

# Verification of genetic integrity and diversity of Nigerian cacao collection through SNP: providing guide to proper cacao field genebank management

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# **BACKGROUND**

- **Cacao is an outcrossing species and its germplasm is conserved in clonal form (on field or in-vitro)**
- **Variety of mislabeled individuals have been estimated at 15 to 44% in global cacao germplasm collection (Motilal and Butler, 2003; Motilal, 2004; Sounigo et al., 2006, Takrama et al., 2005)**
- **The occurrence of mislabeling and offtype in Nigeria cacao field genebank was first reported by Aikpokpodion et al. (2010)**

- **Mislabeleding is one of the key factors contributing to high rate of unproductive progenies produced in the seed garden**
- **It also complicate breeders efforts fidelity assurance and reliable hybrid pods production in the seed gardens (Padi et al., 2015)**
- **Genetic identity of most hybrids generated become untraceable**
- **Availability of off-types affect the accuracy of heritability estimation for black pod disease resistance in cocoa (Adomako, 2006)**

# Objectives

- **To reveal the level of mislabeling in Nigeria cacao field genebank**
- **To verify the true genetic identity of the breeders' active clone collection**
- **To estimate the genetic diversity of Nigerian cacao germplasm**
- **To correct initial mislabeling in the affected clonal plots**

- **To ascertain uniformity within genotypes for their explicit utility**
- **To arrive at securing true basis for expanding Nigeria seed garden**

# Methodology

## Plant Materials

- Leaf samples collected from 1457 trees across 7 plots of Nigeria field genebank

## DNA extraction, quantification and SNP genotyping

- DNA Extraction using: - Modified CTAB
- SNP Genotyping using: - KASP™ assays from LGC genomics

## **Data Analysis:**

- 1. Likelihood-based method implemented in the program CERVUS 3.0 was used for parentage analysis (for clones without reference but with recorded pedigree)**
- 2. Bayesian clustering analysis (for clones without reference nor pedigree information). Model-based assignment test was applied to infer their hidden genetic membership to a known genetic group by STRUCTURE software program**
- 3. Reference profiles of trees maintained in the International Cacao Genebanks (Trinidad & Costa Rica) were used for multilocus matching by GenAlEx 6.2**

# Results

**Table 1: Mislabeling / offtype observed in Nigeria field genebank**

Clone	No of trees sampled	Labeling error range (%)	
		Breeders' active clone collection	Seed garden plots
International clones	462	78	-
C clones	529	0 - 50	0 - 100
T clones	460	10 -100	10 - 100



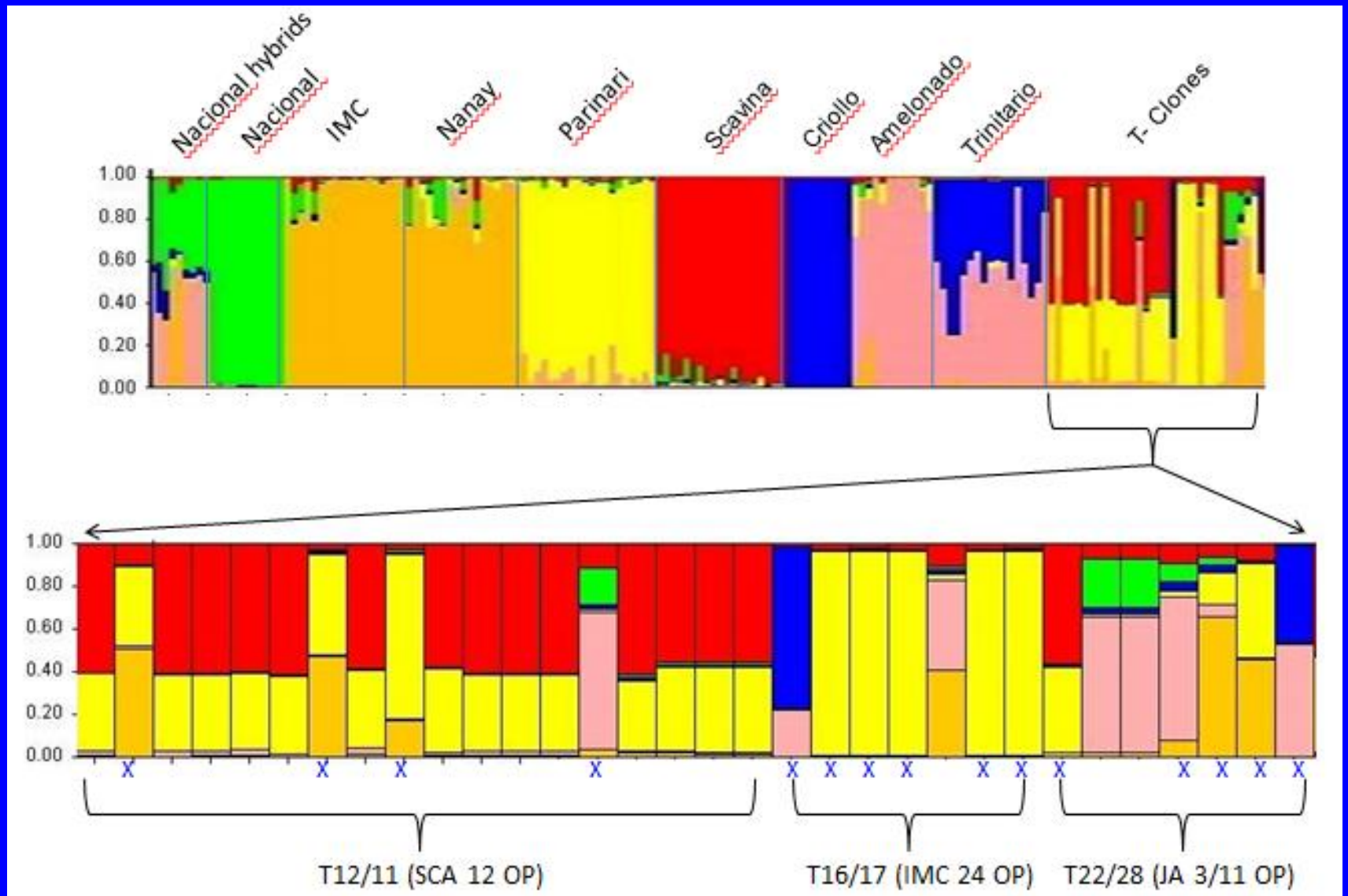
Name	Tree label/ID	Judgement	Genotype	Tc242	Tc372	Tc448	Tc529	Tc560	Tc591	Tc619	Tc645	Tc723	Tc731	Tc823	Tc872	Tc878	Tc998	Tc1442	Tc1458	Tc1484
SCA6	SCA 6 (MCB)	Reference	Malaysia	CT		GG	AA	GG		TT		GG	AA	CC	CC	CG	AA	CT	CC	AG
SCA6	SCA 6 (Marper_D644)	Reference	Trinidad	CT	AA		AA	GG	CC	TT	AG	GG			CC	CG	AA	CT	CC	AG
SCA 6	SCA6_NIG383	Correct	SCA6	CT	AA	GG	AA	GG	CC	TT	AA	GG	AA	CC	CC	CG	AA	CT	CC	AG
SCA 6	SCA6_NIG388	Correct	SCA6	CT	AA	GG	AA	GG	CC	TT	AA	GG	AA	CC	CC	CG	AA	CT	CC	AG
SCA 6	SCA6_NIG458	Correct	SCA6	CT	AA	GG	AA	GG	CC	TT	AA	GG	AA	CC	CC	CG	AA	CT	CC	AG
SCA 6	SCA6_NIG640	Correct	SCA6	CT	AA	GG	AA	GG	CC	TT	AA	GG	AA	CC	CC	CG	AA	CT	CC	AG
SCA 6	SCA6_NIG1834	Offtype	SCA6	TT	AA	CC	CC	TT	AA	CC	AA	GG	GG	AA	GG	CC	AA	CT	CC	AA
SCA 6	SCA6_NIG196	Offtype	SCA6	CT	TT	CG	AC	TT	AC	TT	GG	GT	AG	CC	GG	CG	AA	TT	CG	AG
SCA 6	SCA6_NIG1870	Offtype	SCA6	CT	00	CG	AC	GT	00	TT	AA	GG	AG	00	00	CG	AG	00	00	AG
SCA 6	SCA6_NIG1882	Offtype	SCA6	CC	AA	CC	CC	GT	AA	CC	AA	GT	AG	AA	CG	CG	AG	CC	GG	AG
P7	P 7 (MCB)	Reference	Malaysia	CT		CC	CC	GT		CT		GG	GG	AC	CC	CC	AG	TT	CC	AA
P7	POUND 7 (6B_T4_F410)	Reference	Trinidad	CT	AA		CC	GT	AA	CT	AA	GG			CC	CC	AG	TT	CC	AA
P7	POUND 7 (CRIG, Ghana)	Reference	Ghana	CT	AA	CC		GT	AA	CT	AA	GG	GG	AC	CC	CC	AG	TT	CC	AA
P7	P7_NIG1534	Correct	P7	CT	AA	CC	CC	GT	AA	CT	AA	GG	GG	AC	CC	CC	AG	TT	CC	AA
P7	P7_NIG1552	Correct	P7	CT	AA	CC	CC	GT	AA	CT	AA	GG	GG	AC	CC	CC	AG	TT	CC	AA
P7	P7_NIG1561	Correct	P7	CT	AA	CC	CC	GT	AA	CT	AA	GG	GG	AC	CC	CC	AG	TT	CC	AA
P7	P7_NIG1567	Correct	P7	CT	AA	CC	CC	GT	AA	CT	AA	GG	GG	AC	CC	CC	AG	TT	CC	AA
P7	P7_NIG864	Offtype	P7	CT	AA	CC	00	GT	AC	CT	00	GG	AG	AC	CG	CC	AG	TT	GG	AG
P7	P7_NIG872	Offtype	P7	CT	00	CC	AC	GT	00	CT	AG	GG	AG	AC	CG	CC	AG	CT	CG	00
P7	P7_NIG866	Offtype	P7	CT	AA	CC	AC	TT	CC	CT	AG	GG	AG	AC	CG	CC	AA	CT	CG	AA
P7	P7_NIG1358	Offtype	P7	00	00	00	CC	GT	AC	CC	00	00	GG	00	00	CC	AA	CT	00	AA
P7	P7_NIG861	Offtype	P7	CT	AT	CC	AC	GG	AA	TT	AG	GG	AG	AC	CG	CC	AG	TT	GG	AG

**Fig. 1: Mislabeling (offtype) identified in the international clone collection at CRIN, Nigeria base on 64 SNP markers (of which 17 loci were presented)**

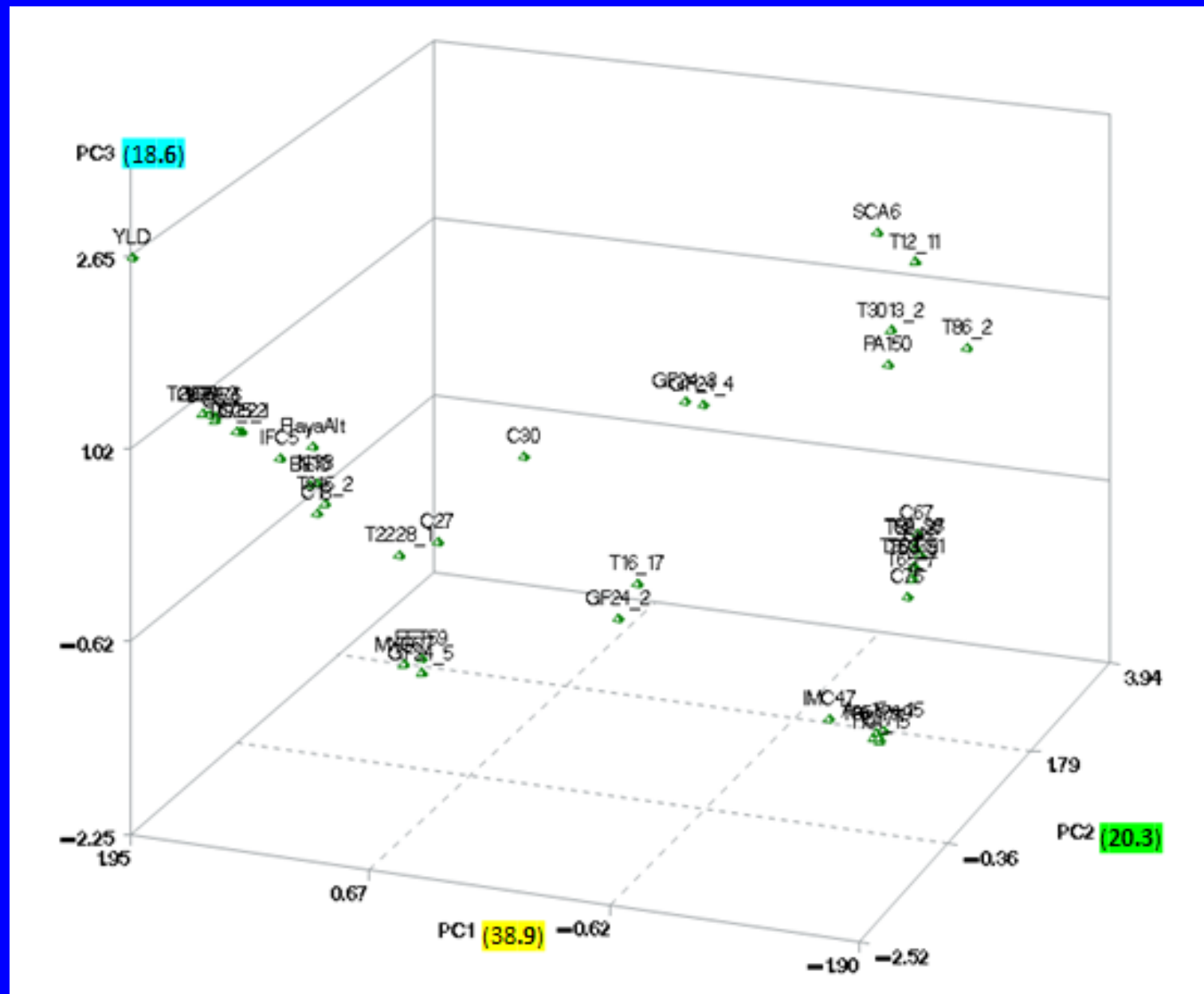
**Table 2: Assigned memberships (Q-value) of correctly identified genotypes of GF24 in Nigeria germplasm.**

<b>Sample ID</b>	<b>Scavina</b>	<b>IMC</b>	<b>Parinary</b>	<b>Nacio nal</b>	<b>Amelo nado</b>	<b>Criollo</b>	<b>Confirmed Pedigree</b>	<b>Identity</b>
<b>GF24_NIG 1843</b>	<b>0.005</b>	<b>0.011</b>	<b>0.005</b>	<b>0.004</b>	<b>0.489</b>	<b>0.486</b>	<b>Trinitario</b>	<b>GF24-Type1</b>
<b>GF24_NIG 421</b>	<b>0.007</b>	<b>0.014</b>	<b>0.006</b>	<b>0.003</b>	<b>0.732</b>	<b>0.238</b>	<b>Trinitario</b>	<b>GF24-Type1</b>
<b>GF24_NIG 538</b>	<b>0.006</b>	<b>0.533</b>	<b>0.013</b>	<b>0.004</b>	<b>0.428</b>	<b>0.016</b>	<b>Amelonad o x IMC</b>	<b>GF24-Type2</b>
<b>GF24_NIG 206</b>	<b>0.429</b>	<b>0.175</b>	<b>0.030</b>	<b>0.017</b>	<b>0.339</b>	<b>0.009</b>	<b>Amelonad o x SCA</b>	<b>GF24-Type3</b>
<b>GF24_NIG 561</b>	<b>0.014</b>	<b>0.014</b>	<b>0.578</b>	<b>0.005</b>	<b>0.388</b>	<b>0.002</b>	<b>Amelonad o x PA</b>	<b>GF24-Type4</b>
<b>GF24_NIG 551</b>	<b>0.012</b>	<b>0.010</b>	<b>0.586</b>	<b>0.005</b>	<b>0.386</b>	<b>0.002</b>	<b>Amelonad o x PA</b>	<b>GF24-Type4</b>
<b>GF24_NIG 1915</b>	<b>0.030</b>	<b>0.008</b>	<b>0.015</b>	<b>0.476</b>	<b>0.436</b>	<b>0.035</b>	<b>Nacional hybrid</b>	<b>GF24-Type5</b>

**K = 6**



**Fig. 2: An example of detecting off-type trees in three “T-clones” of cacao using assignment test.**



**Fig 3: PCA plot of breeders' cacao collection showing their genetic group as revealed by STRUCTURE**

# **Possible sources of errors**

- 1. Errors from the sources of introduction**
- 2. Pre-planting errors (technical / labeling)**
- 3. Post-planting errors (poor management)**
  - Rootstocks overtaking budded scions**
  - Failure to replace dead stands**

# Table 3: Correction plan for mislabeled and offtype trees

Plot / Location	Sample ID	Tree label in plot	Observed pedigree	Correct identity
International clone	GF24-NIG1843	GF 24	Amelonado OP	GF24-Type-1
	GF24-NIG421	GF 24	Amelonado OP	GF24-Type-1
	GF24-NIG533	GF 24	Amelonado OP	GF24-Type-2
	GF24-NIG206	GF 24	Amelonado OP	GF24-Type-3
	GF24-NIG561	GF 24	Amelonado OP	GF24-Type-4
	GF24-NIG551	GF 24	Amelonado OP	GF24-Type-4
	GF24-NIG1915	GF 24	Amelonado OP	GF24-Type-5
Local clone plot	T16/17-NIG328	T16/17	PA	T86/2
	T16/17-NIG329	T16/17	PA	T86/2
	T16/17-NIG340	T16/17	PA	T86/2
	T16/17-NIG314	T16/17	SCA OP	T12/11
	T16/17-NIG319	T16/17	SCA OP	T12/11
	T16/17-NIG360	T16/17	SCA OP	T12/11





**Plate 1: Classification of GF 24 clone into types based on assignment test**  
2016 Next Generation Cocoa Research Symposium



**Clone T86/2  
misabeled as  
T16/17**



# What Next?

- **Fingerprinting of remaining clones (90%) in Nigeria cacao germplasm**
- **Correction of mislabeled / offtype trees in the plots**
- **Need to look into the genetic potentials that may be inherent in the so called 'offtype clones'**
- **Need for support in the area of cacao germplasm maintenance (training, etc)**

# Acknowledgement

- WCF

***Thanks for your  
attention***