International Scholars Journals

African Journal of Crop Science ISSN 2375-1231 Vol. 6 (2), pp. 001-007, February, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Phenotypic variation of cacao (*Theobroma cacao* L.) on farms and in the gene bank in Cameroon

M. I. B. Efombagn[1]*, O. Sounigo[2], S. Nyassé[1], M. Manzanares-Dauleux[3], A. B. Eskes[2]

[1]Institute of Agricultural Research for Development (IRAD), P. O. Box 2067 or 2123, Yaoundé, Cameroon.
[2]CIRAD, UPR31, 34398 Montpellier Cedex 5, France.
[3]Agrocampus de Rennes, 65 Rue de St Brieuc, 35042, Rennes, France.

Accepted 06 September, 2018

A survey was undertaken in the 2 major cocoa producing areas (Southern and Western) of Cameroon to study the morphological diversity existing in cacao farms in relation to genetic diversity in gene bank accessions. A total of 300 farm accessions (FA) were selected in the field which were compared to 77 gene bank accessions distributed into 4 groups (AGs) according to their origin. The 17 quantitative and qualitative descriptors used in this study were related to leaf (flush colour), flower (ligule colour), pod (weight, length, width, apex form, shape, rugosity, colour, husk hardness, basal constriction and pod index) and seed (number, length, width, dry weight and colour) characters. For the qualitative characters evaluated, considerable morphological variation was observed using the Shannon Weaver diversity index (SWDI) within FA and gene bank accessions. Among the FA, a differentiation between southern and western regions was only possible when using quantitative pod traits. Mean quantitative traits values of FA were not too different than those of most gene bank AGs, except for a few traits of agronomical interest (seed weight and pod index). No significant variation was observed for seed traits in all FA groups (southern/western). The morphological structure (quantitative traits) showed spatial differentiation between western and southern FA and a closer relationship between gene bank and some farm accessions. Furthermore, a molecular study done earlier using microsatellite profiles of the same FA did not show any genetic difference between FA of both regions, suggesting that the agro-morphological performance of FA is rather due to non-genetic factors. In contrast, microsatellites have shown that most of the gene bank accessions were genetically distant from the FA, suggesting the low intake of some breeders' genotypes to farmers' fields. The level of diversity found in farmers' germplasm could enhance the gene bank and current breeding programs.

Key words: *Theobroma cacao* L., farm accessions, morphological diversity, breeding.

INTRODUCTION

Cacao (*Theobroma cacao* L.) is a perennial crop of significant economic importance in producing countries of West Africa, South America and Southeast Asia. The traditional classification of *T. cacao* assumes 3 horticultural races or main types: Criollo, Forastero and Trinitario. The conventional classification of cacao into Criollo and Forastero is based on distinct morphological, historical and commercial traits. Trinitario is an interme diate type between Criollo and Forastero, a hybrid group with traits that include the total variation of the species (Motamayor et al., 2008).

In cacao, certain morphological characters of pods and seeds are used as the basis of classification into categories, which may be called varieties, cultivars, types or populations (Wood and Lass, 1985) . Morphological descriptors are useful because they can help the breeders to select the best accessions for the breeding programme (Engels et al., 1980). Phenotypic characterization of the species, usually conducted by gene banks, involves leaf, flower, pod and seed descriptors (Engels et al., 1980; Bekele and Bekele, 1996) . The phenotypic appearance of cacao fruits (pods) plays an important role in the definition of types and populations. Considerable variation is encountered at the level of seed (seed) size.

*Corresponding author. E-mail: efombagn@yahoo.fr. Tel.: (237) 99427646.

Studies on morphological diversity have been carried out on flowers, fruits and leaves of accessions from cacao germplasm (Engels, 1986) which revealed the existence of 2 morphological groups: one composed of the Criollo and Trinitario accessions and the other composed of the Forastero accessions, with a continuous variation between the 2 groups due to several genetic admixtures that occurred in the species. This structure obtained by Engels (1986) was confirmed later by N'Goran (1994) using seed and pod characters. Flower traits used earlier by Enríquez and Soria (1967) and more recently by Lachenaud et al. (1999) allowed the detection of a great variability among cacao cultivars. Globally, all these results showed that morphological markers could allow the structuring of the diversity of different populations in germplasm collections in research stations.

Molecular diversity of cocoa found in Cameroon was previously analyzed by Efombagn et al. (2006, 2008). The need to complement this molecular work with a morphological diversity study became important. Therefore in the current study, there was phenotypic characterization of 300 cacao accessions collected in various farmers' fields, distributed over different cacao growing areas of Cameroon and 77 breeders' accessions available on-station in gene banks of the Institute of Agricultural Research for Development (IRAD), Cameroon. Simple sequences repeat markers were considered verifying if the molecular diversity of farmers and breeders material tallied with the results from the morphological study. The objective was to assess diversity in farmers' and breeders' germplasm material using morphological traits. The level of diversity in farmers' and breeders' popula-tions assessed with qualitative and quantitative phenoty-pic traits for leaves, pods and seeds, as well as the relationships between these 2 types of germplasm were measured. The paper also addressed the exploitation of the phenotypic variation found in gene bank and in farm cacao populations for the purpose of breeding.

MATERIALS AND METHODS

Study site

The study was conducted in the Southern and the Western agro-ecological areas where cacao is grown in the country. Within these 2 areas, the collecting sites ranged from latitude between N02°14.199' and N05°42.924' and longitude between E009°01.430' and E011°20.885'. The Southern part is characterised by heavy shaded cacao plantations with a low level of management and chemical inputs. The average yield varies between 100 and 500 Kg of fermented and dried cocoa per ha (Varlet and Berry, 1997). The soils are ferralitic and acidic and the rainfall pattern includes 2 wet and 2 dry seasons. In the Western area, the climatic conditions for cacao cultivation are relatively favourable and thus cacao plantations are relatively lightly shaded, with a systematic use of chemical inputs (Losch et al., 1992). Yield varies between 600 and 1200 Kg of fermented and dried cocoa per ha. The soils are volcanic and the rainfall pattern includes one wet and one dry season.

Plant material

Cacao accessions used in the study included 300 farm accessions (FA) collected in the Southern (145 FA) and the Western (155 FA) areas and 77 gene bank accessions (GA) as part of the cacao collections of IRAD at the Nkoemvone Research Station (southern Cameroon) (Table 1). Gene bank accessions belong to two genetic groups (GGs) of cacao, or hybrids between the 2 GGs. These GGs include the upper Amazon Forastero (UA) and Trinitario comprising a wide range of hybrids between the Criollo as defined by Cuatrecasas (1964) and Motamayor et al. (2002) and Amazon Forastero, both originating from South America. The cacao trees were randomly selected (by the farmers and the breeders) in the field and assigned accession numbers prior to their transfer and their vegetative propagation in the nurseries on-station. The data on the transferred material were collected in the field in September and October 2004.

Microsatellite profiles of the studied farm and gene bank accessions were used to generate the genetic structure of the cacao material under study. Different steps including PCR (polymerase chain reaction) and capillary electrophoresis were used (Efombagn et al., 2006; 2008).

The 17 morphological and agronomical traits that were recorded in the study are presented in Tables 2 and 3. Plant data including leaf, flower, fruit and seed traits were recorded following the identification of the so-called minimum descriptors from the Bioversity International (formerly called International Board for Plant Genetic Resources Institute (presently called) (Bekele and Butler, 2000; Eskes et al., 2000). These descriptors are reported of being the most discriminative and taxonomically useful ones which preclude redundancy (Bekele et al. (2006). They were also selected for ease of observation, reliability of scoring and for their relation to agronomical value, in the case of seed descriptors. The method used for pod and seed characterization is described by Bekele and Bekele (1996) and Bekele and Butler (2000).

Statistical analysis

Basic statistics and multivariate analysis for quantitative traits: As estimates of diversity study, data analysis for all quantitative morphological characters was done using MINITAB-15 software (Minitab Inc, 2007). Diversity statistics of quantitative traits consisted on descriptive statistics where means and coefficients of variation of different accession groups (AGs) were determined. Analysis of variance (ANOVA) was also performed among different AGs. All the morphological quantitative traits were subjected to Principal Components Analysis (PCA) using the correlation matrix to define the pattern of variation. PCA axes with Eigen values 0.8 were selected to define the variation among accessions for agronomic and morphological traits.

Estimates of diversity study with qualitative traits

For qualitative traits, the phenotypic frequency data were analysed by Shannon-Weaver Diversity Index (SWDI), *H'*, using the formula:

$$H' = -\sum_{i=1}^{K} P_i \ln P_i$$

Where k is the number of phenotypic classes for a descriptor and P_i is the proportion of the total number of accessions (N) in the *i*th class. *H'* was estimated for each of the nine qualitative descriptors (Eskes et al., 2000).

Table 1. Farm and gene bank germplasm used in the study.

Accession status	Accession group (AG)	Genetic group (GG)	Country of origin	Acronym	Number of accessions studied
Gene bank (Nkoemvone Station)	SNK*	Trinitario(Tr)	Cameroon	SNK	36
	ICS		Trinidad	ICS	5
	SNKXICS		Cameroun	SNK600-Tr**	11
	IMC	Upper Amazon Forastero(UA) UA X Tr	Peru	UA	10
	UPA		Ghana		
	UAT		Ghana		
	SNKXUA		Cameroon	SNK600-UAxTr*	15
Total					77
Farm (FA)	FA-South		Southern Cameroon	FA-South	145
	FA-west		Western Cameroon	FA-West	155
Total					300
All					All

*: SNK accessions were selected in farmers' fields in 1950s among the best farmers' trees in term of yield, while SNK600 series were selected on-station in Upper Amazon (UA) x Trinitario (Tr) and in TrxTr crosses, as well as in selfings of Tr (Efombagn, 2008).
**: These two were formerly considered as a common group usually called 'SNK600 series, despite the difference observed in their genetic origin.

RESULTS

Quantitative traits

Among the 300 FA of the study, pods were on average 14.8 cm in length, 7.0 cm in width and 510.6 g in weight (Table 2). Among gene bank accessions, the highest values were recorded in ICS for pod length (18.3 cm), width (8.3 cm) and weight (622.5 g). Farm accessions had a mean seed number of 40.5 for, mean seed length of 23.8 mm, for mean seed width of 13.1 mm and mean individual dry seed weight of 0.92 g. Seed number was lower in the gene bank compared to FA. The highest seed size and weight were recorded for ICS, with 26.6 mm for length, 14.5 mm for width and 1.34 g for weight. In the gene bank, the lowest seed length and weight values of 22.8 mm and 0.93 g, respectively, were found in UA. ICS and UA have registered respectively the lowest (21.2) and the highest (29.0) pod index. The average pod index of the FA was relatively high (26.3) compared to the average of all gene bank AGs. Differences between pod and seeds traits of all AGs of the study were significant, except for seed width (Table 2). Among FA, the accessions from the Western part of the country (FA-West) recorded the highest values for pod traits compared to those of the southern part (FA-South). However, the difference was not significant for seed width between both sets of AGs.

Diversity estimates for qualitative traits

Diversity for each of the nine qualitative descriptors was observed and high diversity values were obtained in both FA and gene bank accessions. Table 3 presents the estimates of SWDI (*H'*) of farm and gene bank accessions. For all the FA, the minimum value of *H'* was 0.42 for pod basal constriction and the maximum value was 0.89 for pod apex form. For all gene bank accessions, the same descriptors recorded respectively the highest and the lowest values, with 0.56 for pod basal constriction and 0.89 for pod apex form. In FA, the *H'* values of flower ligule and leaf colours (not estimated in gene bank accessions) were respectively 0.67 and 0.61 (Table 3).

Principal component analysis

The four principal components (PC1 to PC4) explained 86.0% of the total phenotypic variance. The relationship of these principal components and quantitative variables is given in Table 4. In the first principal component (PC1), all the variables were prominent, except for seed number and width to a lesser extent. In the second principal component (PC2), seed size traits (length and width) were the most significant. A plot of PC1 and PC2 showed the relative grouping of FA and gene bank accessions (Figure 1). No clear separation was observed between FA and gene bank accessions. However, there was a spatial differentiation between the FA of the southern and the western origin.

Relationship between phenotypic and genetic structures

The phenotypic variance revealed by the principal

Table 2. Basic statistics for eight quantitative and agronomic traits in FA and GA.

Trait	Gene bank					Farm			*F* ratio
	SNK	SNK600-Tr	SNK600-UAXTr	ICS	UA	FA-South	FA-West	All FA	
Pod Length (cm)	15.8±2.1	14.1±2.3	13.6±1.6	18.3±1.5	10.8±2.4	13.5±2.8	16.2±2.52	14.8±2.9	2.7*
Pod Width (cm)	7.9±1.0	6.3±0.6	6.6±0.5	8.3±0.5	6.1±1.4	6.0±0.6	8.1±1.05	7.0±1.3	5.4****
Pod Weight (g)	561.2±178.3	515.1±150.9	538.8±101.9	622.5±188.7	471.3±163.9	453.4±134.3	568.1±129.6	510.6±143.7	2.0 *
Seed Number	36.7±4.6	36.7±4.6	40.0±7.6	35.1±1.6	38.8±5.3	39.7±4.5	41.2±5.4	40.4±5.0	2.8*
Seed Length (mm)	22.2±1.9	25.5±1.8	25.5±1.3	26.6±2.1	22.8±2.5	24.0±1.9	23.4±2.5	23.7±1.8	11.1****
Seed Width (mm)	12.3±1.1	13.6±2.3	13.5±1.6	14.5±0.8	13.0±1.6	13.2±1.2	12.9±2.7	13.1±2.1	1.7 ns
Dry weight (1 seed)	1.0±0.3	1.1±0.1	1.2±0.1	1.3±0.0	0.6±0.1	1.0±0.2	0.9±0.2	0.9±0.3	3.1**
Pod index[1]	23.6±3.8	21.8±3.7	23.7±3.3	21.2±2.1	38.0±6.8	26.9±8.6	25.5±6.5	26.3±7.7	2.7*

****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05

[1]: Pod index is expressed as the number of pod needed to produce 1 kg of dried cocoa.

component analysis (Figure 1) was compared with the genetic structure generated by microsatellite analysis (Figure 2) . When the geographic distribution of FA was considered, the phenotypic variation showed a spatial differentiation between western and southern FA (Figure 1), while no genetic clustering could be observed with microsatellite for these 2 FA subgroups (Figure 2). In addition, the proximity between several gene bank accessions and the FA as revealed by phenotypic markers, were not confirmed with microsatellites markers.

DISCUSSION

The study revealed morphological variation within FA and gene bank groups. Pod weight and size, dry seed weight and pod index of the locally selected AGs (SNK and SNK600 series) of the gene bank were higher than those of the farm accessions. Except for seed width, all the quantitative morphological traits were found useful in differentiating farm from gene bank accessions.

There were no differences in seed size (length and width) among SNK accessions and SNK600 series. As observed in a previous study carried on wild cacao trees from French Guiana (Lachenaud and Olivier, 2005), morphological seed descriptors are not always able to discriminate among groups of cacao accessions.

Among AGs of Tr origin, the quantitative characters of the ICS group were higher than those of the locally-selected Tr. Tr differed from UA when all mean values were considered and previous morphological studies have already differentiated these 2 genetic groups (N'Goran, 1994; Bekele et al., 2006). Some AGs like ICS might be of parti-cular interest to breeders because of their supe-rior agronomic traits such as pod index and seed size. Pod characters differed between FA of Southern and Western origin.

According to Lachenaud (2007), variation based on pod traits might be associated with different morpho-geographic groups. Therefore, the difference between the 2 geographic FA groups of our study is rather due to the variation of ecological conditions under which the cacao is grown. Molecular characterization using SSRs markers did not reveal any genetic differences between FA of Southern and Western Cameroon (Efombagn et al., 2008), confirming the hypothesis of an environmental influence on field performances of cacao genotypes.

Based on SWDI (*H'*), all the pod qualitative traits showed variation within farm and gene bank groups of accessions. These descriptors were among the traits found to be most useful for studying the variability of cacao populations (Engels, 1983; Raboin et al., 1993; Bekele et al., 1994; Lachenaud et al., 1999).

When mean values of all the 16 quantitative and qualitative morphological traits of the study were

Table 3. Comparison of Shannon-weaver Diversity Index (*H'*) values for qualitative traits studied in farms and gene bank.

Descriptor	Criterion of comparison			
	Origin		Type of cultivar	
	Farm	Gene bank	Traditional*	Hybrid**
Pod apex shape	0.89	0.89	0.86	0.91
Pod shape	0.69	0.74	0.19	0.86
Pod rugosity	0.65	0.58	0.50	0.70
Pod colour	0.59	0.69	0.56	0.65
Pod husk hardness	0.56	0.68	0.27	0.77
Pod basal constriction	0.42	0.56	0.19	0.56
Cotyledon colour	0.59	0.61	0.57	0.67
Flower ligule colour	0.67	-	0.59	0.73
Leaf flush colour	0.61	-	0.58	0.63

*: Primary germplasm.
**: material resulting from manual or natural pollination in seed gardens or cacao farms.

Table 4. Eigen values r and percentage of variation explained by the first 4 principal components for all the accessions included in the study.

Descriptor	PC1	PC2	PC3	PC4
Eigen values	3.15	1.92	1.08	0.82
Proportion variance (%)	0.39	0.24	0.13	0.10
Cumulative variance (%)	0.39	0.63	0.77	0.87
Pod weight	0.46	- 0.21	0.23	0.18
Pod width	0.38	- 0.34	0.22	0.03
Pod length	0.40	- 0.33	0.23	0.15
Seed number	0.16	- 0.19	- 0.78	0.44
Seed length	0.24	0.51	0.04	0.36
Seed width	0.15	0.56	0.17	0.38
Dry seed weight	0.39	0.30	- 0.03	- 0.58
Pod index	- 0.43	- 0.13	0.43	0.35

considered, the level of diversity between farm and gene bank materials did not vary considerably as shown in Table 2 (field measurements values) and Table 3 (*H'* values). In addition, the genetic diversity study carried out with microsatellite markers (Efombagn et al., 2006) revealed that the level of diversity in farmers' fields was genetically close to that of the accessions maintained in the gene bank. Therefore, the selection process conducted by farmers while growing cacao in Cameroon did not result in a reduction of the diversity existing in cacao farms over the time.

The morphological variation using quantitative traits (PCA) has shown a spatial differentiation between western and southern FA. However, no genetic difference was detected with microsatellite analysis between these two subgroups. The variation found was due to non-genetic factors such as the prevailing cacao growing conditions in both growing regions. According to the PCA, the distribution of FA in relation with gene bank accessions has confirmed that most of the accessions from farmers' fields were close to accessions of SNK and SNK600 series (breeders' material), except for some cultivars from Southern Cameroon. As implication to biodiversity and genetic conservation, the genetic material that harbour genes controlling agronomic traits such as resistance to *Phytophthora* pod rot, is conserved in farmers' fields over several decades through farmers practices. The farmers usually achieve this by selecting pods from their own or neighbouring plantations, prior to the production of seeds for next the plantations (Efombagn, 2006). Quantitative traits of other cacao organs such as leaf and plant growth habit were not used in this study because they were found to have no discriminative value (Ostendorf, 1957). For morphological

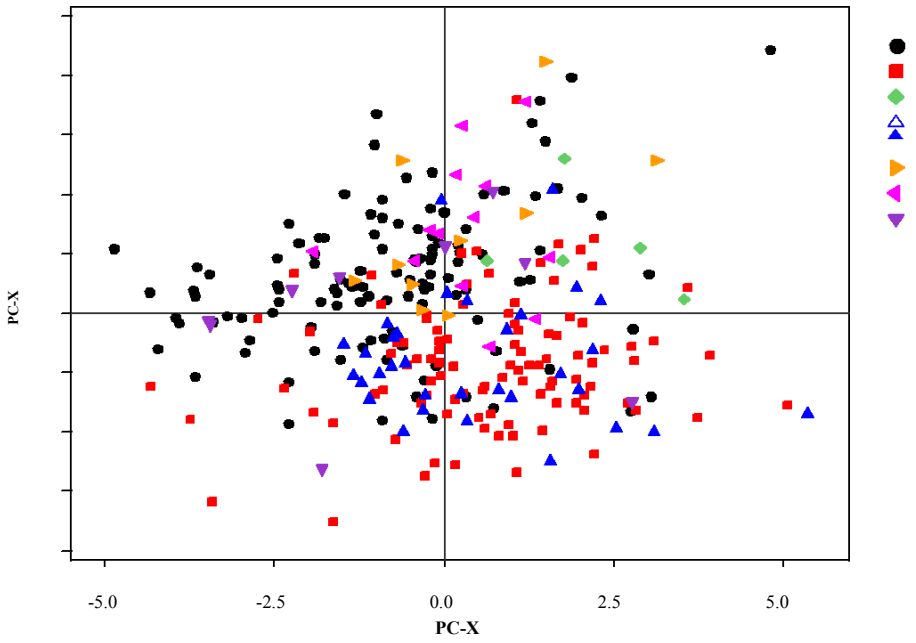

Figure 1. Phenotypic diversity revealed by principal component analysis of all the farm (FA) and gene bank accessions.

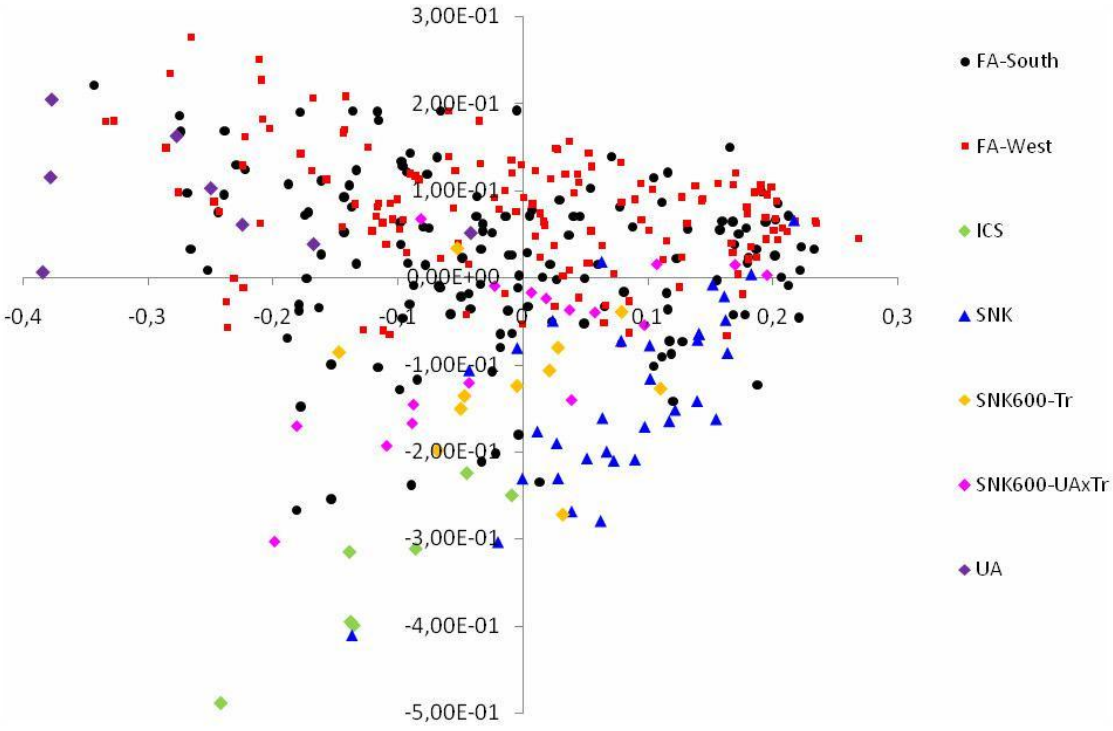

Figure 2. Genetic structure of all the farms and gene bank accessions studied based on microsatellite analysis.

traits of agronomic interest such as seed size, no significant difference was found among farm genotypes. In the cacao selection process in Cameroon, it is therefore suggested to improve seed size by exploiting

gene bank accessions with favourable seed characteristics. However, such traits which are usually quantitative and under polygenic control are under a strong environmental influence (Dias, 2001). Prior to their use in cacao breeding, stability of such quantitative traits should be studied to generate reliable, reproducible data in different ecological conditions (Simmonds, 1981; Engels, 1993) . In future breeding programs, other phenotypic traits of agronomic interest may involve number of pods per tree, which was not investigated in this study, as well as resistance to pest and diseases, and the aromatic and flavour attributes (quality) of cacao.

ACKNOWLEDGEMENTS

The authors would like to thank the CFC/ICCO/Bioversity project titled 'Cacao Productivity and Quality Improvement, a Participatory Approach' for provision of funds and technical support. We also thank the Technicians of IRAD, F. Edoa, I. Badjeck and K.D. Vefonge for their assistance in field data collection.

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