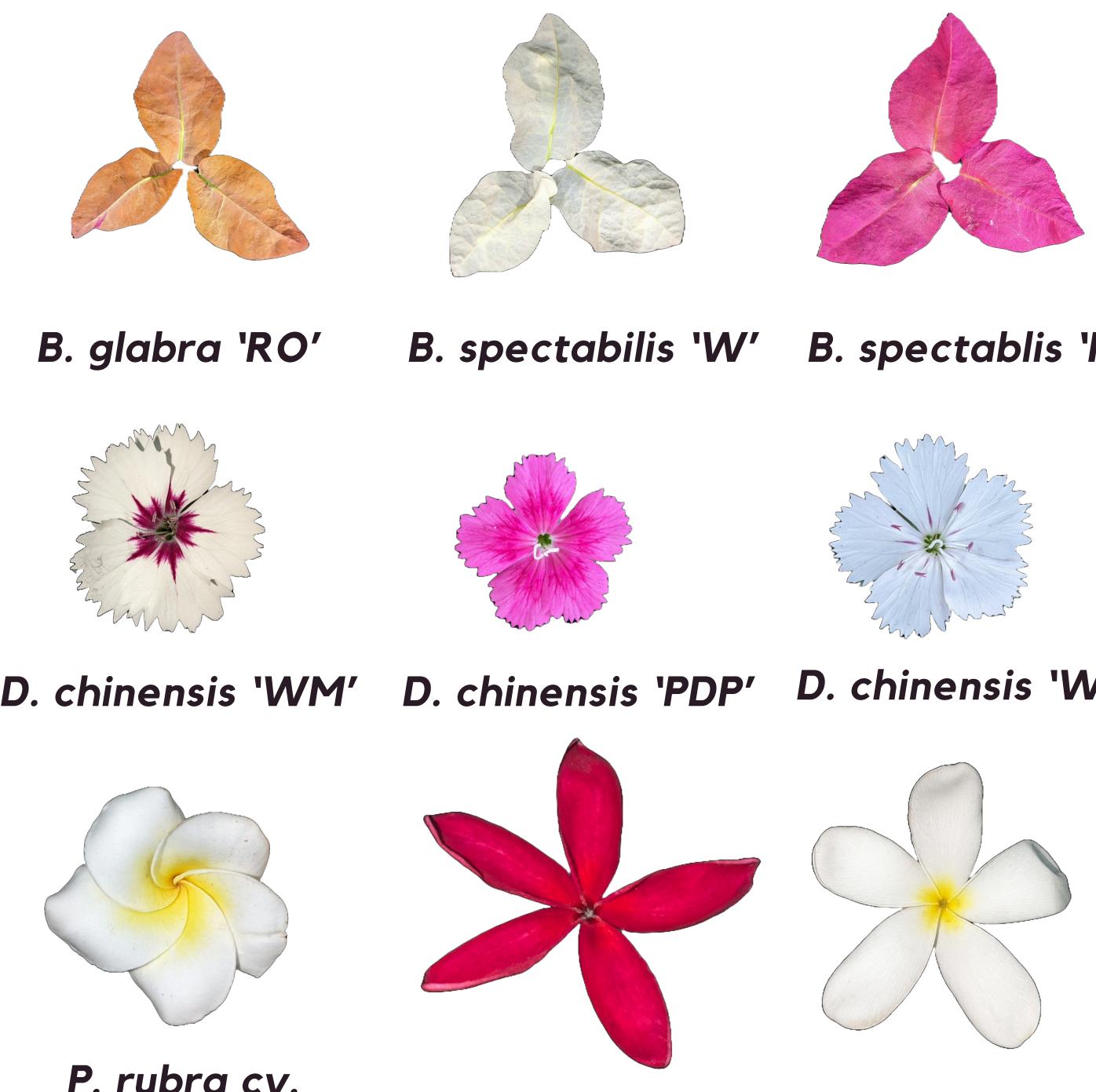


Figure 1. Species selected for DNA barcoding

Differentiating *B. glabra* and *B. spectabilis* morphologically is very challenging.

- *B. glabra* (Reddish Orange bracts)
- *B. spectabilis* (Both White and Pink bracts)
- *D. chinensis* (White-Pink, Pink-Dark Pink and Pure White serrated petals).
- *P. rubra* cv *Acutifolia* (White-Yellow overlapping petals)
- *P. rubra* (Red-Purple Rainbow)
- *P. obtusa* (Long, open White-Yellow).

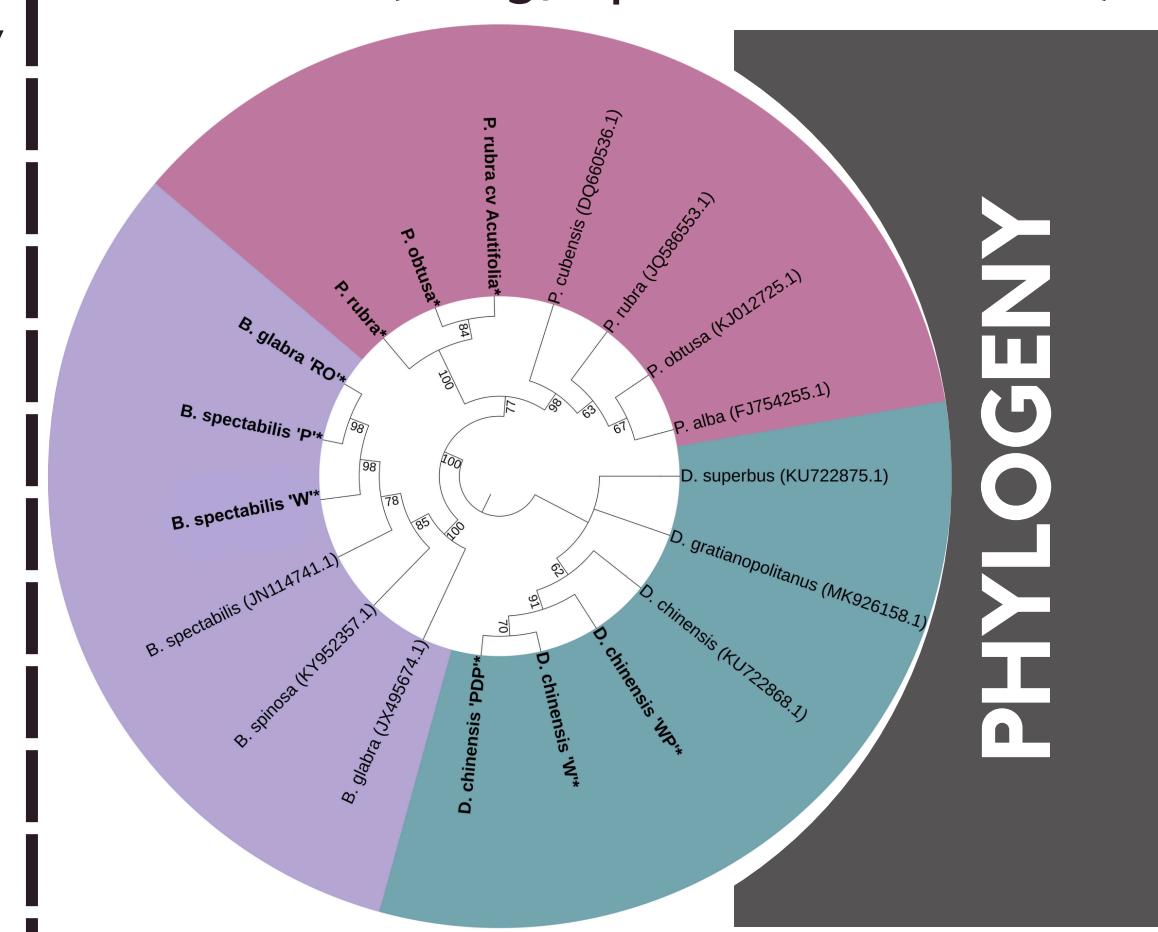


Figure 3. Phylogenetic analysis of *matK* genes (* represents selected species).

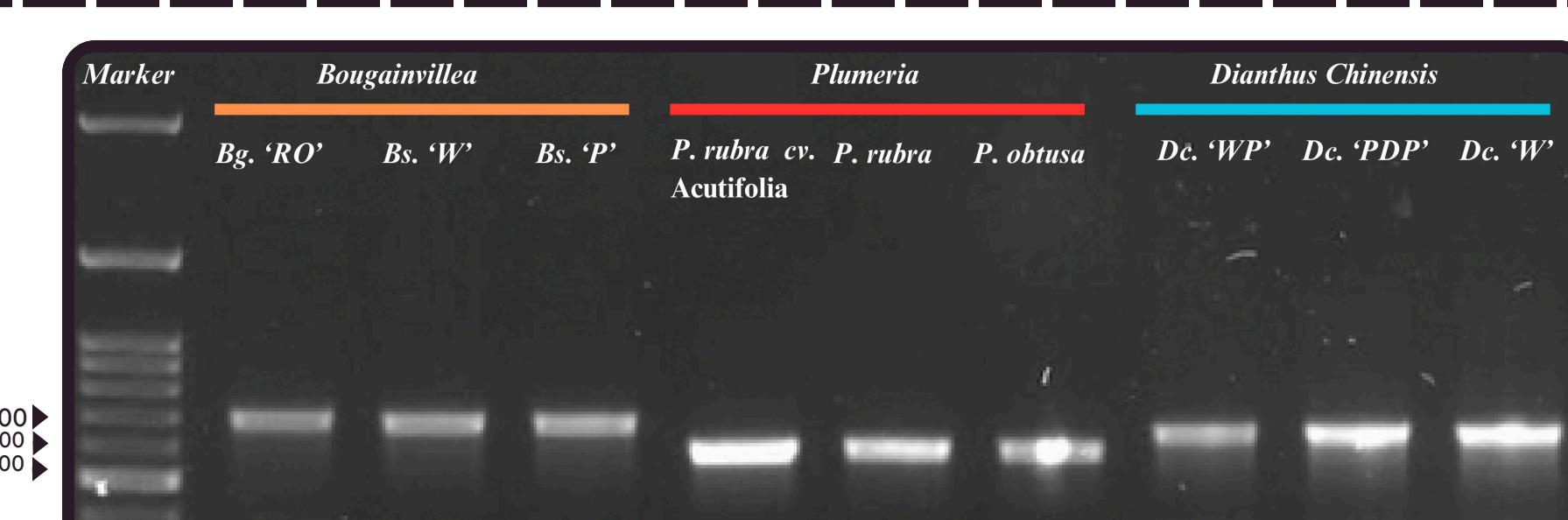


Figure 2. Agarose gel electrophoresis of PCR amplified products for *matK* genes. Clear and distinct bands for all 9 samples collected

Amplicon sizes :

Bougainvillea - **681 nt**
D. chinensis - **637 nt**
Plumeria - **578 nt**

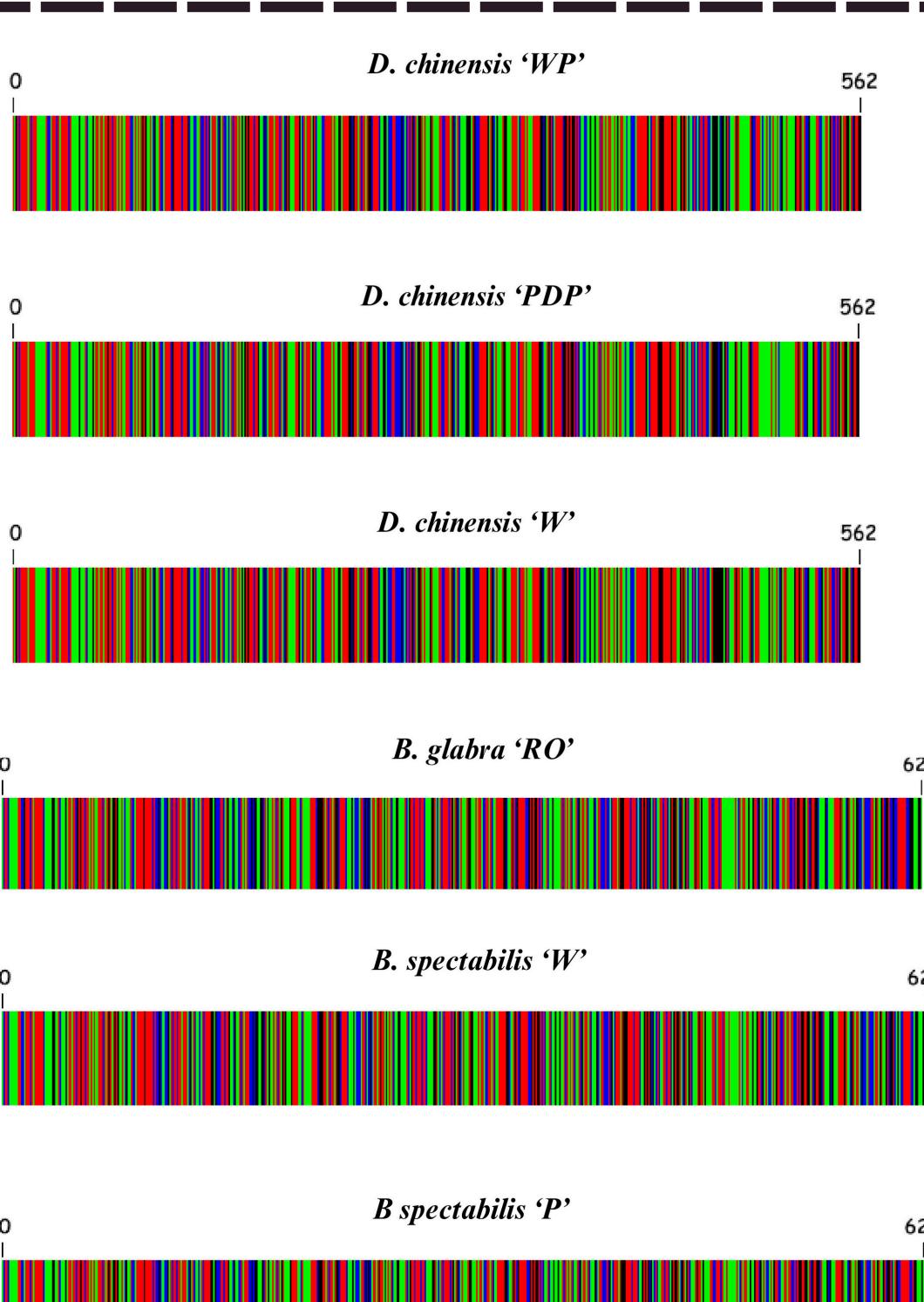


Figure 3. Two-dimensional DNA barcodes generated for the species used in this study.



- Using **M13F** and **M13R** primers, the amplicons were sequenced, yielding good-quality reads trimmed to **544-623 nt** for 2-D barcode generation.
- Multiple sequence alignment revealed **1-3% polymorphism** among species, highlighting *matK* as a marker for differentiating closely related species.
- **SCAN** the QR code for the nucleotide sequence of *matK*.
- Phylogenetic analysis effectively clustered *Bougainvillea*, *Dianthus*, and *Plumeria* species.
- We submitted barcodes to **BOLD Systems** and sequences to **NCBI**, enriching genetic databases.



FUTURE PROSPECT

Effective Differentiation: Combining molecular markers and morphology, using regions like *trnH-psbA* and *trnL-trnF*.

Misidentification Risk: Lack of genetic sequences can lead to errors using BLAST at NCBI.

Library Expansion: This expansion is essential for improving the precision of botanical research and the understanding of genetic diversity within and between species.



REFERENCES

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