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FINGERPRINTING OF CACAO GERMPLASM IN HAWAII

C. Nagai[1], *R. Heinig*[1]
C. T. Olano[2]
J. C. Motamayor[2, 3] *and R. J. Schnell*[2]

[1]Hawaii Agriculture Research Center (HARC), Kunia, HI
[2]USDA-ARS, Subtropical Horticulture Research Station, Miami, FL
[3]Mars Inc., Hackettstown, NJ

SUMMARY

High tonnage and superior quality are required for profitable cacao production in Hawaii. However, Hawaiian cacao plantings are variable in both quality and yield, and are not necessarily adapted to Hawaii's growing conditions. The genotypes of these trees are unknown, and growers are not able to identify the types of cacao trees on their farms. Through the use of DNA marker techniques, we are now able to assist cacao farmers in selection of superior cultivars based on their parentages/pedigrees. About 100 individual trees were fingerprinted using eleven SSR DNA markers. The survey group was found to include Criollo, Trinitario, Forastero and their hybrid types, and 77 trees were genetically unique among the samples. This indicates large genetic variation among cacao trees currently grown in Hawaii. It may not be necessary to import additional of cacao genotypes to supplement locally available germplasm. The variation would allow us to select and produce superior genotypes for growers specifically suited for their production environments.

INTRODUCTION

Cacao (*Theobroma cacao* L.) is native to Central and South America and has been cultivated since prehistoric times in Mexico. The fermented product of cacao, chocolate, has been known and consumed since the 16th century by Europeans. Currently, 70% of cacao grown worldwide is produced in Africa, including the Ivory Coast, Ghana and Nigeria; Brazil is also a leading producer. There are three major types of cacao: Criollo, Forastero and their hybrid, Trinitario. Forastero type is further divided into groups such as Amelonado, Upper Amazon and Lower Amazon (Motamayor et al., 2008). Forastero is the most common cultivar used for blended chocolate (80-90% of the total production). Criollo was originally domesticated by the Mayas (Motamayor et al., 2002) and is highly regarded but is weak in chocolate flavor and tree vigor (Wood and Lass, 1985). Trinitario, descended from an initial hybridization of Criollo and Forastero, is variable in its tree and pod characters as well as in vigor and yield. It is, however, the tree type selected for cultivation in many cacao growing countries and regions.

Commercial production of cacao in Hawaii is increasing, and this trend is expected to continue. The increased acreage is being planted with seedlings from uncharacterized cacao populations from at least three initial introductions of cacao into the islands. An unknown type of cacao was first introduced to Hawaii in the 1850s. In the early 1980s, Hawaii Vintage Chocolate (a joint venture of Kekela Enterprises Inc., Hershey Foods Corp., and Amfac Hawaii) introduced cacao seeds from the Hershey Hummingbird farm in Belize. These seeds were from a selected "hybrid" population originally produced by CATIE institute in Costa Rica and were planted at Malamaki, Hawaii. Molecular marker studies confirmed that they are Trinitario types (Schnell et al., 2005). One hundred of the seedlings were used to establish an orchard at HARC's Kunia Substation in 1985.

Upper Amazon Forastero cacao seeds from the Tagnanan plantation in Mindanao, Philippines, were also introduced in the 1980s for a nursery at Keaau. In 1988, University of Hawaii researchers planted seeds from the Keaau trees at their Waimanalo Station (J. DeFrank, personal communication). Records indicate that the seed was Upper Amazon Forastero (UAF) x Trinitario. Molecular analysis (Schnell et al., 2005) confirmed their identity.

Later, in 1998, Dole Fresh Fruit Co. planted 20,000 cacao seedlings on 17 acres on former Waialua Sugar Company land. This field was rejuvenated in 2004 and commercial cacao production was initiated (Conway, 2005). Small farmers are also growing cacao on all of the principal Hawaiian islands (Bittenbender, 2005). In 2003, a Cacao Chapter was formed within the Hawaii Tree Fruit Growers (HTFG) Association. It now has 24 members, each with 5 acres or less under cultivation. Their interests include genetic identification of currently grown cacao trees and establishment of a 100% Hawaiian cacao brand, as well as marketing options for the industry.

Recently, the production of premium and specialty chocolates made from cacao with especially desirable characteristics has been increasing. Cacao is a great candidate for a high value, low acreage agriculture product. Just as in the coffee industry, the commercial interest in premium quality chocolate is increasingly in demand, and Hawaiian chocolate could be produced for this market if high yields of superior quality cacao can be achieved.

At present, farmers in Hawaii are planting cacao trees derived from seeds imported from various sources with variable bean quality and size. Selection of higher yield genotypes should increase overall cacao yield (Schnell et al., 2005).

Using microsatellite (SSR) markers (non coding repetitive DNA sequences) the Waialua population was identified as Upper Amazon Forastero (UAF) x Trinitario type trees, although there was much variability among them. The marker technique also showed that the parental population came from the University of Hawaii (UH) research station at Waimanalo (Schnell et al., 2005). SSR markers associated with the most productive trees (trees producing 40+ pods per season) in Waialua were identified. Large morphological variation was also observed in pod color, size, shape and texture. This variation was attributed to the hybrid nature of the parents.

Genetic fingerprinting of cacao has been successfully conducted by various techniques such as isozymes (Lachnaud et al., 2004), RAPD, AFLP (Perry et al., 1998) and microsatellite markers (SSR) (Brown et al., 2003; Lanaud et al., 1999; Pugh et al., 2004). The SSR marker system is the most reliable and efficient. Cacao SSR markers have been isolated and characterized, and over 300 markers are available for use. Waialua cacao trees were fingerprinted using 65 primer pairs by R. Schnell (USDA-ARS, Subtropical Horticulture Research Station, Miami) and his international collaborators.

It is extremely important to be able to identify cacao trees in Hawaii. Farmers need to have knowledge of their tree genotypes so that decisions for new planting can be made based on sound genetic information. This study was conducted to identify genetic variation of cacao trees currently grown in Hawaii.

MATERIALS AND METHODS

Plant materials. We collected leaf samples from diverse locations and known sources described by the growers. Leaf samples were collected from selected farms and botanical gardens on the Big Island, Kauai and Oahu from June to August 2006. We collected information on the trees including tree sources, tree age and fruit and tree morphology. Between three and five leaves were collected from each tree for analysis. Healthy, young, fully expanded leaves were preferentially selected. Four growers sent the leaf samples to HARC following HARC's instructions. We did not include samples which had already been analyzed in a previous study.

DNA extraction. Leaves were stored at 4°C prior to DNA extraction following the protocol of Schnell’s lab (Schnell et al., 2005). DNA was extracted from the samples using the FastDNA SPIN Kit (MP Biomedicals, Irvine, CA). The extracted DNA was freeze-dried and shipped to Schnell’s lab, USDA-ARS, Miami, FL for SSR analysis.

Microsatellite markers and Parental analysis. Microsatellite markers used in this study were previously reported by Schnell's group. Primer pairs for 11 selected loci were used to amplify selected microsatellite markers. These primers had been used effectively in previous studies to determine cacao parentage (Schnell et al., 2005). For 2005 samples, a total of 19 primer pairs were used for the analysis.

Diluted DNA was amplified by PCR and capillary electrophoresis (CE) was conducted on an ABI 3730 Genetic Analyzer according to the procedures described by Schnell et al. (2005). The samples were compared to DNA of four major types of cacao: Criollo, Amelonado and various Upper Amazon Forastero (Nanay, Scavina, etc.).

RESULTS

Selection of individual cacao tree samples in Hawaii: A total 103 leaf samples were collected from the selected growers and botanical gardens on the islands of Hawaii, Kauai and Oahu from June to August 2006. On the island of Hawaii, 35 samples were collected from 9 cacao growers' fields in both the Kona and Hilo regions. On Kauai, 2 growers and the National Tropical Botanical Garden provided 12 samples. On Oahu, 56 samples were obtained from 7 growers including 10 samples from Lyon Arboretum. Within a farm, the most distinctive trees were selected for samples. Table 1 shows the list of samples collected including 15 samples collected by Gini Choobua in 2005 for a preliminary analysis. No correlation was found between genotypes of trees based on DNA markers and visual observation of pod morphology such as shape, skin texture (smooth/warty) and color (Table 2). Seed color was a reliable indicator of genotype; all the trees with white-colored seeds were identified as Criollo by SSR markers.

Table 1. List of cacao leaf samples used in study.

Island	Area	Location	Number of samples(a)	Sample prefix
Hawaii	Alae	Menezes Farm 2	5	TM (1-5)
	Hakalau	Menezes Farm 1	4	TM (6-9)
	Hilo	Sharkey Farm	3	TR
	Kona	Choobua Farm	10	GC, CF
	Kona	Kealia Ranch	4	KR
	Kona	Kokoleka Lani Farm	6	MG
	Kona	Konacopia Farms	1	EK
	Kona	Orchards of Kahalu'u	2	BC
	Kona	Twigg-Smith garden	3	TS (1-3)
	Kona	Public land	1	XX
	Kona	Twigg-Smith Farm	2	TS (6-7)
Subtotal			41	
Kauai	Kalaheo	National Tropical Botanical Garden	2	NTBG (1-2)
	Kalaheo	Kikuchi garden	2	NTBG (3-4)
	Kilauea	Ferris property	13	HF
Subtotal			17	
Oahu	Honolulu	Foster Botanical Garden	2	FB
	Kaneohe	Ho'omaluhia Botanical Garden	8	HO
	Kaneohe	Ringuette Farm	3	DR
	Manoa	Lyon Arboretum	14	LA
	Waialua	Dole Food Company, Inc.	24	W
	Waihole Valley	Rapoon Farm	5	R
	Waimanalo	UH Research Station	4	WM
Subtotal			60	
Total			118	

(a) 2005 samples (15) and 2006 samples (103).

Table 2. Examples of tree identification by DNA markers and pod morphology.

Sample	Pod Description	Tree type identification	
		by pod morphology	by DNA markers (SSR)
MG-01	smooth, purple-red pod with rounded tip	Amelonado	UAF
MG-02	rough, green pod with pointed tip	UAF	Trinitario/UAF
MG-03	green Amelonado-type pods	Amelonado	Trinitario/UAF
MG-04	smooth, light green-white pod with pronounced tip	Trinitario	Trinitario/UAF
MG-06	smooth, green pod with medium tip	Trinitario	Trinitario
TS-02	smooth yellow pod	Amelonado	Trinitario
TS-03	warty, furrowed green pod	Trinitario/UAF	Trinitario

Microsatellite DNA (SSR) analysis and genetic group assignment. Out of 103 samples, 77 were genetically unique within the survey group. The samples were placed into four groups; Criollo, Trinitario, Upper Amazon Forastero (UAF) or Trinitario-UAF hybrid; based on the method used by Schnell et al. (2005).

Criollo. A total of 19 samples from four different locations were classified as Criollo. The 12 samples from 2006 were homozygous at 9 SSR loci (Table 3) and 7 samples in 2005 were homozygous at all 11 loci. Two of the trees from 2005 may have been re-sampled in 2006; GC-01 and CF-1 may be the same tree, and HF-2-2-05 may be the same as HF-L1, -L2, -L3 or –L4.

Trinitario. The Trinitario group, which was represented by 47 samples in 14 locations, was the largest of the four identified in this study. The samples were highly variable, with the expected frequencies of the sample genotypes in a Trinitario population ranging from 0.619 to 0.944. Because Trinitario result from crosses between Amelonado and Criollo, the expected genotype frequency is equal to the sum of the frequencies for Amelonado and Criollo. The Amelonado expected frequencies in the Trinitario samples range from 0.247 to 0.936, suggesting that some of the Trinitario trees are in fact more likely to be Amelonado. TS-02 and R-1, for example, have expected frequencies of 0.936 in an Amelonado population.

Trinitario × UAF. A total of 33 samples from 10 locations were Trinitario-UAF hybrids, though the majority (61%) came from trees at Waialua field of Dole Co. This group showed large variation in contribution from the 7 UAF groups with expected frequencies in individual UAF populations ranging from 0.005 to 0.466. Total contribution of UAF ranged 0.243 to 0.727 (Figure 1). Five samples from 2005 were identified as Trinitario-LAF hybrids.

Table 3. Distribution of four major cacao types on three islands of Hawaii.

Type by SSR analysis	Location	Area	Island	Number of samples(a)	Sample number(s)
Criollo	Choobua Farm	Kona	Hawaii	3	GC-01, GC-02, CF-1
	Kealia Ranch	Kona	Hawaii	2	KR-1, KR-2-1
	Kikuchi garden	Kalaheo	Kauai	2	NTBG-03, NTBG-04
	Ferris property	Kilauea	Kauai	12	HF-0, HF-1 , HF-2, HF-3, HF-L1, HF-L2, HF-L3, HF-L4, HF-1-05, HF-2-05, HF-1-2-05, HF-2-2-05
Subtotal				19	
Trinitario	Menezes Farm 1	Hakalau	Hawaii	3	TM-07, TM-08, TM-09
	Sharkey Farm	Hilo	Hawaii	1	TR-01
	Choobua Farm	Kona	Hawaii	5	GC-03, GC-04, GC-05, GC-07, GC-08
	Kokoleka Lani Farm	Kona	Hawaii	2	MG-05, MG-06
	Orchards of Kahalu'u	Kona	Hawaii	1	BC-02
	Twigg-Smith garden	Kona	Hawaii	3	TS-01, TS-02, TS-03
	Public land	Kona	Hawaii	1	XX-01
	Twigg-Smith Farm	Kona	Hawaii	2	TS-06, TS-07
	National Tropical Botanical Garden	Kalaheo	Kauai	2	NTBG-01, NTBG-02
	Foster Botanical Garden	Honolulu	Oahu	2	FB-02, FB-03
	Ho'omaluhia Botanical Garden	Kaneohe	Oahu	8	HO-1, HO-2, HO-3, HO-4, HO-5, HO-6, HO-7, HO-8
	Ringuette Farm	Kaneohe	Oahu	3	DR-1, DR-3, DR-5
	Lyon Arboretum	Manoa	Oahu	9	LA-10, LA-2, LA-3, LA-4, LA-5, LA-6, LA-7, LA-8, LA-9
	Rapoon Farm	Waihole Valley	Oahu	5	R-1, R-2, R-3, R-4, R-5
Subtotal				47	
Trinitario/UAF	Menezes Farm 2	Alae	Hawaii	3	TM-03, TM-05, TM-04
	Menezes Farm 1	Hakalau	Hawaii	1	TM-06
	Sharkey Farm	Hilo	Hawaii	1	TR-02
	Choobua Farm	Kona	Hawaii	1	GC-06
	Kokoleka Lani Farm	Kona	Hawaii	3	MG-02, MG-03, MG-04
	Konacopia Farms	Kona	Hawaii	1	EK-01
	Orchards of Kahalu'u	Kona	Hawaii	1	BC-01
	Lyon Arboretum	Manoa	Oahu	1	LA-11
	Dole Food Company, Inc.	Waialua	Oahu	20	W1010202, W1010305, W1010313, W1010314, W1010505, W1010509, W1010611, W1010702, W1010705, W1010708, W1010711, W1010713, W1010804, W1010808, W1010822, W1020606, W1050704, W1050707, W1070208, W1090601
	UH Research Station	Waimanalo	Oahu	1	WM-11
Subtotal				33	
Trinitario/LAF	Ferris property	Kilauea	Kauai	1	HF-3-05
	Lyon Arboretum	Manoa	Oahu	4	LA-1-05, LA-2-05, LA-3-05, LA-4-05
Subtotal				5	
UAF	Menezes Farm 2	Alae	Hawaii	2	TM-01, TM-02
	Sharkey Farm	Hilo	Hawaii	1	TR-03
	Choobua Farm	Kona	Hawaii	1	CF-2
	Kealia Ranch	Kona	Hawaii	2	KR-4, KR-6
	Kokoleka Lani Farm	Kona	Hawaii	1	MG-01
	Dole Food Company, Inc.	Waialua	Oahu	4	W1010503, W1010709, W1010815, W1010817
	UH Research Station	Waimanalo	Oahu	3	WM-08, WM-10, WM-12
Subtotal				14	
Total				118	

(a) 2005 samples (15) and 2006 samples (103).

UAF. Eleven samples from 6 locations and 3 UH samples were identified as UAF. The expected genotype frequencies of these samples in the 7 UAF populations ranged from 0.274-0.936 (Appendix 1).

Table 4. Types of cacao at various locations in Hawaii.

Location	Area	Island	Criollo	Trinitario	Trinitario x UAF	Trinitario x LAF	UAF	Total
Choobua Farm	Kona	Hawaii	3	5	1		1	10
Dole Food Company, Inc.	Waialua	Oahu			20		4	24
Ferris property	Kilauea	Kauai	12			1		13
Kealia Ranch	Kona	Hawaii	2				2	4
Kikuchi garden	Kalaheo	Kauai	2					2
Kokoleka Lani Farm	Kona	Hawaii		2	3		1	6
Konacopia Farms	Kona	Hawaii			1			1
Menezes Farm 1	Hakalau	Hawaii		3	1			4
Menezes Farm 2	Alae	Hawaii			3		2	5
Orchards of Kahalu'u	Kona	Hawaii		1	1			2
Public land	Kona	Hawaii		1				1
Rapoon Farm	Waihole Valley	Oahu		5				5
Ringuette Farm	Kaneohe	Oahu		3				3
Sharkey Farm	Hilo	Hawaii		1	1		1	3
Twigg-Smith Farm	Kona	Hawaii		2				2
Twigg-Smith garden	Kona	Hawaii		3				3
Foster Botanical Garden	Honolulu	Oahu		2				2
Ho'omaluhia Botanical Garden	Kaneohe	Oahu		8				8
Lyon Arboretum	Manoa	Oahu		9	1	4		14
National Tropical Botanical Garden	Kalaheo	Kauai		2				2
UH Research Station	Waimanalo	Oahu			1		3	4
Total number of samples			19	47	33	5	14	118
Percentage of total number of trees			16.1	39.8	28.0	4.2	11.9	
Percentage of farms with type			26.7	66.7	46.7	6.7	40.0	

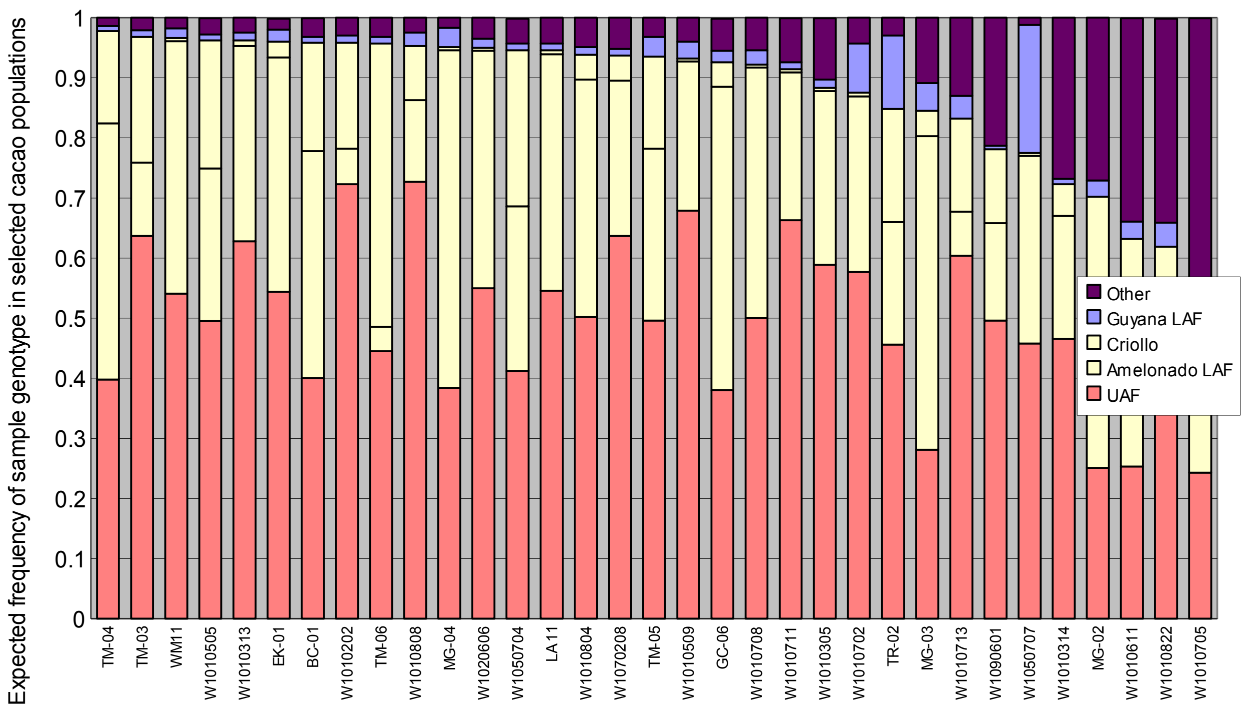
(a) 2005 samples (15) and 2006 samples (103).
UAF = Upper Amazon Forastero
LAF = Lower Amazon Forastero

DISCUSSION

Origin and Distribution of Cacao cultivars in Hawaii. Molecular marker studies of 103 trees from the three islands confirmed that there is a wide range of cacao types in Hawaii, including Criollo, Trinitario and Upper Amazon Forastero and their hybrids (Table 3). Farmers that participated in the project know the genotypes of their cacao trees, and we know the genetic variation of cacao germplasm in Hawaii.

We could not get information on the origins of the seed sources at many farms in this study, but several farmers provided us information on seed origins. Trinitario types were widely distributed at 67% of the farms and all 4 botanical gardens. Trinitario x UAF was found at 47% of the farms and at the Lyon Arboretum. UAF type was located at 40% of the farms but not at any of the botanical gardens (Table 4).

Figure 1. Allelic variation in Trinitario/UAF hybrid samples



Trinitario trees were located at all four of the botanical gardens surveyed on Oahu. The trees at Lyon Arboretum had come from Maunawili (LA-2), Kauai (LA-3) and Fiji (LA-7) and were the seed source for Foster and Ho'omaluhia Botanical Gardens. Foster Botanical Garden planted its trees in 1968, and Ho'omaluhia Botanical Garden planted in 1981. The samples from both gardens were most closely related to LA-3. The trees from Foster Botanical Garden were genetically identical to LA-3 at five or more loci, while seven of the eight trees from Ho'omaluhia Botanical Garden were genetically identical to

LA-3 at ten loci. The remaining sample, HO-7, was identical to the other samples at every locus except Cir6. Neither Cir6 allele was found in LA-3, but both appeared in other Lyon Arboretum trees, suggesting possible cross-fertilization. The fourth botanical garden in this study, the National Tropical Botanical Garden on Kauai, received its Trinitario trees from a different source. NTBG-01 came from a private grower in Kalaheo, Kauai, in 1977. There were no records on the origin of NTBG-02, but it was identical to NTBG-01 at all eight loci for which data were available.

Schnell et al. (2005) determined that the UH Research Station at Waimanalo supplied the Trinitario-UAF hybrid and UAF material planted by Dole Food Company, Inc., in 1998. Waimanalo received its planting material from a nursery in Keaau, Hawaii, which had been planted with Trinitario x UAF hybrid seeds from the Tagnanan estate plantation on Mindanao in the Philippines (Schnell et al., 2005). All the Trinitario x UAF type trees in 8 farms are derived from Keaau seeds (personal communications with growers). UAF trees from 5 additional farms are also likely to have come from Keaau based on their genetic profiles.

Criollo in Hawaii. Criollo is one of the most appreciated cacao types commercially due to its distinct flavor and quality internationally. Criollo trees were lost in many areas due to their susceptibility to diseases. Records show that Criollo was imported by a UH scientist in 1900 and planted at the experimental station at Poamoho (personal communication, Skip Bittenbender). Records indicate two types of Criollo with pod colors of red and yellow. Recently, the original tree (red Criollo) was discovered by S. Bittenbender. The original Criollo tree at Kealia Ranch, KR-1, came from Poamoho, Oahu, sometime in the 1950s (personal communication, Elizabeth Stack, Kealia Ranch). Progeny from Kealia Ranch were planted at Choobua Farm in 2003. The Ferris property Criollo were planted in 2000 using material from two different sources in Hawaii. Ferris Farm had both yellow and red pod-producing trees which were homozygous for all eleven loci analyzed. The Criollo trees at Kikuchi garden were planted in 1993, but the planting material is of unknown origin. An analysis with additional markers will provide additional genetic information about these Criollo.

Selection of superior cultivars in Hawaii. SSR analysis was performed using 11 marker pairs that had been used successfully in previous experiments to identify genetic differences in cacao. It was found that 77 of the samples (75%) were genetically unique, indicating a large variation among the trees tested. Based on these results, it is clear that Hawaii has ample genetic diversity available for selection and propagation of superior genotypes for their particular farming environment. It may not be necessary to import large amounts of foreign cacao seed to supplement the available genotypes. Accidental introduction of disease and insect pests to the islands may be avoided if locally-available plant material is used exclusively.

The varied germplasm available makes it possible to select higher yielding genotypes. We have initiated selection of superior trees with high yield and desirable characteristics such as large seed size and high liquor quality to develop cacao cultivars for Hawaii's environment. Three year data (2007-2009) for pod and bean characters were collected from 350 trees of a UAF x Trinitario population cultivated on the Island of Oahu. All the beans from the pods were micro-fermented and dried with commercial beans. Both pod index and yield estimate showed that variation among trees is large. Superior trees with high pod production and trees with low production were selected, and leaf samples are currently analyzed for SSR markers (Nagai et al., 2009).

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Appendix 1. Proportion of microsatellite alleles shared between sampled individuals and established cacao genotypes.

Sample name	Extraction #	Type by SSR analysis	Parinari[1]	Guyana[2]	Scavina[1]	Chalmers[1]	Nanay[1]	IMC[1]	Nacional[1]	Brazil Wilds[1]	Sum UAF[4]	Criollo	Amelonado	Trinitario[3]	Hawaii[5]
GC-01	TC-10603	Criollo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.042	0.94	0.01	0.95	0.01
GC-02	TC-10604	Criollo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.042	0.94	0.01	0.95	0.01
HF-0	TC-10563	Criollo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.036	0.95	0.01	0.96	0.01
HF-1	TC-10564	Criollo	see HF-0												
HF-2	TC-10565	Criollo	see HF-0												
HF-3	TC-10566	Criollo	see HF-0												
HF-L1	TC-10567	Criollo	see HF-0												
HF-L2	TC-10568	Criollo	see HF-0												
HF-L3	TC-10569	Criollo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.042	0.94	0.01	0.95	0.01
HF-L4	TC-10570	Criollo	see HF-0												
NTBG-03	TC-10613	Criollo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.042	0.94	0.01	0.95	0.01
NTBG-04	TC-10614	Criollo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.042	0.94	0.01	0.95	0.01
BC-02	TC-10579	Trinitario	0.03	0.02	0.01	0.01	0.01	0.02	0.04	0.04	0.159	0.39	0.41	0.8	0.02
DR-1	TC-10524	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.054	0.62	0.31	0.93	0.01
DR-3	TC-10525	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.045	0.47	0.47	0.94	0.01
DR-5	TC-10526	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.043	0.69	0.25	0.94	0.01
FB-02	TC-10615	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.065	0.24	0.68	0.92	0.01
FB-03	TC-10616	Trinitario	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.078	0.15	0.76	0.9	0.01
GC-03	TC-10605	Trinitario	0.04	0.02	0.01	0.01	0.03	0.15	0.01	0.02	0.270	0.14	0.48	0.62	0.09
GC-04	TC-10606	Trinitario	0.02	0.01	0.01	0.01	0.02	0.16	0.01	0.01	0.236	0.29	0.43	0.72	0.04
GC-05	TC-10607	Trinitario	0.01	0.01	0.23	0.03	0.01	0.02	0.04	0.01	0.339	0.38	0.25	0.63	0.03
GC-07	TC-10609	Trinitario	0.01	0.01	0.01	0.02	0.01	0.17	0.02	0.03	0.267	0.03	0.68	0.72	0.01
GC-08	TC-10610	Trinitario	0.02	0.01	0.01	0.02	0.03	0.08	0.01	0.01	0.187	0.31	0.45	0.76	0.05
HO-1	TC-10555	Trinitario	see HO-1												
HO-2	TC-10556	Trinitario	see HO-1												
HO-3	TC-10557	Trinitario	see HO-1												
HO-4	TC-10558	Trinitario	see HO-1												
HO-5	TC-10559	Trinitario	see HO-1												
HO-6	TC-10560	Trinitario	see HO-1												
HO-7	TC-10561	Trinitario	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.073	0.2	0.68	0.89	0.03
HO-8	TC-10562	Trinitario	see HO-1												
LA-10	TC-10522	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.049	0.26	0.67	0.94	0.01
LA-2	TC-10514	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.046	0.25	0.69	0.94	0.01
LA-3	TC-10515	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.050	0.16	0.77	0.93	0.01
LA-4	TC-10516	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.046	0.47	0.47	0.94	0.01
LA-5	TC-10517	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.051	0.34	0.59	0.93	0.01
LA-6	TC-10518	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.044	0.2	0.74	0.94	0.01
LA-7	TC-10519	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.046	0.15	0.79	0.94	0.01
LA-8	TC-10520	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.047	0.1	0.85	0.94	0.01
LA-9	TC-10521	Trinitario	0.03	0.02	0.01	0.02	0.03	0.01	0.01	0.02	0.116	0.4	0.44	0.84	0.02
MG-05	TC-10584	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.048	0.16	0.78	0.94	0.01
MG-06	TC-10585	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.055	0.16	0.77	0.93	0.01
NTBG-01	TC-10611	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.051	0.04	0.9	0.94	0.01
NTBG-02	TC-10612	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.060	0.04	0.88	0.92	0.01
R-1	TC-10571	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.045	0.01	0.94	0.94	0.01
R-2	TC-10572	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.051	0.04	0.89	0.93	0.01
R-3	TC-10573	Trinitario	see R-2												
R-4	TC-10574	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.045	0.04	0.9	0.94	0.01
R-5	TC-10575	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.045	0.07	0.87	0.94	0.01

(cont.) Appendix 1. Proportion of microsatellite alleles shared between sampled individuals and established cacao genotypes.

TM-07	TC-10597	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.059	0.52	0.4	0.92	0.01
TM-08	TC-10598	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.053	0.3	0.63	0.93	0.01
TM-09	TC-10599	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.055	0.33	0.6	0.93	0.01
TR-01	TC-10600	Trinitario	0.01	0.02	0.15	0.01	0.03	0.01	0.01	0.01	0.231	0.27	0.46	0.73	0.02
TS-01	TC-10586	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.056	0.12	0.81	0.93	0.01
TS-02	TC-10587	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.045	0.01	0.94	0.94	0.01
TS-03	TC-10588	Trinitario	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.047	0.25	0.69	0.94	0.01
TS-06	TC-10589	Trinitario	0.05	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.123	0.23	0.62	0.86	0.01
TS-07	TC-10590	Trinitario	0.07	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.136	0.35	0.5	0.84	0.02
XX-01	TC-10577	Trinitario	0.01	0.01	0.01	0.01	0.03	0.02	0.12	0.07	0.272	0.14	0.53	0.68	0.04
BC-01	TC-10578	Trinitario x UAF	0.02	0.01	0.01	0.01	0.06	0.29	0.01	0.01	0.400	0.18	0.38	0.56	0.03
EK-01	TC-10576	Trinitario x UAF	0.02	0.02	0.17	0.01	0.11	0.01	0.22	0.01	0.544	0.03	0.39	0.42	0.02
GC-06	TC-10608	Trinitario x UAF	0.02	0.02	0.18	0.05	0.04	0.07	0.01	0.01	0.380	0.04	0.51	0.55	0.05
LA-11	TC-10523	Trinitario x UAF	0.01	0.01	0.02	0.27	0.03	0.17	0.03	0.02	0.546	0.01	0.39	0.4	0.04
MG-02	TC-10581	Trinitario x UAF	0.03	0.03	0.01	0.01	0.02	0.14	0.02	0.02	0.251	0.26	0.19	0.45	0.27
MG-03	TC-10582	Trinitario x UAF	0.04	0.05	0.03	0.03	0.01	0.01	0.15	0.01	0.281	0.04	0.52	0.56	0.11
MG-04	TC-10583	Trinitario x UAF	0.01	0.03	0.01	0.01	0.03	0.02	0.01	0.31	0.384	0.01	0.56	0.57	0.02
TM-03	TC-10593	Trinitario x UAF	0.04	0.01	0.17	0.01	0.31	0.02	0.08	0.02	0.637	0.21	0.12	0.33	0.02
TM-04	TC-10594	Trinitario x UAF	0.01	0.01	0.31	0.03	0.01	0.02	0.01	0.01	0.398	0.15	0.43	0.58	0.01
TM-05	TC-10595	Trinitario x UAF	0.06	0.03	0.12	0.02	0.03	0.25	0.01	0.01	0.496	0.15	0.29	0.44	0.03
TM-06	TC-10596	Trinitario x UAF	0.35	0.01	0.01	0.02	0.03	0.02	0.01	0.02	0.445	0.47	0.04	0.51	0.03
TR-02	TC-10601	Trinitario x UAF	0.05	0.12	0.01	0.02	0.01	0.33	0.01	0.02	0.456	0.19	0.2	0.39	0.03
W1010202	TC-10527	Trinitario x UAF	0.31	0.01	0.01	0.01	0.01	0.36	0.01	0.02	0.723	0.18	0.06	0.24	0.03
W1010305	TC-10528	Trinitario x UAF	0.05	0.01	0.03	0.01	0.01	0.47	0.01	0.01	0.589	0.01	0.29	0.29	0.1
W1010313	TC-10529	Trinitario x UAF	0.05	0.01	0.37	0.02	0.03	0.03	0.01	0.13	0.628	0.01	0.33	0.33	0.03
W1010314	TC-10530	Trinitario x UAF	0.02	0.01	0.09	0.02	0.02	0.3	0.01	0.01	0.466	0.05	0.2	0.26	0.27
W1010505	TC-10532	Trinitario x UAF	0.16	0.01	0.04	0.02	0.02	0.23	0.01	0.01	0.495	0.21	0.25	0.47	0.03
W1010509	TC-10533	Trinitario x UAF	0.14	0.03	0.05	0.01	0.02	0.41	0.01	0.04	0.679	0.01	0.25	0.25	0.04
W1010611	TC-10534	Trinitario x UAF	0.12	0.03	0.01	0.01	0.01	0.01	0.01	0.07	0.253	0.25	0.13	0.38	0.34
W1010702	TC-10535	Trinitario x UAF	0.11	0.08	0.01	0.01	0.01	0.4	0.01	0.03	0.577	0.01	0.29	0.3	0.04
W1010705	TC-10536	Trinitario x UAF	0.01	0.01	0.03	0.04	0.07	0.05	0.01	0.04	0.243	0.13	0.15	0.28	0.47
W1010708	TC-10537	Trinitario x UAF	0.04	0.02	0.04	0.01	0.01	0.39	0.01	0.01	0.500	0.01	0.42	0.42	0.05
W1010711	TC-10539	Trinitario x UAF	0.03	0.01	0.03	0.22	0.01	0.35	0.01	0.01	0.663	0.01	0.25	0.25	0.07
W1010713	TC-10540	Trinitario x UAF	0.06	0.04	0.11	0.03	0.1	0.2	0.03	0.08	0.604	0.16	0.07	0.23	0.13
W1010804	TC-10541	Trinitario x UAF	0.02	0.01	0.07	0.01	0.01	0.37	0.01	0.01	0.502	0.04	0.4	0.44	0.05
W1010808	TC-10542	Trinitario x UAF	0.27	0.02	0.01	0.01	0.19	0.23	0.01	0.01	0.727	0.09	0.14	0.23	0.03
W1010822	TC-10545	Trinitario x UAF	0.33	0.04	0.01	0.01	0.01	0.01	0.01	0.03	0.396	0.06	0.16	0.22	0.34
W1020606	TC-10547	Trinitario x UAF	0.02	0.02	0.01	0.01	0.02	0.43	0.02	0.04	0.550	0.01	0.4	0.4	0.04
W1050704	TC-10546	Trinitario x UAF	0.01	0.01	0.11	0.01	0.14	0.1	0.02	0.02	0.412	0.26	0.27	0.53	0.04
W1050707	TC-10550	Trinitario x UAF	0.13	0.21	0.01	0.01	0.01	0.27	0.02	0.01	0.458	0.01	0.31	0.32	0.01
W1070208	TC-10548	Trinitario x UAF	0.13	0.01	0.1	0.01	0.04	0.32	0.01	0.05	0.637	0.04	0.26	0.3	0.05
W1090601	TC-10549	Trinitario x UAF	0.02	0.01	0.02	0.02	0.1	0.33	0.01	0.01	0.496	0.12	0.16	0.29	0.22
WM-11	TC-10553	Trinitario x UAF	0.014	0.016	0.138	0.007	0.008	0.274	0.089	0.011	0.541	0.005	0.420	0.425	0.018
MG-01	TC-10580	UAF	0.16	0.01	0.02	0.01	0.02	0.26	0.01	0.21	0.684	0.13	0.05	0.17	0.13
TM-01	TC-10591	UAF	0.01	0.01	0.01	0.01	0.55	0.01	0.01	0.01	0.610	0.01	0.01	0.02	0.37
TM-02	TC-10592	UAF	0.01	0.01	0.02	0.26	0.55	0.01	0.07	0.01	0.925	0.01	0.03	0.04	0.03
TR-03	TC-10602	UAF	0.01	0.01	0.01	0.01	0.11	0.05	0.07	0.02	0.274	0.01	0.18	0.19	0.53
W1010503	TC-10531	UAF	0.02	0.02	0.11	0.01	0.02	0.56	0.01	0.02	0.734	0.01	0.08	0.08	0.17
W1010709	TC-10538	UAF	0.39	0.02	0.03	0.06	0.01	0.3	0.1	0.03	0.922	0.01	0.04	0.05	0.01
W1010815	TC-10543	UAF	0.07	0.06	0.04	0.02	0.22	0.13	0.26	0.09	0.825	0.01	0.06	0.07	0.05
W1010817	TC-10544	UAF	0.07	0.34	0.26	0.02	0.02	0.05	0.02	0.13	0.561	0.01	0.07	0.08	0.02
WM-08	TC-10551	UAF	0.113	0.180	0.235	0.013	0.007	0.162	0.024	0.096	0.650	0.037	0.107	0.144	0.026
WM-10	TC-10552	UAF	0.043	0.007	0.110	0.020	0.040	0.665	0.006	0.008	0.892	0.014	0.068	0.082	0.020
WM-12	TC-10554	UAF	0.393	0.024	0.010	0.008	0.020	0.481	0.010	0.014	0.936	0.011	0.014	0.025	0.015

Data not available for 2005 samples

[1]Upper Amazon Forastero (UAF) genotype
[2]Lower Amazon Forastero (LAF) genotype
[3]equal to the sum of the Criollo and Amelonado genotypes
[4]equal to the sum of the seven Upper Amazon Forastero genotypes
[5]proportion of alleles that are not shared by any of the established genotypes listed