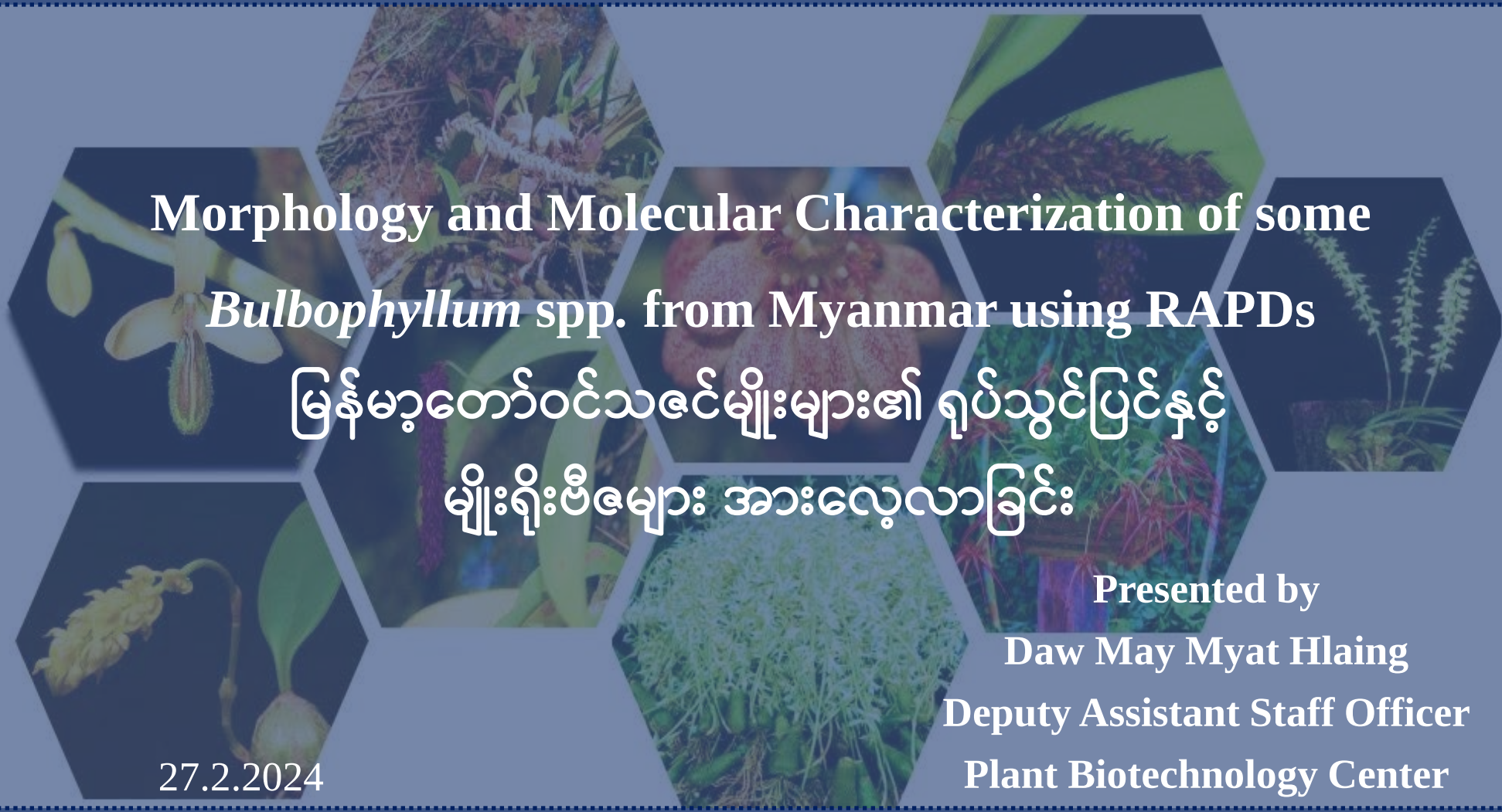
Ministry of Agriculture, Livestock and Irrigation
Department of Agriculture
Horticulture and Plant Biotechnology Division



Morphology and Molecular Characterization of some *Bulbophyllum* spp. from Myanmar using RAPDs

မြန်မာ့တောင်ဝင်သဧင်မျိုးများ၏ ရုပ်သွင်ပြင်နှင့် မျိုးရိုးဗီဇများ အားလေ့လာခြင်း

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Research Outline

- Introduction
- Objectives
- Materials and Methods
- Results and Discussions
- Conclusions

Introduction

***Bulbophyllum spp*.Thouars (Family -Orchidaceae)**

- One of the most **diverse genera** and (Gamisch & Comes,2019)
- Epiphytic and rounded pseudobulbs bearing one or two leaves - small flowers (colored sepals > the petals)
- **2,200** species - all over the **pantropical** regions (Pridgeon etal.,2014)
- Distribution - Bago Region, Tanintharyi Region, Yangon Region, Mon State, Rakhine State and Kachin State in Myanmar (Hundley and Chit KoKo, 1987)
- *Bulbophyllum auricomum* Lindl. **- a royal flower (**Konbaung dynasty (1752–1885)
- Ornamental , decorative, medicinal uses and commercial value

Significant of the study

- Due to the high number of species in the genus *Bulbophyllum*, systematic classification is complicated. In Myanmar, many *Bulbophyllum* species are threatened due to climate change, their habitat destruction and fragmentation and indiscriminate collection.
- Thus, it is urgently necessary to explore the background information about the species.
- The genome characteristics *of Bulbophyllum* are poorly understood. There is a lack of genetic and molecular data for this diverse genus of *Bulbophyllum*

Objectives

1. To assess the genetic relationship among the *Bulbophyllum species*
2. To document the DNA fingerprint of these species for the conservation
3. To explore more information and genetic background of Myanmar *Bulbophyllum* spp. for sustainable conservation and increase use of plant genetic resources

Materials and Methods

- Duration- 6months (Sep 2023- Jan 2024)
- Research Area - Plant Biotechnology Center (Molecular analysis laboratory)
- Collected **17** *Bulbophyllum species* (from Balar farm and Plant Biotechnology Center, Mingalardone Township, Yangon Region)

နတ်သမီးသင်

ရှမ်းသင်ဝါ

Name	Scientific Name	Origin
သင်ညို (Thazin Nyo)	*Bulbophyllum sp.*	Taunggyi District (Hopone Township)
သင်လုံးဝါ (Thazin Lone War)	*Bulbophyllum elassonotum Summerh.*	Taunggyi District
နတ်သမီးသင် (Nat Thamee Thazin)	*Eriodes barbsts (Lindl) Rolfe*	Taunggyi District
သင်ယပ်တောင် (Thazin Yat Taung)	*Bulbophyllum andersonii (Hook.f) J.J. Smith*	Taunggyi District
သင်မွှေးရှာ (Thazin Mwae Shar)	*Bulbophyllum wendlandianum (Krzl) Dammer*	Taunggyi District
သင်ရိုးတုတ် (Thazin Yoe Toke)	*Bulbophullum sp.*	Taunggyi District
သင်ကြိယ် (Thazin Kyal)	*Bulbophyllum nastum*	Taunggyi District
ရှမ်းသင်မွေး (Shan Thazin Mhway)	*Bulbophyllum sp.*	Taunggyi District
သင်နီ (Thazin Ni)	*Bulbophyllum triste Rchb.f*	Southern Shan State
ရှမ်းသင်ဝါ (Shan Thazin War)	*Bulbophyllum suavissimum Rolfe.*	Southern Shan State
သင်နက် (Thazin Nat)	*Bulbophyllum angusteelipticum Sedient*	Southern Shan State
သင်လုံးအဖြူ (Thazin Lone Aphyu)	*Bulbophullum sp.*	Shan State
သင်လုံးခရမ်း (Thazin Lone Khayan)	*Bulbophyllum lilacinum Ridl.1897*	Shan State
တနိုင်းသင် (Ta Naig Thazin)	*Bulbophyllum hirtum (J.E.Sm) Lindl*	Ta Naig Region of Kachin State and Shan State
ကရင်သင် (Kayin Thazin)	*Bulbophullum sp.*	Kayin State
ထားဝယ်သင် (Dawe Thazin)	*Bulbophullum sp.*	Tanintharyi Region
ရခိုင်သင် (Yakhine Thazin)	*Bulbophyllum auricomun Lindl*	Yakhine State, Tanintharyi Region, Mon State

သင်နက်

သင်လုံးညို

Source:Wild orchids of Myanmar Second and Expanded Edition Nyan Tun (Mr. Myanmar) Taunggyi (S.S.S)

Molecular Characterization
&
Morphological Characterization

Materials and Methods

Molecular characterization

- DNA extraction (2.5 % CTAB buffer (Doyle and Doyle, 1987))
- PCR Analyses (12 primers) and Gel electrophoresis (1.5% agarose)
- LAB image software
- NTSYS version 2.1



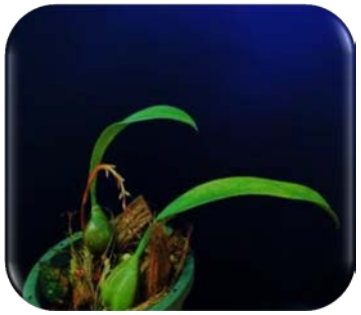


Materials and Methods

Morphological characterization (Vegetative State)

13 Qualitative Traits	
Plant size	Distance between bulb
No. of leaf per pseudobulb	Pseudobulb shape
Leaf apex	Pseudobulb color
Leaf thickness	Pseudobulb appearance
Leaf size	Pseudobulb hair
Leaf color	Pseudobulb length
Covering sheath on pseudobulb	



- **3 Quantitative** traits (leaf length, leaf width, rhizome length)
- SPSS version 25.0

Results and Discussion of Molecular Characterization

Results and Discussion of Molecular Characterization

Table 1: Polymorphism and power of discrimination for RAPD markers

No.	RAPD primers	Size range (bp)	Total no. of band	Total no.of polymorphic band	% Polymorphism	PIC
1	OPA-1	215-1091	11	11	100%	0.88
2	OPA-2	234-2306	10	10	100%	0.88
3	OPA-4	570-1305	17	17	100%	0.94
4	OPA-7	460-2230	12	12	100%	0.90
5	OPA-8	280-1131	17	17	100%	0.93
6	OPA-9	373-1687	18	18	100%	0.94
7	OPA-10	284-1432	22	22	100%	0.94
8	OPA-11	460-2402	20	20	100%	0.94
9	OPA-12	317-1606	17	17	100%	0.93
10	OPA-13	213-1682	16	16	100%	0.91
11	OPA-14	439-1432	11	11	100%	0.90
12	OPA-15	221-1051	12	12	100%	0.91
Total			183	183		

bp=based pairs, PIC=polymorphic information content

- No. of amplified bands higher than other study (Myo MMT et.,al;2011)
- PIC value >50% very informative (Botstein et.al; 1980)

Dendrogram of 17 *Bulbophyllum* species

Thazin Nyo (သင်ညို)

Kayin Thazin(ကရင်သင်)

Dawe Thazin (ထားဝယ်သင်)

Shan Thazin Mhwe (ရှမ်းသင်မွေး)

Thazin Lone War(သင်လုံးဝါ)

Thazin Yoe Toke(သင်ရိုးတုတ်)

Nat Thamee Thazin(နတ်သမီးသင်)

Shan Thazin War (ရှမ်းသင်ဝါ)

Yakhine Thazin(ရခိုင်သင်)

Thazin Yat Taung(သင်ယပ်တောင်)

Thazin Kyal (သင်ကြယ်)

Thazin Mwe Shar(သင်မွှေးလျှာ)

Thazin Nat(သင်နက်)

Ta Naig Thazin (တနိုင်းသင်)

Thazin Ni(သင်နီ)

Thazin Lone Phyu(သင်လုံးဖြူ)

Thazin Lone Kayan(သင်လုံးခရမ်း)

I

II

III

IV

0.01 0.12 0.23 0.34 0.44

Coefficient

Genetic Similarity indices of 17 *Bulbophyllum* spp. using RAPD markers

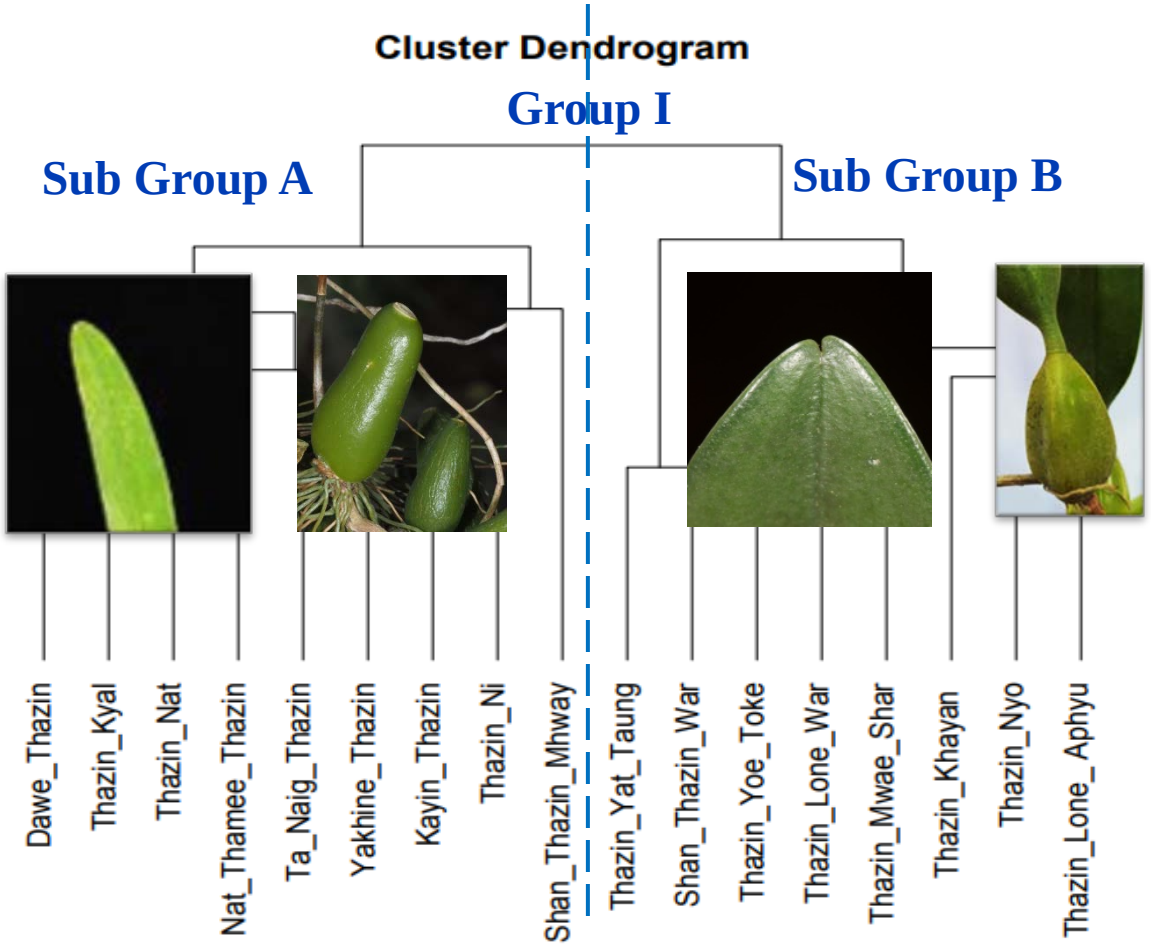
ကရင်သင်္ဃ

ထားဝယ်သင်္ဃ

	Thazin Ni	Shan Thazin War	Yakhine Thazin	Kayin Thazin	Dawe Thazin,	Shan Thazin Mhway	Thazin Nat	Tanig Thazin	Thazin Lone Aphyu	Thazin Lone Khayan
Thazin Ni	0.00	0.00	0.00	0.00	0.00	0.07	0.00	1.00		
Shan Thazin War	0.02	0.06	0.18	0.06	0.07	0.00	0.00	0.00	1.00	
Yakhine Thazin	0.13	0.00	0.05	0.00	0.08	0.00	0.00	0.00	0.07	1.00
. Kayin Thazin	0.40	0.12	0.00	0.06	0.08	0.13	0.00	0.08	0.14	0.00
Dawe Thazin	0.26	0.11	0.00	0.00	0.00	0.06	0.10	0.00	0.14	0.00
Shan Thazin Mhway	0.18	0.05	0.04	0.11	0.00	0.06	0.00	0.07	0.00	0.00
. Thazin Nat	0.06	0.00	0.04	0.11	0.14	0.06	0.00	0.07	0.00	0.00
Tanig Thazin	0.11	0.10	0.00	0.05	0.06	0.00	0.08	0.00	0.11	0.00
Thazin Lone Aphyu	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thazin Lone Khayan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00

Results and Discussion of Morphological Characterization

Results and Discussion of Morphological Characterization



Dendrogram of 17 *Bulbophyllum* varieties using

13 qualitative morphological characters (Vegetative State)

13 Qualitative Traits
Plant size
No. of leaf per pseudobulb
Leaf apex
Leaf thickness
Leaf size
Leaf color
Covering sheath on pseudobulb
Distance between bulb
Pseudobulb shape
Pseudobulb color
Pseudobulb appearance
Pseudobulb hair
Pseudobulb length

Graphical representation of quantitative traits between different varieties of *Bulbophyllum* spp.

Myanmar Name	Leaf length (cm)	Leaf width (cm)	Rhizome length (cm)
Thazin Lone Nyo	bc	cdef	a
Thazin Lone War	a	a	bcde
Nat Thamee Thazin	bcd	g	e
Thazin Yat Taung	bcd	bcde	abcde
Thazin Mwae Shar	bcd	bcd	ab
Thazin Yoe Toke	bcd	cdefg	ab
Thazin Kyal	de	bcde	bcde
Thazin Ni	f	g	cde
Shan Thazin War	b	ab	abcd
Yakhine Thazin	bcd	cdefg	bcd
Kayin Thazin	cde	cdefg	de
Dawe Thazin	cde	cdefg	bcde
Shan Thazin Mhway	ef	fg	abc
Thazin Nat	cde	efg	bcde
Ta Naig Thazin	de	bcde	de
Thazin Lone Aphyu	bcd	cdefg	de
Thazin Lone Khayan	b	bc	a

*a-h: data bearing identical letter within each column are not significantly different (P < 0.05) by Tukey HSD Test

Results and Discussion of Morphological Characterization

Table : Analysis of variance for three measured traits from 17 *Bulbophyllum* spp.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaf length (cm)	Between Groups	4040.884	16	252.555	65.243	.***
	Within Groups	131.613	34	3.871		
	Total	4172.497	50			
Leaf width (cm)	Between Groups	36.374	16	2.273	21.501	***
	Within Groups	3.595	34	0.106		
	Total	39.969	50			
Rhizome length (cm)	Between Groups	45.367	16	2.835	8.561	***
	Within Groups	11.262	34	0.331		
	Total	56.629	50			

***=p<0.001

Name	Flowering Time											
	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
သင်ညို (Thazin Nyo)	■					■						
သင်လုံးဝါ (Thazin Lone War)	■										■	■
နတ်သမီးသင် (Nat Thamee Thazin)											■	■
သင်ယပ်တောင် (Thazin Yat Taung)	■	■									■	■
သင်မွေ့လျှာ (Thazin Mwae Shar)				■								
သင်ရိုးတုတ် (Thazin Yoe Toke)											■	
သင်ကြယ် (Thazin Kyal)	■		■			■						
ရှမ်းသင်မွေး (Shan Thazin Mhway)										■		
သင်နီ (Thazin Ni)	■	■	■									
ရှမ်းသင်ဝါ (Shan Thazin War)	■											
သင်နက် (Thazin Nat)					■							
တနိုင်းသင် (Ta Naig Thazin)	■										■	■
ကရင်သင် (Kayin Thazin)	■											■
ထားဝယ်သင် (Dawe Thazin)	■											■
ရခိုင်သင် (Yakhine Thazin)	■										■	■
သင်လုံးအဖြူ (Thazin Lone Aphyu)												■
သင်လုံးခရမ်း (Thazin lone Khayan)												■

သင်လုံးဝါ

သင်လုံးညို

ရှမ်းသင်ဝါ

သင်မွေ့လျှာ

တနိုင်းသင်

ရခိုင်သင်

သင်နက်

သင်ယပ်တောင်

သင်နီ

သင်လုံးခရမ်း

Source:Wild orchids of Myanmar Second and Expanded Edition Nyan Tun (Mr. Myanmar) Taunggyi (S.S.S)

Conclusion and Suggestion

- All Seventeen *Bulbophyllum species* were genetically distance from each other.
- Most of the *Bulbophyllum species* were morphologically similar (qualitative characters) except for three measured traits such as leaf length, leaf width and rhizome length.
- The RAPD markers showed genetic variability in the studied *Bulbophyllum species* and they are powerful tools for estimating molecular diversity of the genus *Bulbophyllum*.
- Extensive research on morphological and molecular characterization of more *Bulbophyllum species* from different regions is necessary to be able to find out more genetic information in detail.

References

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