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Identification of Genetically Distinct Cassava Clones from On-Farm Plantations to Widen the Thai Cassava Breeding Gene Pool

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is one of the most important economic crops in Thailand. However, the Thai cassava breeding gene pool was genetically narrow with only 11 distinct landraces. An attempt was made here to characterize 266 cassava clones collected from 80 farms in eight provinces using 35 SSR markers. A total of 365 polymorphic alleles were detected in the assayed samples. The molecular analysis of variance revealed that a large SSR variance (19.8%) was present among the farm samples. The genetic relationships of the 266 farm samples revealed by the principal coordinate analysis confirmed the large SSR variation observed among the collected cassava samples. The average dissimilarity (AD) of a cassava sample against the other 265 samples was calculated and the AD values obtained ranged from 0.256 to 0.502 with a mean of 0.319. Based on these AD values, a set of 50 unique cassava samples with AD values of 0.346 or higher was assembled from the on-farm samples to widen the genetic base of the Thai cassava breeding gene pool.

Keywords: Cassava; Distinctness; SSR; *Ex Situ* Conservation; Core Collection

1. Introduction

Cassava (*Manihot esculenta* Crantz) is the fourth most important food crop in the tropics, and is still growing in importance both for food security (especially in Africa) and for multiple commercial and industrial uses (mainly in Latin America and Asia) [1,2]. It also is one of the most important economic crops in Thailand with 80% of the fresh root production of animal feed and starch exported to the European Union and Asian countries [3]. The Thai cassava sector was re-energized when it capitalized on the European market with opportunities for dried chips and pellets, beginning in the 1970s [4]. Last year, 3.3 million acres of cassava were planted and 27 million tons of fresh cassava root were produced across 50 Thai provinces [5,6].

Thai cassava breeding started in 1937 and has contributed to the success of cassava production with an introduction of 20 varieties from Malaysia and the Philippines in the 1930s and 65 varieties from the Columbia and Virgin Islands between 1963 and 1977 [7]. However, the Thai cassava production has greatly increased only after the release of the first Thai cultivar "Rayong 1" in 1975. "Rayong 1" was dominant in cassava production during the 1970s to 1990s and was replaced by "Kasetsart 50" released in 1992. So far, the Thai cassava breeding has officially released 13 bitter-type cassava cultivars [5,8]. However, the unique landrace cultivars hold *in situ* and *ex situ* in Thailand are 11 and 10, respectively, which are much fewer than in other Asian countries such as Vietnam, Malaysia, Indonesia and India [4]. Thus, the Thai cassava breeding gene pool is genetically narrow.

Cassava germplasm has been frequently characterized using many informative molecular markers such as simple sequence repeat (SSR) markers [9-11]. These characterizations revealed not only a high level of genetic diversity but also a strong genetic structure present in cassava germplasm (e.g., [9,12,13]). However, in Thailand little effort has been made to characterize cassava germplasm [14,15], particularly for those cassava clones growing on farms.

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We conducted a study to collect and characterize cassava clones from a large number of farms across Thailand in the hope of assembling a genetically distinct set of clones to widen the breeding gene pool. The specific objectives of this study were to assess the genetic diversity of 266 cassava clones collected from 80 farms in eight Thai provinces using 35 informative SSR markers and to identify the most genetically distinct clones for genetic improvement of cassava. This study was inspired by the core collection concept [16,17] to obtain a small representative subset of the germplasm collection and the average dissimilarity measure of individual plants [18] to identify genetic distinctness.

2. Materials and Methods

2.1. Plant Materials

The cassava samples studied here consisted of 266 out of the 400 clones collected from 80 farms in the cassava planting area ranging from 1.2 to 7.9 acres. The surveyed farms are located in 16 districts and eight provinces (**Figure 1** and **Table 1**). Specifically, 200, 50, 100 and 50 samples were collected from farms of 4, 1, 2, and 1 provinces representing major cassava planting areas in north-eastern, northern, eastern, and western Thailand, respectively. Nakhon Ratchasima and Kamphaeng Phet rank as the first and second largest planting areas of the country [5]. The farm collections were conducted from November 2011 to April 2012. The clone selection was made based on the phenotypic variation within each farm. Information on altitude and location of the farm (latitude, longitude) was also obtained. The collected stems were subsequently re-planted in Nong Lek Subdistrict, Kosum Phisai District, Maha Sarakham Province for further phenotypic and genetic characterizations.

2.2. DNA Extraction and SSR Analysis

The genomic DNA extraction was performed for 400 farm samples based on the young leaf tissue of each collected clone. The modified method of Tai and Tanksley [19] was applied with 100 mg of young leaf tissue collected. The tissue was first ground with a homogenizer, followed by adding 0.7 ml of extraction buffer (100 mM Tris-HCl; pH 8, 50 mM EDTA pH 8, 0.5 M NaCl, 1.25% SDS, 8.3 mM NaOH, 0.38% Na bisulfite) and then mixed by vortexing. The sample was incubated at 65˚C for 20 min and subsequently 0.22 ml of 5 M potassium acetate was added and mixed well. The tube was placed on ice for 40 min, followed by centrifugation for 3 min. The supernatant was transferred to a new tube. The DNA was precipitated by adding 0.7 volume of isopropanol, mixed well and centrifuged for 3 min. The supernatant was poured off and the pellet rinsed with 70% ethanol. The pellet was re-suspended in 300 µl of T5E (50 mM Tris-HCl pH 8, 10 mM EDTA) by briefly vortexing, and incubated at 65˚C for 5 min, followed by vortexing again. 150 µl of 7.4 M ammonium acetate were added and mixed well before centrifugation for 3 min and removal of the supernatant to the new tube. The DNA was precipitated by mixing with 330 µl of isopropanol and centrifuged for 3 min. The pellet was rinsed with 70% ethanol, air dried and re-suspended in 150 µL of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The purity and quality of genomic DNA were assessed after digestion with

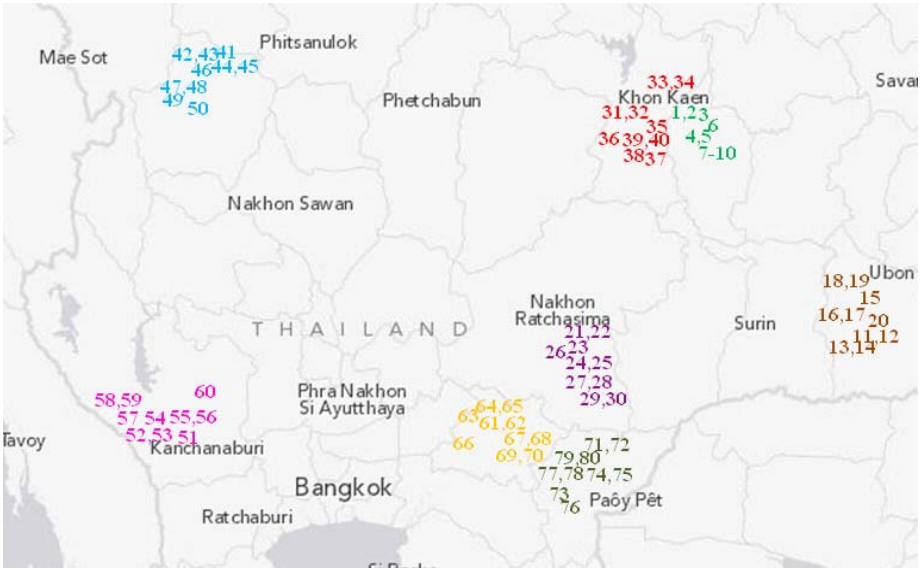

Figure 1. Relative location of the 80 studied farms in eight provinces in Thailand. A unique survey number (1 - 80) represents each farm listed in Table 1 and forms part of the farm name. The farms are colored for different provinces.

Table 1. List of 80 sampled farms in Thailand, their location information and our sample size.

Farm†	Province (label)	District (label)	Township	Lat†	Long†	Alt†	Size
MKN1	Maha Sarakham (1)	Kosum Phisai (1)	Nong Lek	1799879.0	280003.7	160	2
MKN2	Maha Sarakham (1)	Kosum Phisai (1)	Nong Lek	1801273.3	285095.9	153	3
MKH3	Maha Sarakham (1)	Kosum Phisai (1)	Hua Khwang	1800405.6	288947.9	156	4
MBB4	Maha Sarakham (1)	Borabue (2)	Bo Yai	1766248.9	288858.8	195	3
MBB5	Maha Sarakham (1)	Borabue (2)	Bo Yai	1764462.9	285926.9	187	3
MBB6	Maha Sarakham (1)	Borabue (2)	Bo Yai	1763201.9	282791.3	198	5
MWD7	Maha Sarakham (1)	Wapi Pathum (3)	Dong Yai	1757412.9	322126.6	155	3
MWD8	Maha Sarakham (1)	Wapi Pathum (3)	Dong Yai	1757446.5	321769.9	155	3
MWD9	Maha Sarakham (1)	Wapi Pathum (3)	Dong Yai	1757532.6	322544.1	155	4
MWD10	Maha Sarakham (1)	Wapi Pathum (3)	Dong Yai	1757811.1	322308.3	155	3
SKC11	Si Sa Ket (2)	Khukhan (4)	Chai Di	1620007.0	424981.9	154	3
SKC12	Si Sa Ket (2)	Khukhan (4)	Chai Di	1636347.8	408591.0	144	4
SKC13	Si Sa Ket (2)	Khukhan (4)	Chai Di	1636224.3	408740.0	143	4
SKC14	Si Sa Ket (2)	Khukhan (4)	Chai Di	1636772.9	409937.8	144	3
SKC14	Si Sa Ket (2)	Khukhan (4)	Chai Di	1636772.9	409937.8	144	5
SKK16	Si Sa Ket (2)	Khukhan (4)	Kanthararom	1630967.7	401514.0	150	4
SKK17	Si Sa Ket (2)	Khukhan (4)	Kanthararom	1632470.1	402297.5	150	3
SKK18	Si Sa Ket (2)	Khukhan (4)	Kanthararom	1632439.2	402357.2	149	3
SKK19	Si Sa Ket (2)	Khukhan (4)	Kanthararom	1631762.4	402563.8	149	4
SKK20	Si Sa Ket (2)	Khukhan (4)	Kanthararom	1631147.5	402651.0	151	4
NNN21	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Hua Raet	1622264.4	212443.3	227	2
NNN22	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Hua Raet	1621832.8	212528.1	228	2
NNN23	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Hua Raet	1620972.1	212488.0	227	2
NNN24	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Hua Raet	1620860.8	211498.5	223	4
NNN25	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Hua Raet	1623981.0	215547.4	225	3
NNN26	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Hua Raet	1624675.7	216633.3	226	2
NNN27	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Takai	1624624.5	226482.2	215	3
NNN28	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Takai	1624766.2	222113.1	216	3
NNN29	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Takai	1625335.5	220742.5	209	3
NNN30	Nakhon Ratch (3)	Nong Bun Mak (5)	Nong Takai	1625976.8	221139.0	221	5
KMT31	Khon Kaen (4)	Mancha Khiri (6)	Ta Sala	1800170.2	239551.7	189	3
KMT32	Khon Kaen (4)	Mancha Khiri (6)	Ta Sala	1800292.5	239612.5	185	4
KMT33	Khon Kaen (4)	Mancha Khiri (6)	Ta Sala	1800691.6	239676.7	184	3
KMT34	Khon Kaen (4)	Mancha Khiri (6)	Ta Sala	1801171.6	240692.4	188	3
KMN35	Khon Kaen (4)	Mancha Khiri (6)	Nong Paen	1791152.2	250737.2	164	3
KBB36	Khon Kaen (4)	Ban Phai (7)	Ban Phai	1781360.6	257374.9	168	3
KBB37	Khon Kaen (4)	Ban Phai (7)	Ban Phai	1781422.1	257375.6	167	3
KBB38	Khon Kaen (4)	Ban Phai (7)	Ban Phai	1781545.4	257347.2	165	3
KBB39	Khon Kaen (4)	Ban Phai (7)	Ban Phai	1781607.9	257258.7	166	3

Continued

KBB40	Khon Kaen (4)	Ban Phai (7)	Ban Phai	1781638.9	257229.3	166	5
KSM41	Kamphaeng Phet (5)	Sai Ngam (8)	Maha Chai	1826830.7	590379.8	55	3
KSM42	Kamphaeng Phet (5)	Sai Ngam (8)	Maha Chai	1827048.0	590882.9	55	4
KSM43	Kamphaeng Phet (5)	Sai Ngam (8)	Maha Chai	1827050.2	591416.5	55	3
KMN44	Kamphaeng Phet (5)	Mueang Kam (9)	NTPT	1820490.9	580265.1	60	5
KMT45	Kamphaeng Phet (5)	Mueang Kam (9)	Thep Nakhon	1812795.1	567360.6	68	4
KMS46	Kamphaeng Phet (5)	Mueang Kam (9)	Sa Kaeo	1822593.3	565906.9	70	4
KMS47	Kamphaeng Phet (5)	Mueang Kam (9)	Sa Kaeo	1822654.8	565936.4	70	3
KMS48	Kamphaeng Phet (5)	Mueang Kam (9)	Sa Kaeo	1822757.1	558820.4	74	3
KMS49	Kamphaeng Phet (5)	Mueang Kam (9)	Sa Kaeo	1822900.5	565876.3	69	4
KMS50	Kamphaeng Phet (5)	Mueang Kam (9)	Sa Kaeo	1822961.9	565876.2	70	4
KML51	Kanchanaburi (6)	Mueang Kan (10)	Lat Ya	1563924.7	544488.3	49	3
KML52	Kanchanaburi (6)	Mueang Kan (10)	Lat Ya	1564538.8	544277.3	45	3
KML53	Kanchanaburi (6)	Mueang Kan (10)	Lat Ya	1565521.9	544245.6	58	3
KML54	Kanchanaburi (6)	Mueang Kan (10)	Lat Ya	1566351.0	544064.3	69	3
KML55	Kanchanaburi (6)	Mueang Kan (10)	Lat Ya	1566780.9	543943.7	73	4
KSS56	Kanchanaburi (6)	Sai Yok (11)	Sing	1555480.6	525250.2	59	3
KSS57	Kanchanaburi (6)	Sai Yok (11)	Sing	1555541.9	525130.2	56	4
KSS58	Kanchanaburi (6)	Sai Yok (11)	Sing	1555910.4	525009.9	58	3
KSS59	Kanchanaburi (6)	Sai Yok (11)	Sing	1555633.4	524470.4	57	4
KSS60	Kanchanaburi (6)	Sai Yok (11)	Sing	1556893.3	524799.0	71	4
PPN61	Prachin Buri (7)	Prachantakham (12)	Nong Kaeo	1565767.5	773777.6	10	2
PPN62	Prachin Buri (7)	Prachantakham (12)	Nong Kaeo	1565500.5	774680.7	11	4
PPN63	Prachin Buri (7)	Prachantakham (12)	Nong Kaeo	1565987.9	774255.3	13	4
PPB64	Prachin Buri (7)	Prachantakham (12)	Ban Hoi	1568666.4	777346.7	23	3
PPB65	Prachin Buri (7)	Prachantakham (12)	Ban Hoi	1568900.1	779024.4	16	3
PKN66	Prachin Buri (7)	Kabin Buri (13)	Nonsi	1556049.5	784929.9	23	3
PKN67	Prachin Buri (7)	Kabin Buri (13)	Nonsi	1556559.7	786545.4	25	3
PKN68	Prachin Buri (7)	Kabin Buri (13)	Nonsi	1556206.6	787960.4	29	3
PKN69	Prachin Buri (7)	Kabin Buri (13)	Na Khaem	1553446.1	793996.8	20	4
PKN70	Prachin Buri (7)	Kabin Buri (13)	Na Khaem	1553180.8	794990.7	28	3
SWW71	Sa Kaeo (8)	Watthana Nakhon (14)	Watthana Nakhon	1520298.7	210886.3	72	3
SWW72	Sa Kaeo (8)	Watthana Nakhon (14)	Watthana Nakhon	1520174.7	210975.1	71	3
SWW73	Sa Kaeo (8)	Watthana Nakhon (14)	Watthana Nakhon	1520051.0	211033.9	71	3
SWW74	Sa Kaeo (8)	Wang Nam Yen (15)	Wang Nam Yen	1492127.2	194504.3	143	3
SWW75	Sa Kaeo (8)	Wang Nam Yen (15)	Wang Nam Yen	1491845.0	192303.7	90	3
SWW76	Sa Kaeo (8)	Wang Nam Yen (15)	Wang Nam Yen	1491444.5	192359.3	90	4
SKN77	Sa Kaeo (8)	Khao Chakan (16)	Nong Wa	1517748.5	181599.3	55	3
SKN78	Sa Kaeo (8)	Khao Chakan (16)	Nong Wa	1516584.9	181164.1	58	3
SKN79	Sa Kaeo (8)	Khao Chakan (16)	Nong Wa	1515969.0	181216.7	63	3
SKN80	Sa Kaeo (8)	Khao Chakan (16)	Nong Wa	1514953.7	181234.4	50	3

[†]The farm abbreviation is composed of the first letter of the province, district and township and the unique survey number. Lat = latitude (UTM). Long = longitude (UTM). Alt = altitude (m). Nakhon Ratch = Nakhon Ratchasima. Mueang Kam = Mueang Kamphaeng Phet. Mueang Kan = Mueang Kanchanaburi. NTPT = Nikhom Thung Pro Thale.

RNaseA (Sigma). Extracted DNA was quantified with a Thermo Scientific NanoDrop™ spectrometer (Fisher Scientific, USA) and agarose gel electrophoresis. The extracted genomic DNAs were stored at −20˚C until further use. Two independent DNA isolations were done for each sample.

Twenty-four genomic SSR and 17 expressed sequence tags(EST)-derived SSR markers were selected based on marker type, informativeness and linkage group from the published literature [12,20-22] for the SSR analysis. An initial screening of 400 collected clones was performed with three genomic SSR and three EST-SSR markers for clone-wise polymorphism to assess clone duplication on the same farm. The effort confirmed 266 less likely duplicated clones from 80 farms. These less likely duplicated clones and 16 cultivar samples were finalized for analysis with 41 SSR markers. The polymerase chain reaction (PCR) was performed in a total volume of 10 µl reaction mixture containing 40 ng DNA template, 0.4 U *Taq* DNA polymerase (Vivantis), 1 µl of 10XPCR buffer S (160 µM $(NH4)_2SO_4$, 500 µM Tris-HCl, pH 9.1, 17.5 µM $MgCl_2$ and 0.1% Triton; Vivantis), 0.2 mM dNTPs (Vivantis), and 0.02 µM each of forward and reverse primers in a 0.20 ml PCR tube. The amplification was performed using Agilent Technologies Sure Cycler 8800 (Germany). The amplification regime consisted of 95˚C for 3 min; then 36 cycles at 95˚C for 30 s, 55˚C for 40 s, and 72˚C for 1 min; and a final extension at 72˚C for 10 min. The PCR products were analyzed by a 1.5% agarose gel electrophoresis, ethydium bromide stained and visualized by Electrophoresis Gel Photodocumentation System (Vilber Lourmat, Japan). In addition, the PCR amplification products were separated on 6% (19:1) polyacrylamide gel and revealed SSR bands by gel silver staining modified from Bassam *et al*. [23]. The 100 bp DNA ladder plus (Vivantis) was used as a molecular size standard. The PCR reactions were done independently twice. DNA fragments amplified by SSR primer pairs were identified based on their sizes in base pairs measured with DNA ladders and compared with the sizes reported in the literature. The scored alleles were assessed for consistency with duplicated samples. Only repeatable amplified DNA fragments were manually scored as 1 for presence or 0 for absence of a DNA fragment for each sample.

2.3. Data Analysis

The SSR data were analyzed for the level of polymerphismwith respect to primer and farm by counting the number of polymorphic alleles and generating summary statistics of allelic frequencies. The numbers of alleles detected by all primer pairs were plotted against their frequencies of occurrence in all assayed samples. As cassava ploidy is uncertain (*i.e*., either diploid or autotetraploid [24,25]) and only two of the 41 SSR markers may fit a di-allelic profile, Shannon's entropy was calculated following Reyes-Valdes and Williams [26] to estimate the diversity content per locus, as this estimate does not require strict genetic assumptions such as marker inheritance and sample ploidy. The entropy-based diversity content (eDC) provides a measure of the effective number of alleles per marker locus [26].

The analysis of molecular variance (AMOVA) was performed using the GenAlEx v6 software [27] to assess genetic diversity of assayed samples. Significance of resulting variance components and inter-group genetic distances was tested with 9999 random permutations. A principal coordinate analysis (PCoA) of the 282 cassava samples was performed using NTSYS-PC 2.01 [28] based on the similarity matrix of 365 SSR alleles, and plots of the first three resulting principal components were made to assess the accession associations.

The average dissimilarity of each sample against the other 265 samples was estimated following Fu [18] and using the SAS software that was written by Dr. Yong-Bi Fu, Plant Gene Resources of Canada. This average dissimilarity measures the overall genetic difference between a sample of interest and the remaining 265 samples assayed. Based on the average dissimilarity values, a set of 50 unique cassava samples with the highest average dissimilarity values were selected from the farm samples.

3. Results and Discussion

3.1. SSR Variation

The SSR analysis revealed that six of the 17 EST-derived SSR markers displayed monomorphic bands for all 266 samples and thus they were removed from further analysis. The other 35 markers revealed a total of 2 monomorphic and 365 polymorphic alleles in the 266 samples (**Table 2**). The number of alleles detected per locus ranged from 2 to 21 and averaged 10.4. The mean allele frequency for all alleles at a locus ranged from 0.446 to 0.994 and averaged 0.609. Interestingly, 11 EST-derived SSR markers detected only 61 alleles that are much fewer than the 24 genomic SSR primer pairs (304). The most informative primer pair was the genomic SSRY235 on linkage group G with an eDC value of 5.23 and 18 alleles detected, followed by the genomic GA5 on linkage group Q with an eDC value of 4.92 and 21 alleles detected (**Table 2**). The less informative primer pairs were two EST-derived EME254 and EME637 with eDC values smaller than 0.20. Some of these primer pairs should sample SSR alleles in both transcribed and non-

Table 2. Thirty-five SSR markers assayed in 266 on-farm cassava samples and the estimates of entropy-based diversity content per locus (eDC).

Marker[†]	Type[†]	Linkage group[†]	Number of alleles	Size range (base pair)	eDC[‡]
SSRY3	G	D[a]	10	115 - 250	2.87
SSRY5	G	J[a]	6	120 - 254	1.51
SSRY8	G	I[a]	11	250 - 289	3.41
SSRY11	G	nd[a]	12	193 - 414	2.41
SSRY13	G	N[a]	11	175 - 185	2.68
SSRY28	G	U[a]	10	164 - 214	2.54
SSRY34	G	M[a]	13	281 - 312	0.97
SSRY40	G	D[a]	18	245 - 455	4.05
SSRY43	G	U[a]	12	230 - 345	2.48
SSRY143	G	O[a]	16	165 - 285	3.87
SSRY161	G	E[a]	12	175 - 210	2.95
SSRY164	G	H[a]	14	148 - 216	3.88
SSRY186	G	nd[a]	13	223 - 336	3.44
SSRY235	G	G[a]	18	180 - 368	5.23
SSRY324	G	nd[a]	15	175 - 320	2.93
GA5	G	Q[b]	21	125 - 239	4.92
GA12	G	nd[b]	15	125 - 185	2.14
GA21	G	nd[b]	12	104 - 150	3.18
GA126	G	K[b]	9	180 - 225	1.76
GA127	G	K[b]	13	220 - 266	3.20
GA131	G	G[b]	9	110 - 135	2.09
GA134	G	nd[b]	16	250 - 325	4.45
GA136	G	nd[b]	12	150 - 280	3.15
GA140	G	nd[b]	6	170 - 185	1.48
MeESSR15	E	nd[d]	7	150 - 228	0.41
MeESSR19	E	nd[d]	10	208 - 363	0.79
MeESSR29	E	nd[d]	6	170 - 185	1.84
EME164	E	3[c]	10	170 - 230	3.09
EME171	E	6[c]	4	150 - 165	0.79
EME212	E	10[c]	8	193 - 250	0.74
EME240	E	6[c]	5	180 - 210	0.97
EME254	E	2[c]	3	250 - 257	0.01
EME445	E	18[c]	3	255 - 260	1.00
EME637	E	9[c]	2	185 - 189	0.19
EME189	E	2[c]	3	195 - 230	0.58
Total or mean			365		2.34

[†]Information on markers, type and linkage group was obtained from a) Mba *et al*. [20]; b) Chavarriaga-Aguirre *et al*. [12]; c) Kunkeaw *et al*. [22]; and d) Raji *et al*. [21] Genomic (G) and expressed sequence tag-derived (E) marker types are specified. nd = not determined yet. [‡]eDC was calculated following Reyes-Valdes and Williams [26].

transcribed chromosomal regions and provide an adequate measure of genetic diversity.

3.2. Genetic Diversity

The molecular analysis of variance revealed that there was a large SSR variance (19.8%) present among farm samples and 80.2% residing within farm samples (**Table 3**). Based on the farm-specific proportions of the total SSR variation, the 10 most genetically diverse farms were KML54, KBB40, SKK16, SKC15, SKK19, NNN30, PPN63, KMT33, SKN77, and SKN79 (results not shown). The genetic relationships of the 266 farm samples shown in **Figure 2** confirmed there was large SSR variation present among the collected cassava samples. Two PCoA components explained a total of 28.3% SSR variation.

The large SSR variation observed on the farm samples is not surprising for two reasons. Firstly, cassava is an outcrossing species with a multi-locus outcrossing rate estimated at 91.5% [29]. Our results are consistent with those reported for cassava germplasm from other countries using SSR markers (e.g., [9,12,13,30]). Secondly, some studies have shown that the high genetic diversity could be maintained through gene flow and recombination (e.g., [31]). The accumulation of fixed somatic mutation in cassava transmitted through vegetative propagation can be another important factor attributed to the intra-varietal polymorphism [31,32].

Table 3. Results of the molecular analysis of variance for the 266 on-farm cassava clones collected from 80 farms based on the 365 SSR markers.

Source	*df*	Sum of squares	Variance component	Percent of variation	*p*-value
Among farms	79	6589.05	11.29	19.75	<0.0001
Within farms	186	8534.43	45.88	80.25	
Total	265	15123.48	57.17		

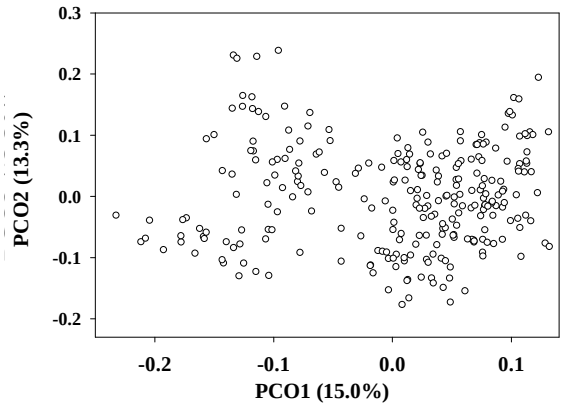

Figure 2. Genetic relationships of the 266 on-farm cassava clones as revealed in a PCoA plot based on 365 SSR alleles.

3.3. Unique Cassava Germplasm

The average dissimilarity (AD) of a cassava sample against the other 265 samples was calculated and the AD values obtained ranged from 0.256 to 0.502 with a mean of 0.319 (**Figure 3**). Based on these AD values, a set of 50 unique cassava samples with AD values of 0.346 or higher was assembled from the on-farm germplasm collection (**Table 4**). This unique set represented 18.8% of the collected and assayed clones from 39 farms across eight provinces, and 12.5% of the collected clones from 80 farms. The number of clones (in the unique set) per province fluctuated, depending on the sampling method [33]. However, the present study showed that the unique set comprised of cassava samples from all 8 provinces. It is interesting that there were 11 (22%) clones collected from farms in Khu Khan District, Si Sa Ket Province, which were among 50 most genetically unique clones.

The ADs obtained from this study are limited to only the 266 on-farm clones assayed. The AD values would change if more clones were assessed. This measure can recognize the distinctiveness, but not necessarily the relatedness, of cassava clones [18]. For example, two closely related clones that are quite distinct from the remaining clones could have similar higher levels of AD than the others and both clones would have been identified as genetically distinct. It is important to recognize these limitations when using the relative measure of genetic distinctiveness reported here.

This unique set of cassava clones differs in local adaptations from germplasm introduced from the International Centre for Tropical Agriculture and from other countries. Extensive investigation of the unique set is an efficient approach to enhancing evaluation and utilization for crop germplasm [34]. Our identified unique set could be further explored along with agronomic trait evaluations for genetic introgression or hybridization to widen the genetic base of the Thai cassava breeding gene pool.

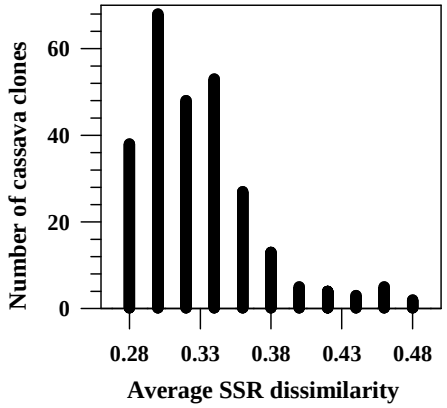

Figure 3. Distribution of average SSR dissimilarities for the 266 on-farm cassava clones.

Table 4. List of 50 most genetically unique cassava clones collected from 80 farms in Thailand with the largest average dissimilarity (AD) values.

C-label†	A-label†	Province	District	Township	Farm†	SL†	AD†
02-21	49	Si Sa Ket	Khukhan	Chai Di	SKC15	1	0.502
08-43	263	Sa Kaeo	Khao Chakan	Nong Wa	SKN79	3	0.469
04-42	125	Khon Kaen	Ban Phai	Ban Phai	KBB39	1	0.460
08-50	266	Sa Kaeo	Khao Chakan	Nong Wa	SKN80	3	0.452
06-20	181	Kanchanaburi	Mueang Kanchanaburi	Lat Ya	KML54	3	0.450
01-07	4	Maha Sarakham	Kosum Phisai	Nong Lek	MKN2	2	0.446
07-12	211	Prachin Buri	Prachantakham	Nong Kaeo	PPN63	2	0.445
08-49	265	Sa Kaeo	Khao Chakan	Nong Wa	SKN80	2	0.439
04-49	131	Khon Kaen	Ban Phai	Ban Phai	KBB40	4	0.433
01-26	16	Maha Sarakham	Borabue	Bo Yai	MBB6	1	0.429
04-03	101	Khon Kaen	Mancha Khiri	Ta Sala	KMT31	2	0.411
03-01	71	Nakhon Ratchasima	Nong Bun Mak	Nong Hua Raet	NNN21	1	0.406
02-28	55	Si Sa Ket	Khukhan	Kanthararom	SKK16	3	0.401
08-20	247	Sa Kaeo	Wang Nam Yen	Wang Nam Yen	SWW74	3	0.401
06-31	189	Kanchanaburi	Sai Yok	Sing	KSS57	1	0.398
04-12	108	Khon Kaen	Mancha Khiri	Ta Sala	KMT33	2	0.395
02-45	66	Si Sa Ket	Khukhan	Kanthararom	SKK19	4	0.391
02-41	63	Si Sa Ket	Khukhan	Kanthararom	SKK19	1	0.385
06-11	176	Kanchanaburi	Mueang Kanchanaburi	Lat Ya	KML53	1	0.385
04-32	120	Khon Kaen	Ban Phai	Ban Phai	KBB37	2	0.378
02-01	34	Si Sa Ket	Khukhan	Chai Di	SKC11	1	0.377
06-16	179	Kanchanaburi	Mueang Kanchanaburi	Lat Ya	KML54	1	0.373
02-04	35	Si Sa Ket	Khukhan	Chai Di	SKC11	2	0.372
01-15	9	Maha Sarakham	Kosum Phisai	Hua Khwang	MKH3	4	0.369
05-46	166	Kamphaeng Phet	Mueang Kamphaeng Phet	Sa Kaeo	KMS50	1	0.368
01-16	10	Maha Sarakham	Borabue	Bo Yai	MBB4	1	0.365
07-21	217	Prachin Buri	Prachantakham	Ban Hoi	PPB65	1	0.365
02-11	41	Si Sa Ket	Khukhan	Chai Di	SKC13	1	0.364
03-30	85	Nakhon Ratchasima	Nong Bun Mak	Nong Hua Raet	NNN26	2	0.364
05-15	142	Kamphaeng Phet	Sai Ngam	Maha Chai	KSM43	3	0.362
02-39	62	Si Sa Ket	Khukhan	Kanthararom	SKK18	3	0.361
06-38	194	Kanchanaburi	Sai Yok	Sing	KSS58	2	0.361
02-50	70	Si Sa Ket	Khukhan	Kanthararom	SKK20	4	0.360
05-14	141	Kamphaeng Phet	Sai Ngam	Maha Chai	KSM43	2	0.360
06-06	173	Kanchanaburi	Mueang Kanchanaburi	Lat Ya	KML52	1	0.360
02-09	39	Si Sa Ket	Khukhan	Chai Di	SKC12	3	0.358
02-48	68	Si Sa Ket	Khukhan	Kanthararom	SKK20	2	0.358
05-47	167	Kamphaeng Phet	Mueang Kamphaeng Phet	Sa Kaeo	KMS50	2	0.357
06-03	171	Kanchanaburi	Mueang Kanchanaburi	Lat Ya	KML51	2	0.356
08-16	245	Sa Kaeo	Wang Nam Yen	Wang Nam Yen	SWW74	1	0.356
08-35	257	Sa Kaeo	Khao Chakan	Nong Wa	SKN77	3	0.356
01-05	2	Maha Sarakham	Kosum Phisai	Nong Lek	MKN1	2	0.354
07-15	213	Prachin Buri	Prachantakham	Nong Kaeo	PPN63	4	0.354
06-22	183	Kanchanaburi	Mueang Kanchanaburi	Lat Ya	KML55	2	0.353
05-21	148	Kamphaeng Phet	Mueang KamphaengPhet	Thep Nakhon	KMT45	1	0.352
01-48	32	Maha Sarakham	Wapi Pathum	Dong Yai	MWD10	2	0.351
05-44	164	Kamphaeng Phet	Mueang Kamphaeng Phet	Sa Kaeo	KMS49	3	0.348
07-42	230	Prachin Buri	Kabin Buri	Na Khaem	PKN69	2	0.348
01-06	3	Maha Sarakham	Kosum Phisai	Nong Lek	MKN2	1	0.347
05-11	140	Kamphaeng Phet	Sai Ngam	Maha Chai	KSM43	1	0.347

†C-label = collection label (*i.e.*, the first number for the province followed by the numbering within the province). A-label = assay label for this study. Farm label is given in **Table 1**. SL = Sample label.

It should be evaluated across different ecosystems to determine the genotype by environmental effects for important traits. All the collected clones are currently vegetative conservation in the field and some of these are propagated *in vitro*. These materials will not only allow for on-farm yield assessments, but are also useful for testing pests and pathogens. The unique set of the cassava clones also provide a valuable addition to the *ex situ* collection of cassava germplasm for long-term conservation in Thailand.

3.4. Concluding Remarks

This SSR analysis represented a large effort to characterize on-farm cassava clones in Thailand, detected major SSR variation present in the cassava samples collected from 80 farms in the eight provinces, and established a set of 50 most genetically unique cassava clones to widen the genetic base of the Thai cassava breeding gene pool.

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