Testing the Utility of Intergenic Spacer Regions to Identify Distinct Plumeria Taxa

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Introduction

In Hawaii, Plumeria cultivars contribute to the floriculture, landscape, and tourist industries. However, many of the cultivars suffer from insect, fungal, and viral afflictions, thereby diminishing flower yields and rendering plants unmarketable or unfit for export. Thus, a Plumeria breeding program will help to overcome these problems, while simultaneously creating novel Plumeria cultivars for Hawaii's floriculture industry.
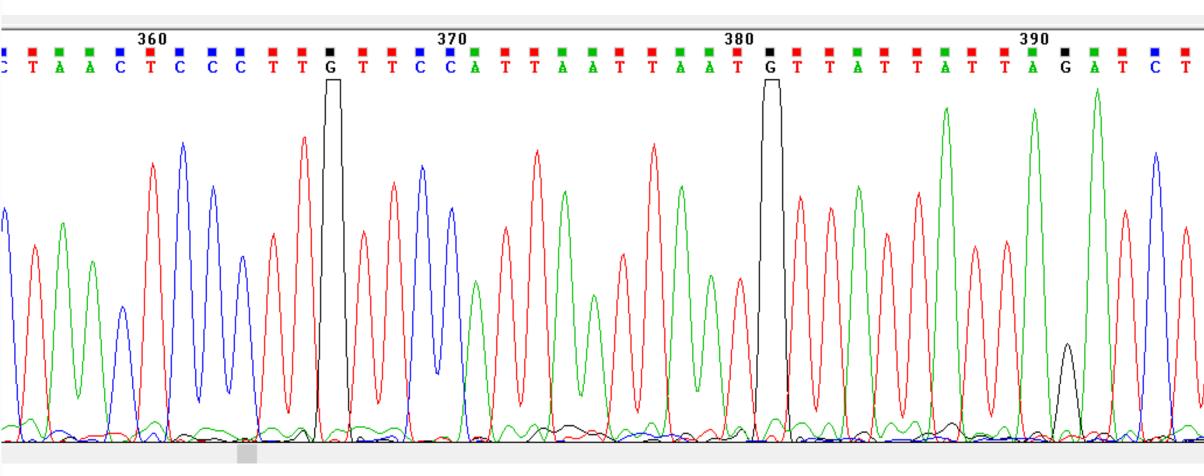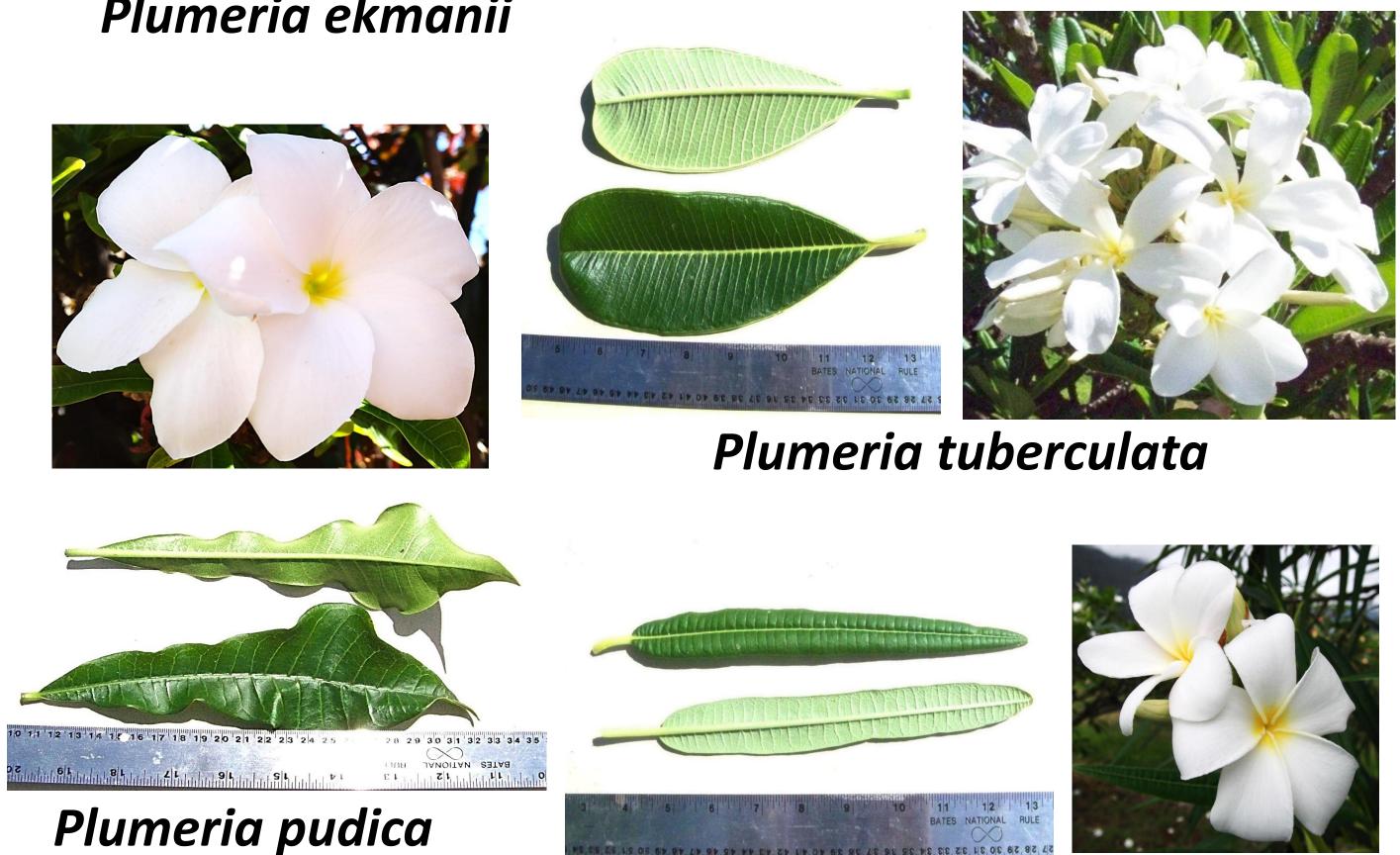
Information about genetic relationships from molecular studies can allow us to develop better breeding strategies to introduce horticulturally important traits from *Plumeria* spp. into cultivated Plumeria varieties. Recent studies have employed the use of intergenic spacer (IGS) regions to distinguish species and assess genetic similarity.

| Gene A | Intergenic Spacer Region | Gene B |

Objective: **Identify IGS regions that distinguish Plumeria species and assess genetic relationships.**

Methods

DNA Extraction (OMEGA E.Z.N.A.® SP Plant DNA Kit)
PCR (Bioline MyTaq™ Red Mix)
Sequence Editing & Assembly (Chromas Lite & CAP3)

6 Chloroplast Intergenic Spacer (IGS) Regions:
- psbJ-petA
- rpl32-trnL
- ndhF-rpl32
- psbD-trnT
- trnQ-rps16
- trnV-ndhC

Molecular Evolutionary Genetics Analysis (MEGA 6.0):
Neighbor-Joining
Maximum Parsimony

Basic Criteria for IGS Utility:
✔ Outgroup taxa are identified & Distinct taxa aggregate
✔ Maximum **intra**specific divergence is less than minimum **inter**specific divergence

Materials

5 Distinct *Plumeria* taxa:

Plumeria ekmanii

Plumeria caracasana

Plumeria tuberculata

Plumeria pudica

Plumeria alba

Results

Unique sequences within IGS regions can help to verify authenticity of *Plumeria* spp.

	psbJ-petA IGS		rpl32-trnL IGS	
Plumeria Taxa	Intraspecific	Interspecific	Intraspecific	Interspecific
P. ekmanii	0.000	0.002	0.005	0.014
P. pudica	0.000	0.001	0.001	0.006
P. alba	0.001	0.004	0.005	0.009
P. caracasana	0.000	0.001	0.001	0.006
P. tuberculata	0.000	0.004	0.06	0.047

Sequence Divergences

Maximum intraspecific divergence is less than minimum interspecific divergence.

Results

Combined NJ Tree of psbJ-petA & rpl32-trnL (Topology View).

Combined MP Tree of psbJ-petA & rpl32-trnL (Topology View).

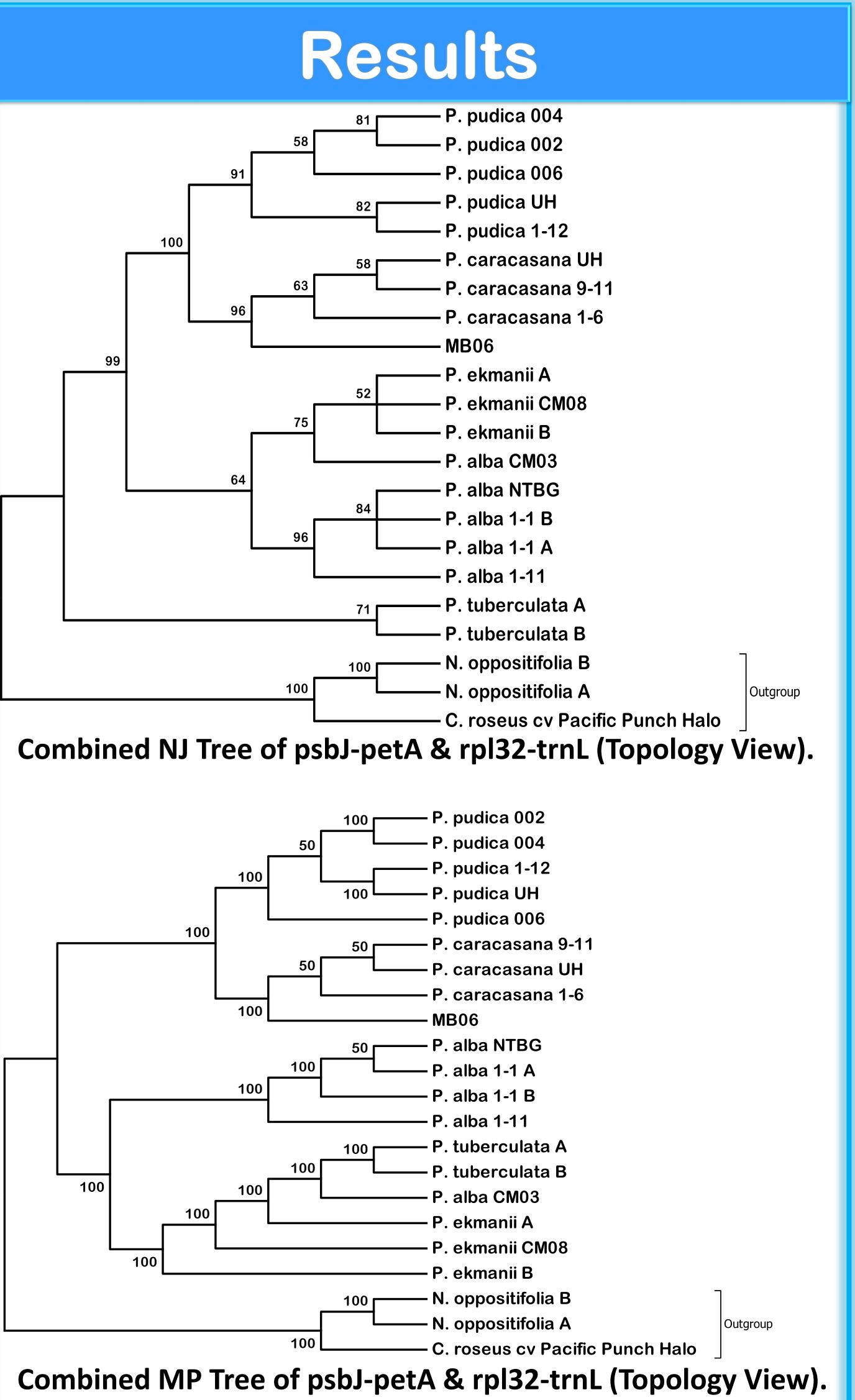## Conclusions

IGS regions **psbJ-petA** and **rpl32-trnL** were the most successful at recovering outgroup taxa while aggregating distinct *Plumeria* taxa. The combination of both regions yielded similar tree topologies to trees generated from analyses of individual regions. Thus, these regions can be used to further assess genetic relatedness among *Plumeria* spp. and cultivars to develop better breeding strategies.

Acknowledgements