



GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF ABACA (*Musa textilis* Nee) USING MICROSATELLITE MARKERS

Orlex B. Yllano^{1,2,3}, Antonio G. Lalusin¹, Sheryl D. Castro¹, Antonio C. Laurena^{1,2} and Evelyn Mae T. Mendoza^{1,2}

¹Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, University of the Philippines, Los Banos

²Molecular Biology and Biotechnology Program, University of the Philippines, Los Banos, Laguna, Philippines

³Biology Department, CAST, Adventist University of the Philippines, Puting Kahoy, Silang, Cavite, Philippines

Abaca or Manila hemp (*Musa textilis* Nee)

- An indigenous species extensively cultivated in the Philippines
- Indo-Malay region was determined to be the primary center of origin of the genus *Musa*
- The presence of related species of *Australimusa* section in the area makes the Philippines the center of great diversity of the genus



(Chakravorti, 1951; Chandraratna, 1953; Halos, 2008)

From the Philippines, abaca was introduced to the following areas:



Map from CIA World Factbook

(Buchanan, Copeland 1876; 1911; Corpuz, 1997; Dempsey, 1963; Spencer, 1953)

Abaca Contribution to the Philippine Economy

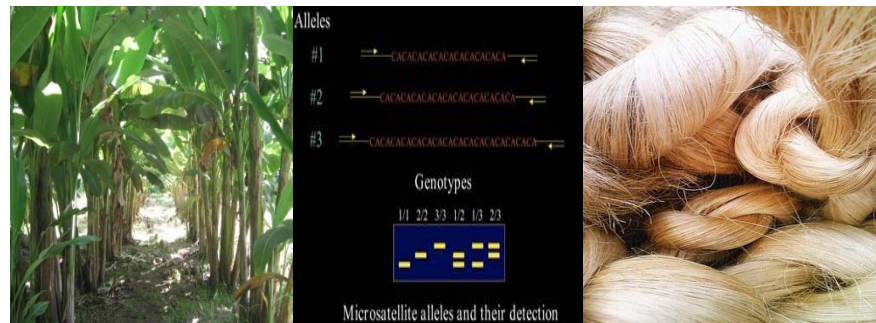
- Is one of the most economically important crops for fiber production in the Philippines
- The Philippines supplies about 84% of the current world abaca requirement (2002)
- It amounts to 80 M USD per year for local and international markets (1996-2000)



(PAI, 2003; SW, 2006; Ecofriend.org, 2008)

Abaca Paradox

- Even though Abaca is the most economically important crop for fiber production,
 - Abaca is still under researched



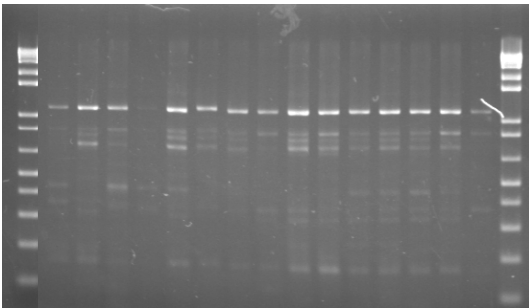
Challenges?

Insufficient knowledge on:

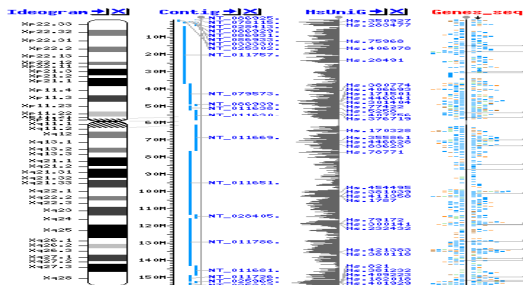
Biodiversity



Genetic variability



Genome organization



Marker Systems

Morphological markers

Isozymes

RAPD

AFLP

STS

RGA

SNP

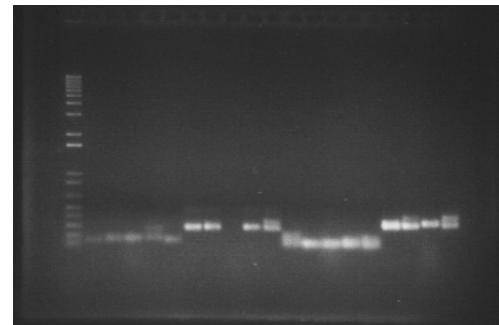
RFLP

SNP

SSR*

Microsatellites

- Robust genetic markers
- PCR-based
- Inherited in a Mendelian model
- Codominant
- Hypervariable



(Powell et al., 1986; Saghai-Marroof et al., 1994)

Microsatellite Repeats

Repeat units

AAAAAAAAAAAA	= (A) ₁₁	= mononucleotide (11bp)
GTGTGTGTGTGT	= (GT) ₆	= dinucleotide (12bp)
CTGCTGCTGCTG	= (CTG) ₄	= trinucleotide (12bp)
ACTCACTCACTCACTC	= (ACTC) ₄	= tetranucleotide (16 bp)

Microsatellite

b) Homozygous microsatellite

...CGTAGCCTTGCATCCTTCTCTCTCTCTCTCTATCGGTACTACGTGG...(46 bp)
...CGTAGCCTTGCATCCTTCTCTCTCTCTCTCTATCGGTACTACGTGG...(46 bp)

5' flanking region

microsatellite locus

3' flanking region

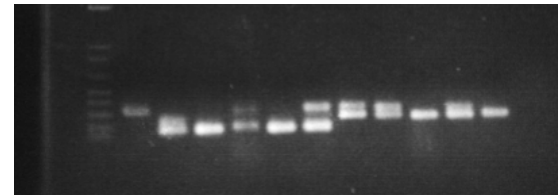
c) Heterozygous microsatellite

...CGTAGCCTTGCATCCTTCTCTCTCTCTCTCT ATCGGTACTACGTGG...(46 bp)
...CGTAGCCTTGCATCCTTCTCTCTCTCTCTCTCTCTATCGGTACTACGTGG.. (50 bp)

5' flanking region

microsatellite locus

3' flanking region



Applications of SSRs

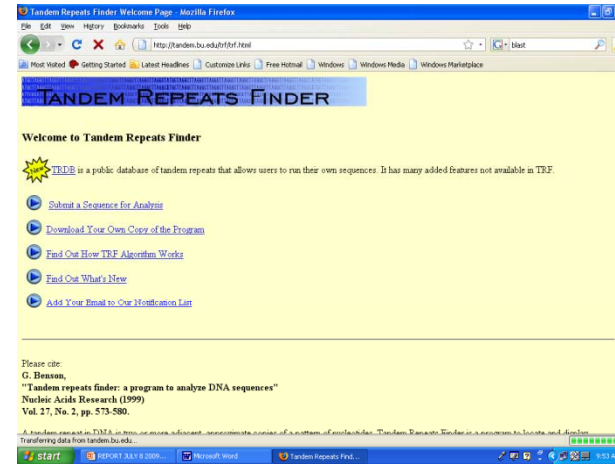
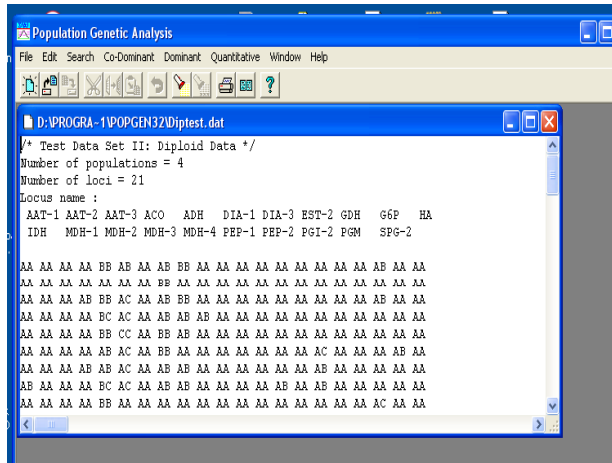
- **genetic fingerprinting and diversity analysis**
- **intra- or interpopulation structure analysis**
- **positional cloning; map-based cloning**
- **QTL mapping**
- **gene tagging**
- **characterization of transformants**
- **hybridity testing**
- **germplasm characterization and identification of proprietary**
- **analysis of herbarium samples**
- **estimation of genome size**
- **forensic analysis**
- **molecular systematics**
- **diagnostics**

(Tautz, 1989; Powell et al., 1996, de Vienne, 2003)

Objectives of the Study

- To assess the genetic diversity of abaca;
- To determine the phylogenetic relationships of different abaca accessions in Luzon, Visayas and Mindanao, Philippines.

Analysis



Current multiple alignments: none

BLAST Assembled Genomes

Choose a species genome to search, or [list all genomic BLAST databases](#).

- Human
- Mouse
- Rat
- Arabidopsis thaliana*
- Oryza sativa*
- Bos taurus*
- Danio rerio*
- Drosophila melanogaster*
- Gallus gallus*
- Pan troglodytes*
- Microbes*
- Apis mellifera*

Basic BLAST

Choose a BLAST program to run.

- nucleotide blast** Search a **nucleotide** database using a **nucleotide** query
Algorithms: blastn, megablast, discontinuous megablast
- protein blast** Search **protein** database using a **protein** query
Algorithms: blastp, psi-blast, phi-blast
- tblastx** Search **protein** database using a **translated nucleotide** query
- tblastn** Search **translated nucleotide** database using a **protein** query
- tblastx** Search **translated nucleotide** database using a **translated nucleotide** query

Primer3 (v. 0.4.0) Pick primers from a DNA sequence.

[Primer3plus interface](#) [More primer/oligo tools](#) [disclaimer](#) [Primer3 Home](#)
[Old \(0.3.0\) interface](#) [cautions](#) [FAQ/Wiki](#)



Results

Genetic Diversity

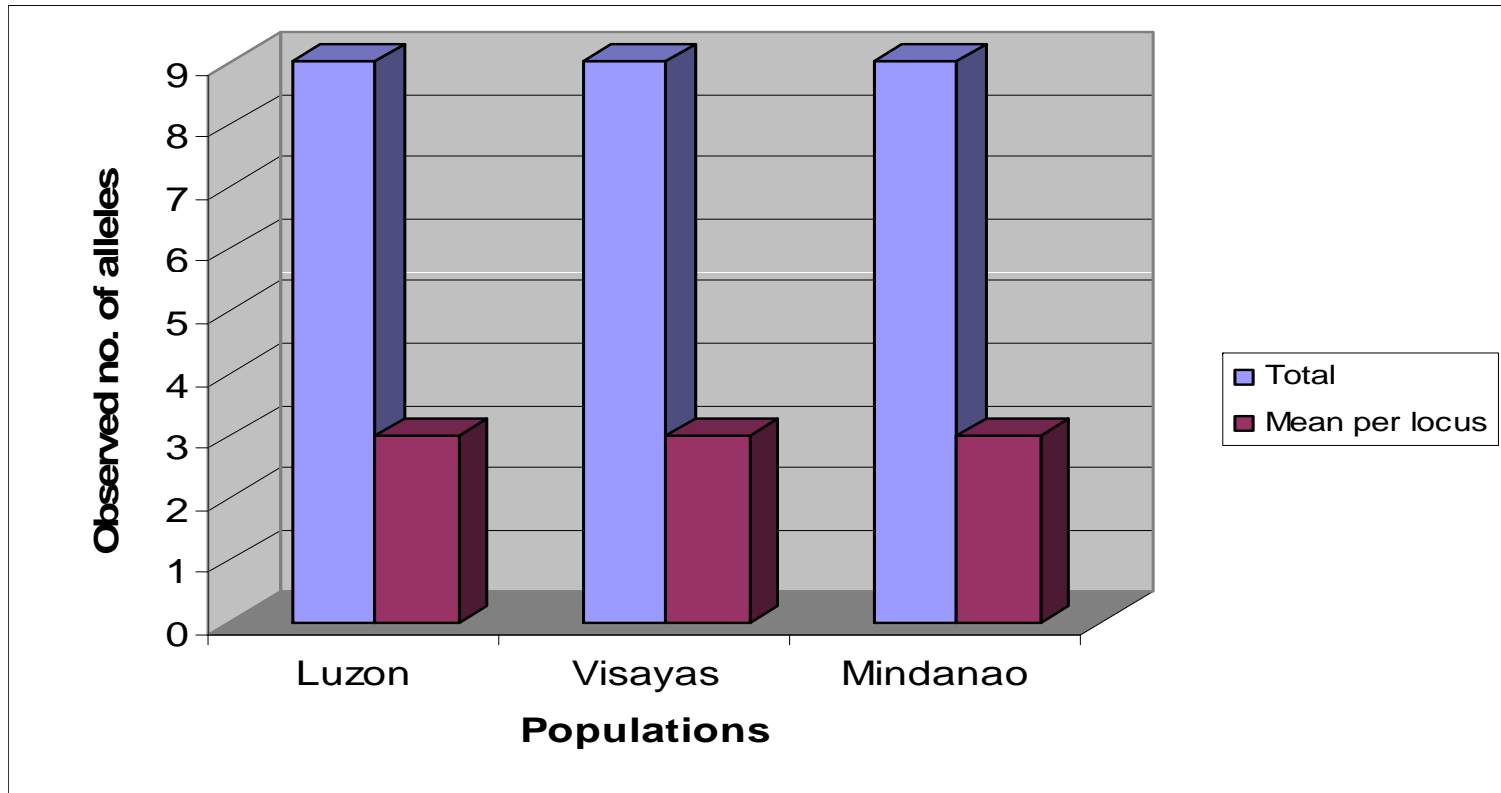


Figure 2. The observed number of alleles in different populations.

Genetic Diversity

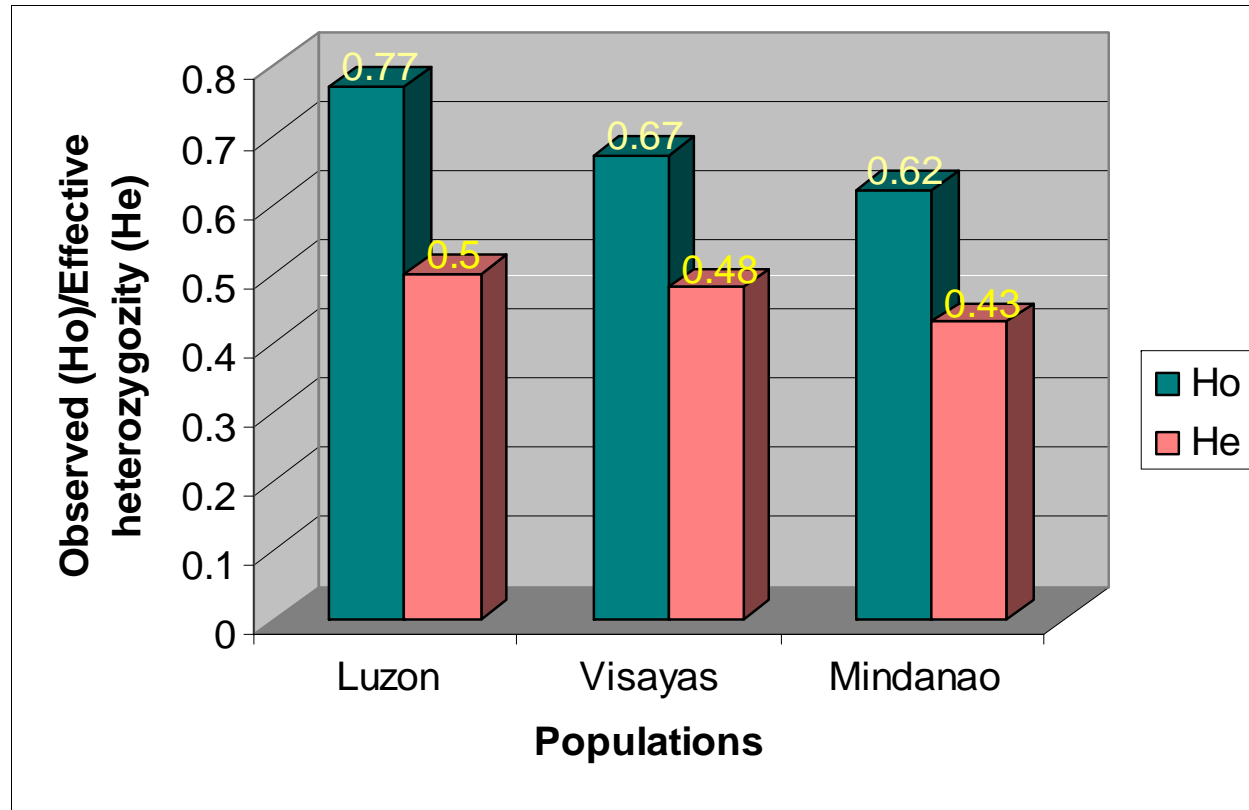


Figure 3. The observed heterozygosity and effective heterozygosity.

Table 1. Test for Hardy-Weinberg Equilibrium (Chi-Square)

Populations	Loci	Chi-Square P-value
Luzon	MOSAT 39	0.0064*
	MOSAT 40	0.0001*
	MOSAT 45	0.0001*
Visayas	MOSAT 39	0.0006*
	MOSAT 40	0.0001*
	MOSAT 45	0.0014*
Mindanao	MOSAT 39	1.0000
	MOSAT 40	0.0985
	MOSAT 45	0.1910

*value lower than 0.05

Genetic Diversity

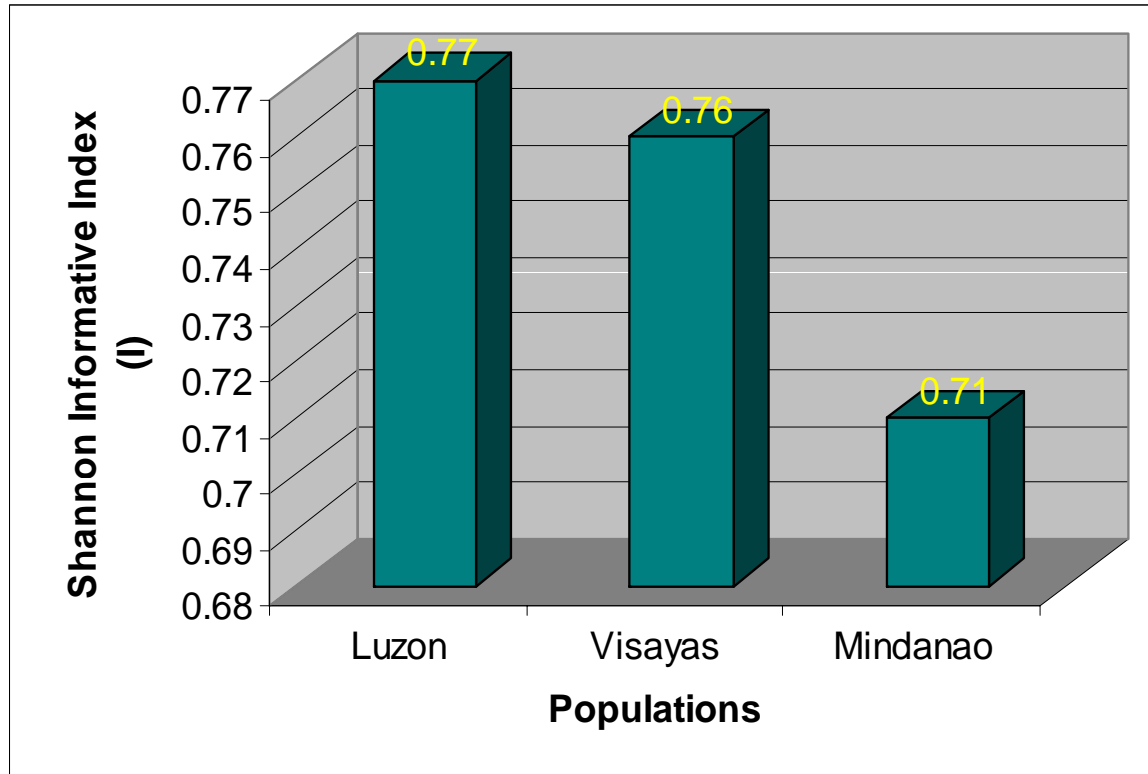


Figure 4. The Shannon Diversity Index of abaca in Luzon, Visayas and Mindanao.

Fixation Index

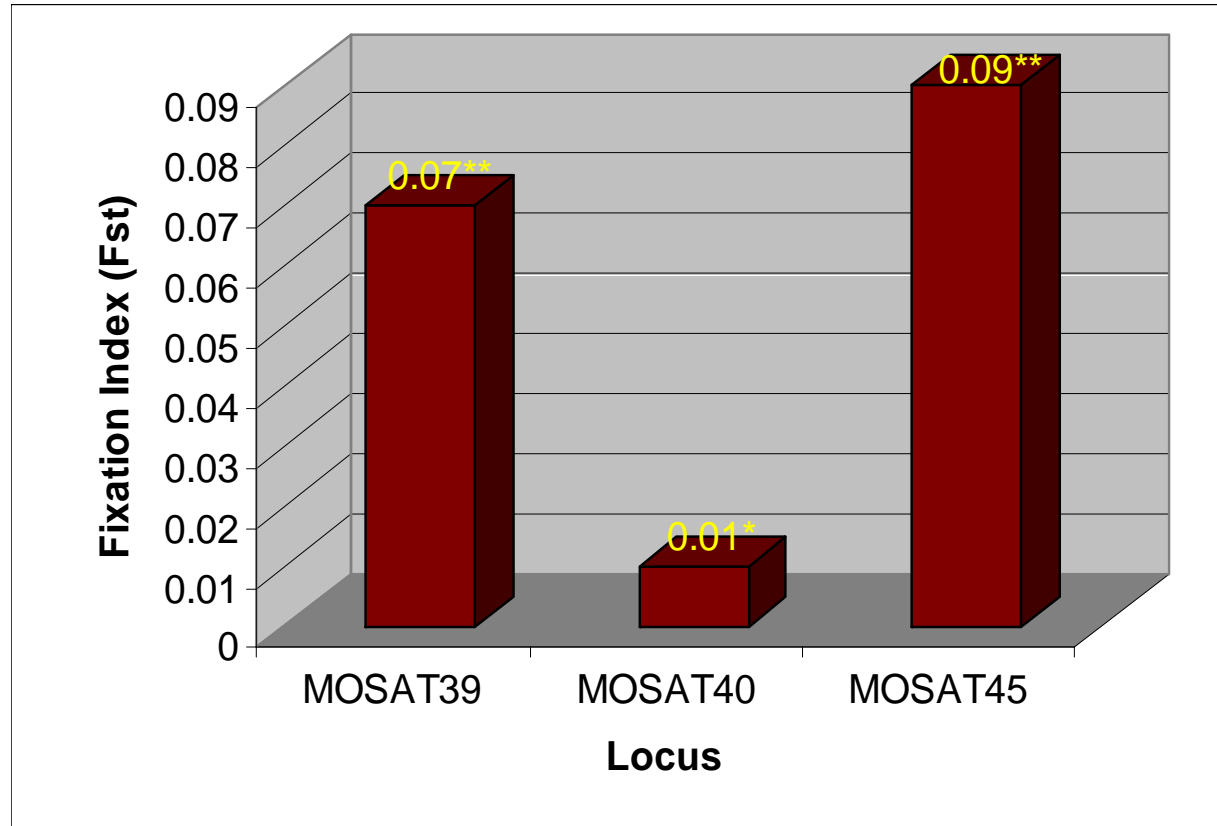


Figure 5. The Fixation Index among loci.

[**Moderate genetic differentiation (0.05-0.15); *Little genetic differentiation (0-0.15)]

Phylogenetic Relationships of Different Abaca Accessions in the Philippines

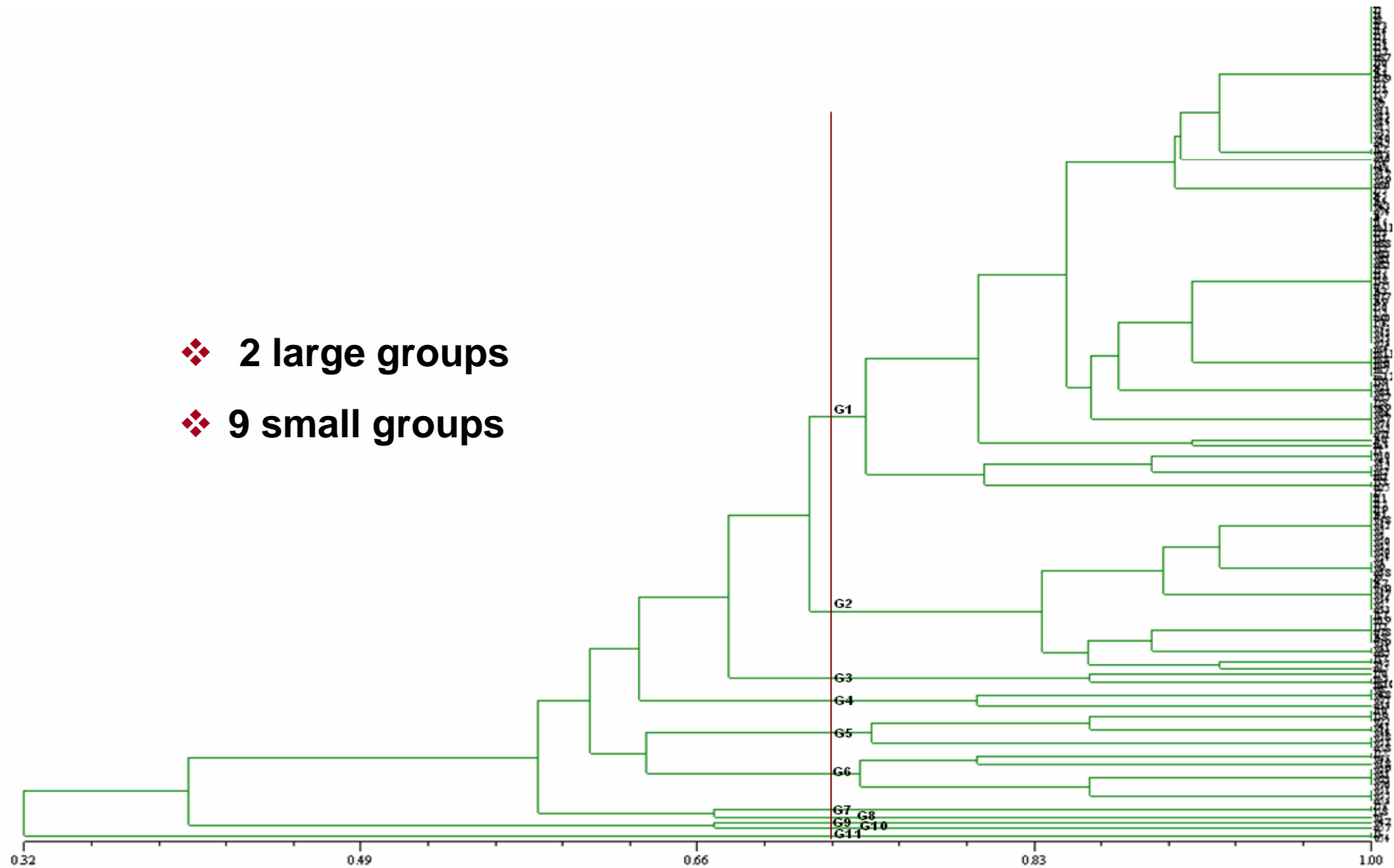
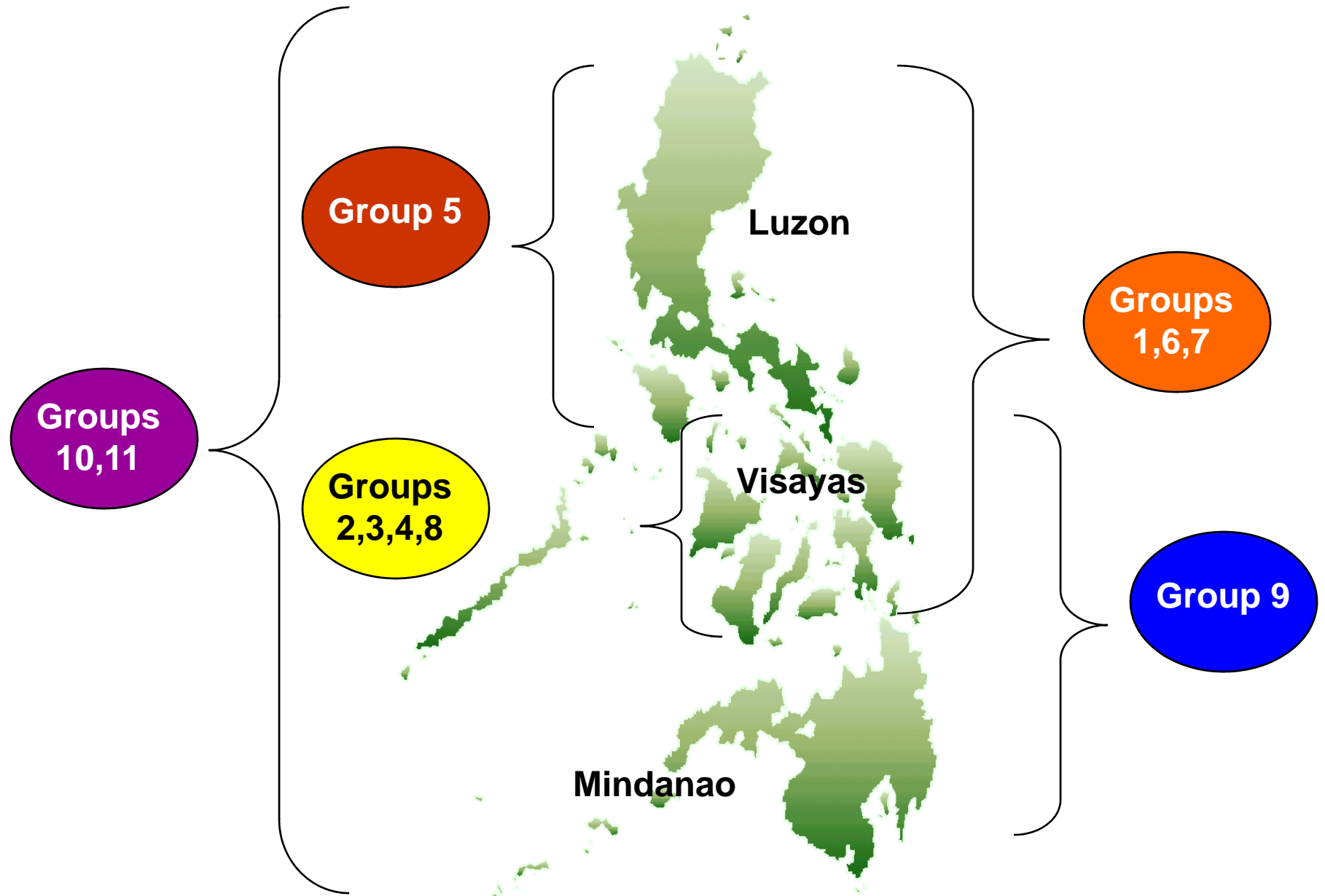


Figure 6. Dendrogram (UPGMA) of 158 *M. textilis* from Luzon, Visayas and Mindanao using Nei's (1978) unbiased genetic identity.

Distribution of abaca accessions



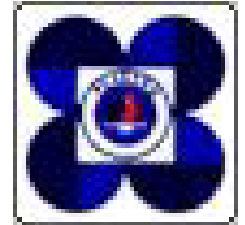
Summary, Recommendation and Future Directions

- **Abaca populations in Luzon, Visayas and Mindanao were considerably diverse with Shannon diversity index of 0.75;**
- **Among the three populations, Luzon had the highest genetic diversity (0.77), followed by Visayas (0.76) and Mindanao (0.71) population;**
- **Phylogenetic grouping using Nei's unbiased genetic identity (UPGMA) revealed 11 groups consisting of 2 large groups and 9 small groups;**
- **Low to moderate genetic differentiation were noted among the loci.**
- **Additional molecular markers are currently screened for marker- assisted breeding of abaca in the Philippines.**

Acknowledgement



- Institute of Plant Breeding (IPB), CA, UPLB
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- Japan Society for the Promotion of Science (JSPS)
- Nagoya University



arigato gozaimasu!

Thank you very much!