INGENIC Newsletter

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IN THIS ISSUE

From the Editor's Desk::

The INGENIC Committee is pleased to release the 11th issue of its newsletter. This issue will be distributed electronically and also as a hardcopy. To accelerate the availability of its content, several papers were posted on the INGENIC website (http://ingenic.cas.psu.edu) during 2007 as soon as they were ready for publication. We look forward to continued support to maintain this form of communication among all of the stakeholders in the cocoa community.

The past two years were eventful for the cocoa community. The 15th International Cocoa Research Conference, held in Costa Rica on October 9-14, 2006, was very productive and enlightening. The *IN* Meetings, held right after the Conference, continued in the same vein. INGENIC and INCOPED held their 5th Workshops, and INAFORESTA was launched with its first. We hope these groups will continue to serve the needs of cocoa farmers and researchers with active support from the consumers. On Sunday October 14th, prior to the INGENIC Workshop, there was a meeting of the INGENIC Biomol Group where twelve presentations were made that were placed on the INGENIC web-site.

INGENIC would like to express its appreciation to the dedicated local Workshop Organising Committee from the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), led by Drs. Wilbert Phillips and Carlos Astorga, for facilitating the smooth running of the Workshop, the hospitality and enjoyable ambience. The visit to CATIE, Turrialba, and the dinner and festivities that followed were memorable. The INGENIC Committee also wishes to express its gratitude to the Biscuit Cake, Chocolate and Confectionery Association (BCCCA), UK, Bundesverband der Deutschen Susswarenindustrie, Germany, the Technical Centre for Agricultural and Rural Cooperation (CTA), The Netherlands, the United States Department of Agriculture, USA, and the World Cocoa Foundation for financial support and sponsoring participants. CATIE, the Cocoa Producers' Alliance and Mars Inc., USA and UK are gratefully acknowledged for co-sponsoring the Joint Welcoming Cocktail for the three Workshops.

Another welcome development was the formation of CACAONET, a global network for cacao genetic resources. In this issue, two reports are included that incorporate discussions coordinated by INGENIC on CACAONET's proposed conservation strategy.

The progress in cocoa breeding and genetics, disease control and molecular biology has been significant in recent years. The second CFC/ICCO/Bioversity International project on cocoa productivity and quality improvement is well underway, and the meetings such as that held in Ecuador in August have been fruitful. The scientists, assistants and partners involved in the CFC/ICCO/Bioversity International projects are commended for the successes achieved thus far. We wish to specially recognise the sterling contribution of our dearly departed colleague and friend, Dr. Aliyu Abdul-Karimu, formerly of CRIG.

There is a good level of communication among stakeholders involved in the various international cocoa projects, and farmers are already seeing the rewards of these partnerships. This is exemplified in the report, included in this issue, on the Regional Breeding initiative in South-East Asia, an on-going effort to promote exchange of information and germplasm to combat Cocoa Pod Borer.

Congratulations are extended to Dr. Wilbert Phillips, CATIE, Turrialba, Costa Rica, who was awarded the Costa Rican National Award of Science by The Ministry of Science and Technology, for his studies on *Moniliophthora roreri* and frosty pod rot (moniliasis), including discovery of clones with resistance to this disease.

The INGENIC Committee reiterates its commitment to promoting communication among all partners in the international cocoa breeding efforts. It is grateful for the continued financial and or logistical support of the BCCCA, CIRAD, CPA, CRIG, CRU, MCB, Pennsylvania State University, UESC and WCF.

We invite you to start thinking about topics and presentations for the 6th INGENIC Workshop to be held in Bali, Indonesia in November, 2009. You can e-mail suggestions for a topic to Drs.Bertus Eskes or Michelle End.

Please submit contributions for the next issue of the newsletter to me at Frances.Bekele@sta.uwi.edu or louisebekele@yahoo.co.uk as soon as possible. If sufficient contributions are submitted before June 30, 2008, the next issue of the newsletter will be released on time.

Readers are invited to visit the INGENIC website to learn more about recent INGENIC activities including those of the INGENIC Biomol Group. You are also invited to participate in the featured discussion groups.

We look forward to another year of useful interactions and discussions on further partnerships and collaborative research activities among our readership.

With best wishes from the editor of your INGENIC Newsletter and the other members of the INGENIC Committee! Happy New Year!

For the INGENIC Committee,

Frances Bekele

Editor

Preliminary Characterisation and Evaluation of Cocoa on-farm Genetic Diversity in the North-west Region of Guyana

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Abstract

In 2001, 65 trees on 21 certified organic cocoa farms in north-west Guyana, County of Essequibo, on the border with Venezuela, were characterised using 22 qualitative and quantitative morphological traits from the Cocoa Research Unit's (Trinidad) descriptor list. The mean cotyledon weight recorded was 1.04±0.46 g and mean pod index (PI) was 36.3±21.96. Ten accessions were found to have cotyledon weight ≥1.2 g and PI ≤21. Pale-coloured beans, which have no or low concentrations of anthocyanin pigment, were found among 14 accessions. Cocoa trees were also screened for incidence of black pod disease (BP, caused by *Phytophthora* spp.), and witches' broom disease (WB, caused by *Moniliophthora perniciosa*). Forty-seven percent of the total number of pods sampled was infected with *Phytophthora*. Rapid screening of high-yielding and fine or flavour cocoa selections for resistance to BP using the detached pod inoculation method was carried out under laboratory conditions at the National Agricultural Research Institute, Mon Repos Field Research Unit (NARI/MRFRU). On an 8-point disease rating scale, mean cocoa pod disease resistance to BP was 5.5±1.2. Using the pod and bean technical coefficients of yield, the total dry bean loss to BP was estimated at 0.1 kg per tree. WB was not observed on-farm in the north-west region of Guyana although it is reported in other parts of the country. However, when seedlings of high-yielding and fine/flavour cocoa selections from farms in north-west Guyana were inoculated with the pathogen under controlled conditions at NARI/MRFRU, they showed susceptibility to WB. Preliminary results of morphological characterisation and evaluation of on-farm cocoa in north-west Guyana revealed wide phenotypic diversity, with Trinitario being the most important of the genetic groups among the on-farm cocoa sampled, and established the presence of fine-flavour cocoa and the absence of cocoa with symptoms of WB. The trees with desirable traits will be sourced for pods and/or budwood for nursery propagation in support of the organic cocoa expansion in north-west Guyana.

Introduction

In Guyana, particularly the north-west in the county of Essequibo, developments in cocoa during the past 60 years can be attributed to two noteworthy events. Firstly, in 1948, the post-World War II Evan's Settlement Commission report led to the formation of the 'Cacao Development Research Scheme' in 1950. In 1951, demonstration plots of shaded cocoa systems and a cocoa plant nursery were established in all three of Guyana's counties, namely Essequibo at the Hosororo Field Station in north-west Guyana, Demerara and Berbice. Research and development focused on introduction and evaluation of improved cocoa varieties, and fertilizer studies. Secondly, the visit of HRH Prince Charles to Guyana, in February 2000, led to the development of an organic cocoa project in north-west Guyana. The general objective of the project was to increase the number of job opportunities and improve the socio-economic well-being of indigenous people and farmers in hinterland areas of Guyana by rehabilitating the cocoa industry (Chesney 2002).

The five-year (1950-55) Cocoa Development Research Scheme in the then British Guiana contributed to the commercial production of cocoa in all three of Guyana's counties namely, Essequibo, Demerara and Berbice. Acreage expanded from about 400 ha in 1950 to 800 ha by 1969 and production of dry cocoa beans increased from about 20 t to 200 t over the corresponding period. Most of the production was in Demerara and Berbice. Production plots in north-west Guyana were at a higher elevation and the only means to commute between north-west Guyana and Demerara/Berbice was by light aircraft or by river systems; an arduous 26-hour journey. Cocoa expansion faced serious challenges from diseases, including *buba* disease or cushion gall disease, WB in Demerara and Berbice, and BP everywhere (Chesney 2002).

Sources of the existing cocoa genetic diversity in north-west Guyana include old Dutch plantations in the Berbice River, the International Cocoa Genebank, Trinidad (ICG,T), Venezuela and Brazil (Bartley 2005; Johnston 1997; Chesney 1997). During 2001 and 2002, on-farm cocoa genetic diversity in north-west Guyana was characterised and evaluated to facilitate the selection of trees on farms for nursery propagation and production expansion in support of certified organic cocoa production for export.

Site conditions

The climate of north-west Guyana is wet tropical with mean annual rainfall of 2750 mm (range 1528-3358 mm) and mean annual air temperature of 26.5^{0}C (temperature minima range: 21.2-22.6 ^{0}C; temperature maxima range: 29.3-31.5 ^{0}C). Diurnal relative humidity ranges from 92.6-99.9% (morning) to 69.0-89.4% (afternoon). Soils are reddish-brown laterite classified as Hosororo gravely clay (Ministry of Agriculture 1975). Cocoa farms are found on lower slopes and at the bottom of laterite hills. There were less than 50 farms on 30 ha-equivalent of land. Average farm size was 1.8 ha with one-third of the original 750 trees ha^{-1}. The age of cocoa trees ranged from 30-80 years-old. A wide variety of shade trees, dominated by Inga and *Pentaclethra*, were found (Corbin 2001).

Morphological characterisation of on-farm cocoa

During 2001, the cocoa descriptor list adopted by the Cocoa Research Unit (CRU), The University of the West Indies, Trinidad (Bekele and Butler 2000) was used to characterise 65 cocoa trees on 21 farms in the localities of Wanaina, Bumbury, Hosororo and Mabaruma Settlement in north-west Guyana. Data were collected based on 22 qualitative and quantitative traits. Trees, from which samples were taken, were labelled and geo-referenced to facilitate future identification. Photographic records of each accession were created, and these were visually assessed at CRU to determine similarities to clones conserved in the ICG,T. Cocoa yield and fine flavour potential were analyzed using the cluster analysis program of Minitab ver. 13 (Minitab Inc. 2000).

The results of the cluster analysis showed that the 65 accessions could be grouped into 12 clusters. In determining the presence of 12 clusters, the morphological characteristics, in order of statistical importance, were pod type, seed number, seed pigment, seed dry weight, pod length, and pod diameter (Table 1). One-third of the clusters contained six or more accessions, representing two-thirds of the total number of accessions sampled.

Table 1: Distribution of 65 on-farm cocoa accessions into varietal groups based on six parameters

Parameter	Mean (s.d.) for all accessions	Clusters			
		1	3	4	8
Number of individuals in cluster		7	10	22	6
Predominant pod types	amelonado & angoleta	cundeamor	angoleta	amelonado	amelonado
Bean number	3.8 (9.1)	37.1 (3.0)	39.9 (7.5)	34.5 (9.6)	28.3 (7.9)
Cotyledon pigmentation	3.2 (2.0)	3.1 (2.3)	3.2 (2.3)	3.1 (1.8)	4 (1.1)
Cotyledon dry weight (g)	1.0 (0.5)	1.2 (0.5)	1.1 (0.4)	0.8 (0.3)	1.4 (0.4)
Pod length (mm)	165.9 (31.7)	181.5 (7.2)	180.8 (18.7)	144.3 (15.9)	165.9 (10.7)
Pod diameter (mm)	81.3 (10.8)	79.6 (11.5)	80.7 (6.5)	78.0 (9.5)	90.3 (6.1)

Key: Pod type based on modified scoring of classes: (0=cundeamor; 1=amelonado; 3=angoleta; 5=calabacillo; 7=other); cotyledon pigmentation (0=white; 1=light violet; 3=medium violet; 5=dark violet)

The dominant pod type was amelonado. Mean bean number per pod was approximately 35 with mean cotyledon colour being medium violet. Light coloured beans were present among predominantly violet coloured beans in the pods. Pale or light coloured beans, which have no or low concentrations of anthocyanins, are thought to be of Criollo or Trinitario origin, and are associated with fine-flavour quality. Fourteen cocoa accessions were observed to have pale coloured beans mixed with violet coloured beans. No pod was observed to contain a predominance of light coloured beans.

Mean pod index was 36.3 (range: 10.2 to 124.5). Based on yield potential indicators of cotyledon weight ≥1.2 g and pod index ≤21, 10 accessions were found to have good yield potential. Preliminary results based on pod and bean characteristics show that the majority of the accessions closely resemble Trinitario, some with red pods, (59%), with hybrids (14%), unknowns (14%) and Forastero (13%) making up the remainder.

Evaluation of on-farm cocoa

During the period September 2001 to June 2002, 18 cocoa farms were surveyed and the trees were monitored for symptoms of cocoa diseases. Rapid screening of selected accessions for resistance to black pod (BP) disease, using the detached pod inoculation technique (Iwaro *et al*. 2000), and witches' broom (WB) disease, using a seedling inoculation technique (Suarez-Capello 2000), was carried out at the Mon Repos Field Research Unit Plant Nursery of the National Agricultural Research Institute. Monthly rainfall recorded at the Hosororo Station during the months of September, October, November 2001 and June 2002 was 293.7, 189.8, 171.8 and 327 mm, respectively.

Forty-seven percent of the total number of pods (1098) sampled from 139 trees was naturally infected in the field with *Phytophthora.* Using the pod and bean yield technical coefficients, the dry bean loss to disease would be approximately 0.1 kg per tree sampled. Infection caused by other pathogens was confirmed using Koch's postulates (Wheeler 1976) in a greenhouse experiment.

With respect to the screening for BP resistance, on an eight point disease rating scale, cocoa pod disease resistance to BP varied from 3.7 (moderately to partially resistant) to 7.5 (susceptible to highly susceptible) with a mean of 5.5±1.2 (partially resistant to moderately susceptible). Rapid screening of selected accessions for resistance to WB revealed that all accessions developed multiple symptoms of the disease. Stem swelling was the most common symptom and cankers without broom formation, the least common.

On-farm monitoring and laboratory screening identified seven pathogens on the cocoa plants. Infected pods were removed from trees and infection testing was done without wounding. The main organism identified was *Phytophthora,* the causal agent of BP. Diagnostic testing confirmed the identity of *Phytophthora.* Based on farm data collected between August 2001 and June 2002, the potential dry bean loss due to BP is 0.1 kg per tree per year. The other pathogens *(Fusarium, Aspergillus, Penicillum, Colletotrichum* and *Rhizopus)* were considered secondary or opportunistic pathogens and were thought to be of no economic importance to cocoa farming in north-west Guyana.

Symptoms of WB were not observed on any of the farms where sampling was done. However, artificial inoculation of seedlings grown off-farm resulted in successful infection of the aforementioned seedlings. It would appear that the peculiar ecological and environmental conditions as well as careful plant management in the past have kept the pathogen out of the growing environment. A plausible inference is that the pathogen is absent, or if present, is in a non-virulent form, or the ecological conditions in the north-west growing environments suppress the development of the disease. This hypothesis should be tested as soon as possible. The north-west Guyana cocoa farms are geographically separated from cocoa farms in Demerara and Berbice by great distances, hills, rivers, and travel from other cocoa producing areas to the north-west is limited to small, light aircraft and an arduous 26-hour journey by river.

Conclusion

The preliminary evaluation of 65 on-farm cocoa trees confirmed the presence of diversity, and good yield and quality in this population of cocoa. The ecological characteristics of the localities provide ideal conditions for small-holder farming of organic cocoa. Further evaluation of resistance/tolerance to black pod and witches' broom diseases using molecular techniques is recommended. Further investigation on the absence of WB on the north-west Guyana farms is recommended.

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Ortet Selection in Cacao Progenies from the Kérindioutou River in French Guiana

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During a collection survey conducted in 1990 in the upper reaches of the Oyapok River, the border between French Guiana and Brazil, three populations of geographically distant cocoa trees were identified and collected (Lachenaud & Sallée 1993). One of them ("KER"), the largest, which stretched for about 10 km on both banks of the Kérindioutou River (the name of the Oyapok in that zone, see Figure 1) seemed to be very uniform in the appearance and size of its pods. However, it could have been divided into several sub-populations, since the cocoa trees did not occupy the riverbanks in a continuous manner. Five sub-sets were noted, of unequal size and density. Pods or budwood were taken from 14 mother-trees and they were all planted in a collection at CIRAD's Paracou-Combi station, at Sinnamary (French Guiana). The seedlings, grown from the pods, were then planted in a small isolated plot, and the budded plants in a budwood garden.

Various observations and studies have been carried out on that planting material. Those relative to floral descriptors and isozyme electrophoresis have already been published (Lachenaud *et al*. 1999; Lachenaud *et al*. 2004). They showed that the "KER" population was original and stood out clearly from the other populations of wild Guianan cocoa trees studied as well as from two Amelonado control clones. This article describes certain agronomic descriptors with a view to selecting representative KER clones with interesting agronomic traits to be used for further studies and to represent the KER populations in collections.

Materials and Methods

During the survey in July 1990, pods were harvested from seven mother-trees belonging to four sub-populations (KER 1, KER 2, KER 8 and KER 11). The seedlings obtained, which were thus seven open-pollinated progenies, were planted in March 1992 in an isolated plot at a density of 1333 (3 m x 2.5 m spacing) in a totally randomized single-tree plot design. At the time of planting, there were 15 trees per family for the KER 1 and KER 2 progenies, and 10 for the five other progenies (hence 80 trees in all). The trees were individually harvested from April 1995 to October 2000 and by that date there remained 63 trees in the trial.

The agronomic criteria studied were:

- juvenile growth, *i.e.* the increase in cross-section (in cm^2) 20 cm from the ground, between 1 and 2 years in the field, for the 64 trees surviving the first two years,
- the trunk cross-section (in cm^2) 50 cm from the ground, at 6 years,
- the numbers of healthy and rotten pods collected,
- the total weight of healthy pods,
- the average weight of one healthy pod,
- the production : vigour ratio (cropping efficiency) at 6 years.

The data were conventionally analysed (using ANOVA) for the "sub-population" (KER 1, 2, 8 and 11) and "family" factors. Homogeneity of variances was verified using the Levene test (at 5%).

Results

Table 1 shows the results presented by sub-population. No significant difference was noted between the sub-groups for any of the variables and the same applied for the progenies (data not shown).

Table 1: Agronomic traits of the four sub-populations originating from the Kérindioutou River, between March 1992 and October 2000, in plot A2 at Paracou-Combi.

S-pop.	Juv. gr.	Sec98	CE 98	Total	TW	APW
KER 1	11.85	42.08	0.0136	6.61	1.77	330
KER 2	11.64	51.54	0.0275	9.13	2.65	286
KER 8	8.37	31.21	0.0076	7.00	1.78	245
KER 11	10.89	49.47	0.0151	7.59	1.97	270
Total	11.03	46.70	0.017	7.70	2.07	280

Juv. Gr. = juvenile growth, Sec98 = trunk cross-section in 1998, CE 98 = cropping efficiency in 1998, Total = total number of pods per tree, TW = total weight of healthy pods, in kg, and APW = average pod weight (in g, for trees that produced at least one healthy pod).

Under these conditions, mass ortet selection was carried out based on classes for the CE98 or TW criteria. The small number of pods precluded valid selection for field resistance to pod rot diseases or pod size. The ortets chosen are indicated in Table 2.

Table 2: Selected ortets

Name	TW (kg of pods per tree)	CE98 (kg pods/cm^2)
KER 2-L	14.15	0.160
KER 2-P	12.80	0.145
KER 11-1-L	9.65	0.059
KER 11-4-L	9.05	0.046
KER 1-P	8.80	0.046

Discussion and Conclusion

The low overall production in the plot should be noted. On average, only eight pods were counted per tree in the first five harvesting years. That might have been attributable to the previous crop cover (coffee) and, to a degree that is difficult to determine, to thefts and imperfect monitoring. The absence of a control of known performance prevented any conclusion from being drawn. Nevertheless, it can be said in relation to that low production or late start to production, that the vegetative development of the trees was also relatively limited. For instance, at 4 years the average diameter was 5.56 cm (50 cm from the ground), as opposed to 8.50 cm at 3.5 years one metre from the ground in a neighbouring plot of Amelonado and "local" Trinitario trees (= progenies of germplasm formerly grown in French Guiana). Also worth noting is the low average weight of one pod (280g), compared to that of cocoa trees originating from the Camopi and Tanpok valleys (367g, Lachenaud *et al*. 2000). These facts might reflect a particularly unsuitable environment for cocoa, which also seemed to be confirmed by the performance of a clone from the same area, clone KER 6, sampled in budwood form and budded (hence grafted) in a budwood garden approximately 300 m from plot A2, which produced more pods over the same period weighing an average of 481 g (with extremes of 272 and 738 g). This suggests that this clone was growing under more favourable conditions than the KER seedling populations.

Nevertheless, as the KER material is self-incompatible (Lachenaud & Oliver 2005), relatively uniform and growing in an isolated plot, it could not be ruled out that a certain degree of inter-incompatibility might partly explain the low production recorded. That possibility would have to be further tested.

Perhaps due to the poor general performance of the plot where the KER populations were planted, the open-pollinated progenies and the sub-populations did not display any significant differences for the set of criteria studied, thereby vindicating the fact that only a single uniform population is being considered for further studies, named as the "KER" population. Of the trees with the most worthwhile performance for the efficiency and production level criteria, five can be cloned and, along with clones of the mother-trees KER 3, KER 5, KER 6, KER 7 and KER 9, they are proposed to represent the "KER" population in collections or in genetic diversity studies, or serve as parents in breeding programmes.

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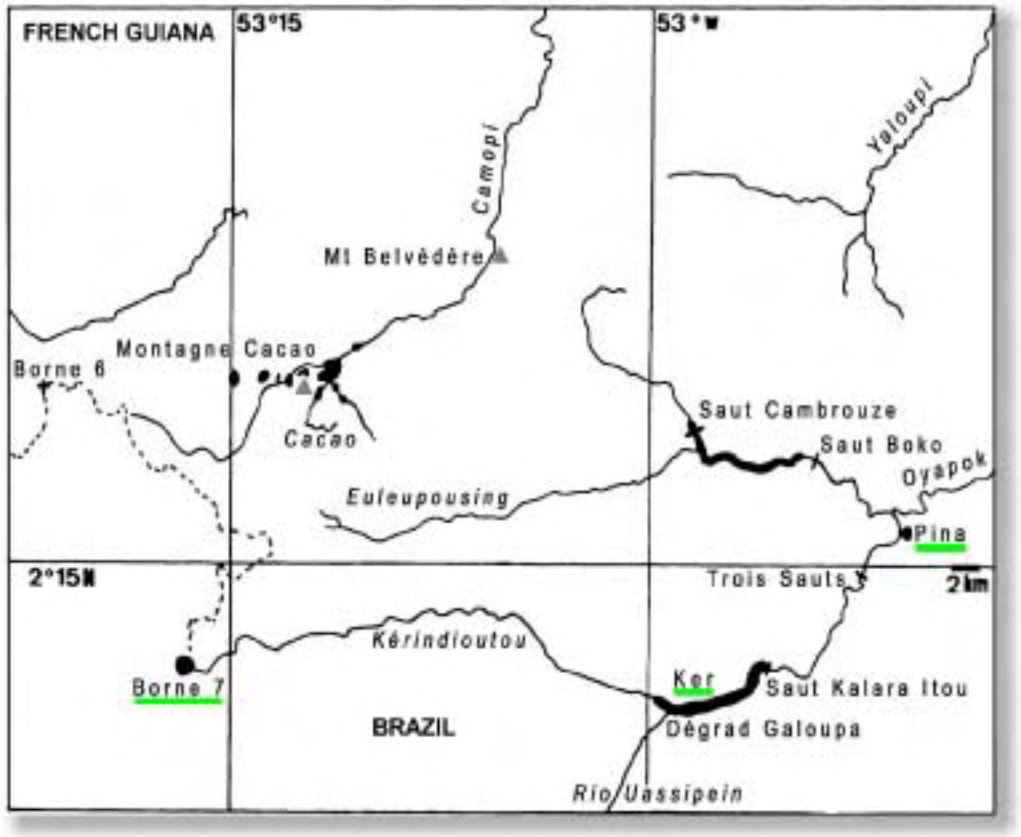
Figure 1: Location of certain natural cocoa tree populations in south-eastern French Guiana (the Borne 7, KER and Pina populations were surveyed in 1990)



Efficacy of Simplified Methods to Assess Pod Production in Cocoa Breeding Trials

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Scope

Dry cocoa yield is determined by three components: bean weight per pod, number of pods per tree (pod production) and number of trees per hectare. In cocoa breeding trials, dry bean yield is usually assessed over a five to eight-year period by measuring the total wet bean weight, which is multiplied by a conversion ratio (of about 0.4) to obtain dry bean weight (Toxopeus 1969). This entails harvesting pods, opening these pods and weighing the wet beans during each round of harvesting. In some countries, only pod counts are made at each harvesting round, and the pod index (number of pods required to obtain one kilogram of dry cocoa beans) is used to obtain the amount of dry bean yield. This depends on the correct estimation of the pod index, through repeated measurements, because of the significant variation due to harvesting season and genotype. Although the pod index is not correlated with production, it is an important trait in itself since farmers prefer a low pod index as less work is involved in harvesting and pod breaking.

Instead of weighing of wet beans, Lachenaud (1984 and 1991) has proposed weighing of the whole pods. Dry bean yield is then obtained by multiplying the pod weight with a conversion ratio of 0.0875. This conversion ratio is based on an average pod weight x wet bean ratio of 0.25 and an average wet bean x

dry bean ratio of 0.35. These ratios may each vary quite substantially with the season and also with the genotype (Toxopeus 1969), although the average ratios may be quite similar for segregating hybrid varieties of a similar type, *e.g.* Upper Amazon x Amelonado crosses commonly tested in variety trials in Côte d'Ivoire. The method based on counting the healthy and diseased pods and weighing the healthy pods has been used as the standard method for estimation of dry cocoa yield in variety trials at the CNRA in Côte d'Ivoire since 1984 (Lachenaud 1991).

The output of the breeding programme is based on the quality of the material placed in the trials and also on the size of the trials, *i.e.* the total number of genotypes that can be reliably observed. Maintenance and evaluation of breeding trials are expensive and labour-intensive; therefore financial resources are often the limiting factor for the size of the breeding programme. Currently, there is a generalised interest in participatory selection approaches including the establishment and evaluation of a large number of on-farm variety trials.

The evaluation of such trials is even more laborious and time-consuming than those on-station, if the traditional evaluation methods are to be applied. The objective of the current study is to assess the feasibility of assessing the pod production of different varieties using simplified methods, while maintaining the basic quality of the observations required for successful cocoa breeding and selection.

Materials and Methods

Observations were made during the 2002/03 and 2003/04 harvesting campaigns on a total of 476 trees belonging to 20 hybrid families (crosses between Upper Amazon and Trinitario genotypes) planted in a variety trial established in 1993 at the Divo Research Station of the CNRA. The hybrid families, with at least 15 trees each, were planted in a completely randomised design with single-tree plots. Five methods were compared to evaluate pod production:

Method 1. Pod counting as part of the standard method adopted routinely by the CNRA (Lachenaud 1984). For the 2002/03 and 2003/04 cropping seasons, three monthly harvesting rounds were conducted during the main cropping seasons and one harvesting round for the mid-season crops.

Method 2. Counting of all pods older than two months (longer than about 14 cm) just before the beginning of the harvest of the main crop (September) and before the mid-season crop (May).

Method 3. Scoring of the number of pods on a 0 to 5 point scale (0 = no pods and 5 = trees with more than 50 pods per tree).

Method 4. Counting of the number of active flower cushions (with cut peduncles still visible) on the main stem and branches, up to the height of 2 metres above ground level, just after the harvest of the mid-season crop (June 2003, not observed in 2004).

Method 5. Scoring of the number of active flower cushions using a 0 to 5 point scale (0 = no active flower cushions and 5 = trees with more than 20 active flower cushions).

Method 2 aims at estimation of actual pod production, whereas Methods 3, 4 and 5 would allow only estimations of relative pod productions to compare varieties. During the 2003/04 main harvest campaign, Methods 2 and 3 were also applied to estimate levels of infection with *Phytophthora* pod rot (Ppr).

For Methods 1, 2 and 4, two technicians were required to carry out the observations, whereas for Methods 3 and 5 one technician did all the observations. The observations for the simplified methods (2 to 5) were carried out independently (*i.e.* at different intervals than for the observations for Methods 2 and 4).

Results

The number of hours for a technician to carry out the observations per ha and year has been estimated to be 270, 178, 22, 58 and 18 for the Methods 1 to 5, respectively.

The average number of healthy pods harvested per tree according to the Methods 1 and 2 in the 2002/03 main and mid crops were 31.6 and 32.5 and 5.4 and 7.1, respectively. This shows a very good agreement between actual pod production estimated by Methods 1 and 2.

For both harvesting campaigns (2002/03 and 2003/04), similarly high coefficients of correlation were obtained between the results of Method 1 and those of Methods 2 and 3 (Table 1). For the cumulated data over the two harvesting campaigns (2002/03 and 2003/04), the r-values were even higher (0.77 to 0.99).

Method 2 (counting of pods before the main and mid crops) was observed to be better correlated with Method 1 than Method 3 (scoring of the number of pods before the main and mid crops). Interestingly, for Method 3 the r-values were lower for family means than for individual tree measurements. The correlations for the main crop data alone were as significant as when the counts or scores of both the main and mid crops were considered (data not shown). This is likely due

to the low number of pods harvested in the mid crop in relation to the main crop.

For the 2002/03 harvesting campaign, all simplified methods (2 to 5) were compared to the pod production as estimated by the standard method in the same year and also with cumulated pod production over a 6-year period (Table 2). The coefficients of correlation were the highest for Method 2, both for individual tree and family means. For individual trees, the second best method was 3, but surprisingly, for family means the second best method was 5 (scoring of number of active flower cushions). The coefficients of correlation were quite low for family means estimated with Method 3 and for individual tree means estimated with Methods 4 and 5. Similar results were obtained for the 2003/04 harvesting campaign (data not shown).

During the 2003/04 main harvesting campaign, the incidence of Ppr infected pods was evaluated by the standard method and estimated by Methods 2 and 3. According to the standard method, the average infection was 16%, with a variation from 9 to 31% among the 20 families. Table 3 shows significant coefficients of correlation between the standard and simplified methods, with relatively high values for family means. The r-values were lower, but still significant, when comparing the 2003/04 estimated Ppr incidence to the average Ppr incidence over the 6-year period.

Table 1: Rank coefficients of correlation between the standard method and two simplified methods used to estimate yield of healthy pods in two harvesting campaigns (with four harvesting rounds per campaign)

Simplified methods	Individual tree (T) or family means (F)	Method 1 (standard)		
		2002/03	2003/04	Total
2	T	0.91 ***(1)	0.94***	0.95***
	F	0.92***	0.97***	0.99***
3	T	0.80***	0.81***	0.87***
	F	0.74***	0.65**	0.77***

(1)Level of significance at P=0.01 (**) and P= 0.001 (***)

Table 2: Rank coefficients of correlation between the standard method (2002/03 campaign and accumulated pod production over a six-year period (1996-1999 + 2002-2005) with four simplified methods used to estimate yield of healthy pods in one harvesting campaign (2002/03)

Simplified methods (2002/03 campaign)	Individual tree (T) or family means (F)	Method 1 (standard)	
		2002/03	1996-1999 + 2002-2005
2	T	0.91 ***(1)	0.83 ***
	F	0.92***	0.92***
3	T	0.80***	0.71***
	F	0.74***	0.67**
4	T	0.57***	0.61***
	F	0.78***	0.86***
5	T	0.49***	0.54***
	F	0.84***	0.89***

(1) Level of significance at P=0.01 (**) and P= 0.001 (***)

Table 3: Linear coefficients of correlation between Ppr incidence evaluated by two simplified methods during the 2003/04 main crop and Ppr incidence evaluated with the standard method for the 2003/04 main crop and for a six-year average of Ppr incidence (only main crop)

Simplified methods 2003/04	Individual tree (T) or family means (F)	Method 1 (standard)	
		2003/04 main crop	1996-1999 + 2002-2005 main crops
2	T	0.69**(1)	0.56**
	F	0.89***	0.73***
3	T	0.71***	0.52**
	F	0.82***	0.43*

(1) Level of significance at P=0.01 (**) and P= 0.001 (***)

Discussion

The estimation of the amount of labour necessary for the different methods shows a major reduction when scoring is performed instead of counting. In addition, counting of flower cushions is much less labour-intensive than pod counting.

The results show that counting of pods before the main and mid crops (Method 2) can give a reliable estimate of the real number of pods harvested. This was shown over two harvesting campaigns (years). Accumulation of data over two years increases the correlation between the simplified methods and the standard method, at least for Method 3.

The high correlation (r = 0.92) of one-year pod production for family means with cumulated pod production over 6 years is surprising. It would suggest that one year yield data of adult cocoa trees can provide an indication for long-term pod production. To verify if this conclusion can be generalised, two other trials with 4 and 10 years of yield data were analysed. The coefficients of correlation between one-year yield and cumulated yield increased from about 0.45, for the first year, to 0.90-0.95 for the second and fifth years onward, respectively, for the two trials analysed. This suggests effectively that for estimation of long-term pod production, it may not be necessary to make observations over all the years.

Scoring of the number of pods using a 0 to 5 point scale (Method 3) tended to give slightly lower coefficients of correlation with the standard method than pod counting (Method 2), and this was especially so for family means.

The counting and scoring of active flower cushions at the end of the mid crop (Methods 4 and 5) appear to be promising new methods for estimating average family pod production for the same harvesting campaign (Table 2) and for cumulated pod production (r = 0.78 to 0.89). However, the correlation at individual tree level was much lower (r = 0.49 to 0.61). This would suggest that the error variance at the individual tree level for scoring of active flower cushions is larger than for family means.

Counting and scoring of rotten pods before harvest campaigns seemed to be informative for the actual degree of infection during the harvest campaign, especially at the level of mean family values. This is less so for the cumulated degree of infection over several years.

Applications of simplified methods

Estimation of actual pod production by pod counts before the main harvesting periods

The results indicate that individual tree and average hybrid family pod productions and Ppr incidence can be reliably estimated under the conditions of cocoa production in Divo by counting healthy and rotten pods older than 2 months at the beginning of the harvesting season (Method 2). This method would result in a substantial reduction in the amount of work and time spent by technicians to obtain reliable observations on actual pod production in large selection trials and especially so in distant breeding or on-farm trials. Of course, the method has to be accompanied by the reliable estimation of the pod index to convert the pod production into actual dry bean yield for each variety tested.

Similar results with Method 2 might be expected in other cocoa producing regions, where pod production is concentrated in a main and a mid season crop. The possibility of adapting this method to locations with a more continuous cropping pattern remains to be shown.

In such locations, the counting of the number of pods on the trees will need to be done more frequently (*e.g.* at three-month intervals).

Estimation of relative pod productions

In most breeding trials (*e.g.* population breeding trials, observation trials, on-farm trials), it is not necessary to obtain actual yield estimates. What is most important to breeders is to be able to select the best varieties.

This can be done by relative yield estimates (in comparison to the average for the trial or to well-known control varieties). In our study, Methods 3, 4 and 5 were compared for their efficacy in estimating relative pod production.

For relative pod production of individual trees, Method 3 (scoring of pod production on a 0 to 5 point scale before the main crop) was the best for assessing short and long-term pod production (r = 0.80 and 0.71, respectively). For family means, Method 5 (scoring of the number of active flower cushions) was the best to assess short and long-term pod production (r = 0.84 and 0.89, respectively). Methods 3 and 5 only required 22 and 18 hours to evaluate one ha of cocoa trials, which represents a saving of more than 85% of time compared to the standard method and to Method 2.

Transformation of relative pod production into relative dry bean yields

The relative scores for pod numbers (Method 3) or for active flower cushions (Method 5) should be transformed into relative dry cocoa yield by using the pod index. This can be done by multiplying the average family score with the relative pod index (average pod index for the trial/mean pod index of the family). An average score of 3 for the amount of pods or of active flower cushions for a family with pod index of 20, in a trial with average pod index of 30, would become 3 x (30/20) = 4.5 for relative dry bean yield.

For comparison of relative dry bean yield of individual trees by using Method 3, it is required that information be available on the relative pod index. This can be obtained by weighing 10 pods, or preferably recording the wet bean weight of 10 pods of selected trees during the same harvesting round. The pod weight or wet bean weight can be transformed into a pod index (PI) by using the average conversion ratios published by Lachenaud (1991), *i.e.* 0.25 for pod weight x wet bean ratio and 0.35 for the average wet bean x dry bean ratio.

Conclusions

The results suggest that simplified methods can be efficient in the evaluation of relative pod production, as well as for the estimation of the proportion of diseased pods (in our case this was *Phytophthora*, but the same may apply to other diseases such as *Monilia* and Witches' Broom). This would allow for savings of up to 80% in time and financial resources to select new varieties in hybrid or clonal trials or to select promising trees within segregating populations. The use of simple and cost-efficient methods will be especially valuable in the evaluation of trials established at distant sites (such as on-farm trials), for which it is very difficult to collect yield data at monthly intervals.

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High Yielding Cocoa Varieties of the Central Plantation Crops Research Institute, India

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Introduction

The cocoa breeding programme of the Central Plantation Crops Research Institute (CPCRI) encompasses germplasm collection, evaluation, selection and hybridization. The hybridization activities were conducted from 1983 onwards to develop high-yielding varieties with specific characters. The hybrids were established in four progeny trials to assess their performance. One clonal trial was conducted with the objective of developing high-yielding clones. The major objectives of these programmes were to develop superior hybrids and clones with distinct evidence of heterosis and medium canopy size that can easily be accommodated in arecanut and coconut gardens. These activities resulted in the identification of two hybrids; I-56 x II-67 and ICS 6 x SCA 6, and a clone, NC- 45/ 53, that have high yields, an early bearing habit, medium canopy size and superior bean qualities.

1. I-56 x II-67

This hybrid variety is a product of a progeny trial conducted with seventeen hybrids (seedling progenies) along with their parents (seven clones) and a control progeny (open-pollinated I-56 seedlings) (Table 1). This trial was laid out during 1984-85 in a randomized complete block design replicated thrice with a plot size of eight plants at a spacing of 2.7 m x 5.4 m under arecanut shade at CPCRI, Regional Station, Vittal, Karnataka.

Growth and yield were recorded from the second year of planting, and conclusions were drawn from six years of pooled data and also from the annual performance of the hybrids. Seven superior hybrids were identified with dry bean yields in excess of 1.3 kg. The hybrid, I-56 x II-67, produced the highest dry bean yield of 1.48 kg/plant/year compared to all other hybrids and parents. The parents involved in this cross were originally obtained from Landas Estate, Malaysia in 1968. This hybrid showed a heterosis value of 39.6 percent above the mid-parent value and a genetic gain of 74.2 per cent yield over that of the control progeny, which produced only 0.74 kg/tree/year. The beans of I-56 x II-67 weighed 1.00 g (dry weight) and had a pod index value of 23.8. This hybrid also showed vigorous growth with a girth of 50.7 cm 12 years after planting. Detailed descriptions of morphological, pod, bean and quality characters are presented in Table 3.

2. ICS 6 x SCA 6

A set of nine bi-parental crosses ("F_1" hybrids), along with "F_2 progenies" of four Malaysian hybrids imported from Malaysia in 1968, and a control (F_2 progeny of PA 7 x NA 33) were planted at two sites during 1986-87 (Table 2). These hybrids were established under arecanut (at Vittal) with a spacing of 2.7 m x 5.4 m and under coconut (at Kasaragod) in a randomized block design (RBD) with three replications and eight plants/plot with a spacing of 5.4 m x 5.4 m in a quincunx system. Pod and bean values were recorded for all the treatments over a period of five years. Eight trees were identified as high yielders and have been planted in a comparative yield trial. With regard to the average performance of all the progenies, the ICS 6 x SCA 6 hybrid has shown superiority in almost all of the five years of observation, with a yield of 1.15 kg dry beans per plant per annum, and with a canopy of $10m^3$ at the age of 10 years after planting. The range for the adult tree production over the two environments for individual progeny trees of this cross is 0.54 to 1.76 kg/tree/year.

3. NC-45/ 53

Eight high-yielding trees of Nigerian origin (NC-102, NC-119, NC-73, NC-63, NC-13, NC-116, NC-53 and NC-8) that were derived from seeds obtained from Nigeria during 1974, were selected during 1985, multiplied clonally (by side-grafting) and used in a clonal trial. This experiment was laid out in a RBD along with two checks represented by the I-14 open-pollinated seedling progeny and I-14 clone. Thirty-six clones of each of the 10 treatments were planted during 1985 in three replicated blocks in a quincunx system comprising of 12 plants (plot size) with a spacing of 5.4 m x 5.4 m.

Annual growth and yield parameters were assessed after stabilization of yield *i.e.* after six years of planting. Based on the pooled data and the yield stability indices over 6 years (1991-1997), the clone NC-45/53 was selected. This clone had the highest yield per plant per annum (0.93 -1.73 kg) with a lower value for coefficient of variation for individual tree yield. NC-45/53 has an additional quality of being self-compatible, and had an average dry bean yield of 1.33 kg, with $10m^3$ canopy, in 5 rounds of harvest at the age of 10 years. Nine other superior Nigerian clones with a yield potential of >90-pods/tree/year were also identified in this trial.

4. Conclusions and Perspectives

The two hybrids (I-56 x II-67 and ICS 6 x SCA 6) and the clone NC-45/53 have been selected and found to be suitable for arecanut gardens of coastal Karnataka and coconut gardens of Kerala. They are under investigation to determine their adaptation in other areas such as the Tamil Nadu and Andhra states of south India and north-eastern India, respectively. They have been selected under the following conditions: lateritic to loamy soil with pH of approximately 5 to 6 and with good drainage, temperature range of 26-38^{o}C, minimum precipitation of 1000 mm with a protective irrigation supplement for 5-6 months. They come into bearing in the 2nd year after transplanting, if clones are used, and 3 years

Table 1: Parents and progenies involved in the trails

1No.	Hybrids	No.	Hybrids	No.	Parents
1	I-14 x NC-42/94	10	I-56 x III-105	18	I-14 (Jerangau Red Axil)
2	I-14 x IV-20	11	II-67 x NC-42/94	19	I-56 (PA 7 x NA 32)
3	I-14 x I-56	12	II-67 x IV-20	20	II-67 (Landas-364*)
4	I-14 x III-35	13	III-35 x NC-42/94	21	III-35 (Amelonado x NA 33)
5	I-14 x II-105	14	III-35 x IV-20	22	III-105 (Amelonado x PA 7)
6	I-56 x NC-42/94	15	III-105x NC-42/94	23	IV-20 (Landas-357)
7	I-56 x IV-20	16	III-105 x IV-20	24	NC-42/94 (T86/2)
8	I-56 x II-67	17	III- 105 x I-56	25	I- 56 check
9	I-56 x III-35				

Table 2: Progenies involved in the trial

No.	Hybrid	No.	Hybrid
1	ICS 1 x SCA 6	8	NA 31 x ICS 6
2	ICS 1 x SCA 12	9	ICS 89 x SCA 6
3	NA 31 x ICS 1	10	PA 7 x NA 32 (F_2)
4	ICS 6 x SCA 6	11	Amelonado x PA 7 (F_2)
5	ICS 6 x SCA 12	12	Amelonado x NA 32 (F_2)
6	ICS 6 x NA 33	13	Amelonado x NA 33 (F_2)
7	IMC 67 x ICS 6	14	PA 7 x NA 33 (F_2 selfed, control)

after planting in the case of the hybrids. The selections respond well to the recommended cultural practices.

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Table 3: Characteristics of hybrids I-56 x II-67 and ICS 6 x SCA 6 (12 years after planting)

Hybrid	I-56 x II-67	ICS 6 x SCA 6
Variety name	Vittal Cocoa Hybrid – 1 (VTLCH 1)	Vittal Cocoa Hybrid - 2 (VTLCH 2)
Breeding method	Introduction, evaluation and selection of hybrid progenies or clones, followed by hybridization between selected genotypes, evaluation and selection.	Introduction, evaluation and selection of introduced clones, followed by hybridization, evaluation and selection.
Pedigree	I-56 = a PA 7 x NA 32 progeny tree II-67 = Landas 364 clone	ICS 6 = Imperial College Selection 6 SCA 6 = Scavina 6
Source of material	Landas Estate, Malaysia. Parental clones were originally introduced from Landas estate, Malaysia and utilized in the hybridization programme	Kew Gardens, England via Lalbaugh Garden, Bangalore
Compatibility	Self-Incompatible and cross-compatible with other trees of the same variety	Self-Incompatible and cross-compatible with other trees of the same variety
Dry bean yield	1.48 kg/ tree/ year	1.15 kg/ tree/ year
Special features	Heavy bearer and vigorous	Early and heavy bearer, tolerant to black pod disease (BPD)
Plant habit	Erect	Intermediate
Girth (cm)	50.7	42.0
Height at first branching (m)	1.29	1.43
Height of canopy (m)	4.56	4.00
Canopy volume (m^3)	16.82	13.60
Leaf traits	Base - Obtuse Apex - Short acuminate Petiole - Non-pulvinated Colour of young leaf - Intermediate	Base - Obtuse Apex - Short acuminate Petiole - Pulvinated Coloor of young leaf - Pale purple
Pods/ tree/ per year	46.93	70.0
Pod	Weight - 300 g, length - 16.7 cm, breadth- 8.7 cm	Weight - 260 g, length - 14.6 cm, breadth - 10 cm
Fruit	Shape - Obovate Basal constriction - Nil Apex - Mammilate Surface - Intermediate to smooth Ridge and furrow prominence - Slight Hardness - Hard	Obovate Slight Mammilate Intermediate to slight rugose Slight Hard
Husk thickness	At ridge 1.0 cm, at furrow 0.8 cm	At ridge 1.06 cm, at furrow 0.8 cm
Colour pattern	Green (immature), Yellow (ripe)	Green (immature), Yellow (ripe)
Number of beans/ pod	42.0	35.0
Bean weight (dry)	1.00 g	1.10 g
Fat content	53.6%	53.6%
Shell (testa) percentage	13.0%	10.9%

Table 4: Characteristics of the NC-45/ 53 clone (12 years after planting)

Name	Vittal Cocoa Clone – 1 (VTLCC-1)	**Petiole colour of young leaf**	Purple and pulvinated
Breeding method	Introduction - Evaluation - Selection - Evaluation – Selection ("ortet selection")	**Fruit shape**	Obovate
Source	Nigeria	**Basal constriction**	Slight
Pedigree	P-10 x P-1 (unknown selections)	**Apex form**	Mammilate
Compatibility	Self-compatible	**Pod surface**	Intermediate to smooth
Dry bean yield	1.33 kg/tree/year	**Hardness**	Hard
Special features	Heavy bearer	**Colour pattern**	Green (immature), Yellow (ripe)
Plant habit	Intermediate	**No. of pods/ tree**	75
Girth (cm)	39.10	**No. of beans/ pod**	36.7
Total height (m)	4.16	**Single pod weight**	321 g
Canopy volume (m^3)	12.86	**Fat content**	42.50 %
Leaf character	Base - acute	**Bean Size**	1.05 g
Leaf apex	Long acuminate	**Shell percentage**	12 %

Figure 1: I-56 x I-67

Figure 2: ICS 6 x SCA 6

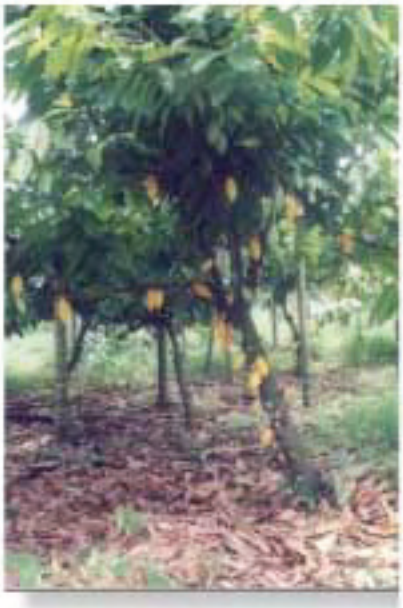

Figure 3: NC 45/53

Clonal Propagation by Somatic Embryogenesis: Preliminary Results

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Introduction

Cocoa breeding programmes are increasingly being based on selecting clones within segregating progenies (hybrid clones). Although, hybrid varieties have demonstrated good adaptation to most regions, particularly to pioneer production regions, with earlier and better yields, the degree of disease resistance and the within-family uniformity in those hybrids are insufficient. Moreover, it is not always easy to produce and distribute true-to-type improved seeds. Selecting clones from within hybrid families may lead to rapid genetic gains, particularly for resistance to diseases and for productivity. In addition, using clonal varieties offers the advantage of significantly improving the vegetative uniformity of plots, and makes it possible to actually reproduce improved varieties on the farm. Among vegetative propagation methods, somatic embryogenesis has been used for several years by different laboratories worldwide. This method makes it possible to obtain plants with architecture comparable to that of the seedlings (orthotropic axis and jorquette). The *Firclone* project, implemented through collaboration of CIRAD with INIAP in Ecuador and with SAO (*Société Agricole de l'Ouest*), a private cocoa producing company in Côte d'Ivoire, set out to compare clones reproduced by somatic embryogenesis, by budding and by cuttings in Côte d'Ivoire and Ecuador in terms of agronomic traits.

Material and methods

Plant material

Forty interesting genotypes were chosen at each site based on their agronomic characteristics. In Côte d'Ivoire, trees from Upper Amazon x Amelonado or Upper Amazon x Trinitario hybrids were chosen for their production, architecture and bean quality. In Ecuador, genotypes were chosen for their quality, level of resistance to witches' broom disease and yield potential from the collection of Nacional clones at INIAP (EET, CCAT, EB and SNA). Those genotypes were then tested for their response to somatic embryogenesis, and the best were multiplied along with two control clones (SCA 6 and IMC 67) to establish two field trials, one in Côte d'Ivoire and one in Ecuador.

Propagation methods

The *in vitro* propagation method used by INIAP in Pichilingue and by CIRAD in Montpellier was somatic embryogenesis from floral parts (Lopez-Baez *et al*. 1994; Alemanno *et al*. 1996; Li *et al*. 1998). Through *Firclone* project funding, the *in vitro* culture facilities at the Pichilingue station were renovated with the installation of 100m² of laboratory space furnished with modern equipment. In Côte d'Ivoire, a portable sterile hood, designed by CIRAD, was used in the field to prepare the floral parts prior to their transportation to the CIRAD laboratory in Montpellier. Immature flowers were collected and the petals and staminodes were used as primary explants. Secondary cultures were initiated from primary somatic embryos to produce secondary somatic embryos (Maximova *et al*. 2001).

In all, 1615 *in vitro* plantlets were produced at CIRAD, and 1903 at INIAP. The plantlets were then acclimatized in the greenhouse, and hardened under shade for several months.

The same genotypes were propagated in the nursery by plagiotropic rooted cuttings and by patch budding of plagiotropic budwood on two types of rootstock with different vigour. Open-pollinated seeds from selected trees were used to produce seedling controls.

Field experiment

Two trial plots were planted in May, 2004. The experimental design was a split-plot complete blocks design, with as main factor clonal forms (5 types), and as secondary factor the genotype (8 in Côte d'Ivoire and 12 in Ecuador). The genotypes planted were chosen according to the availability of *in vitro* plantlets in the nursery. Each experimental plot comprised 5 trees per genotype, planted in a row. The planting density was 1111 trees ha^{-1}.

Results and Discussion

Efficiency of the propagation methods

Somatic embryogenesis

Primary embryogenesis with staminodes gave better results than with petals for the material from Côte d'Ivoire, but the opposite was observed for the material from Ecuador. Embryogenic response was generally better in Ecuador. The results varied considerably for the genotypes, and some materials did not produce any embryos.

Secondary embryogenesis was always more efficient than primary embryogenesis. However, responses varied according to the genotypes. Figure 1 shows the performance of the best clones chosen for one of the trials. Most of the embryos were capable of becoming plants, but many of the plantlets were abnormal and not viable; the maximum conversion rate reached 45% at CIRAD and 100% in Ecuador. The final success rate of acclimatization varied with genotype from 0 to 100% in Ecuador (Figure 2). In Montpellier, an average of 12% of the secondary embryogenic explants resulted in acclimatized plants compared to 45% in Ecuador. After the hardening period, the success rate in relation to the percentage of secondary embryogenesis was lower in Côte d'Ivoire (5%) than in Ecuador (25%) due to the stress caused by transporting the plants from Montpellier. The architecture of the

aerial part of the *in vitro* plants in the nursery was similar to that of seedlings. However, greater secondary root development was often found in *in vitro* plants.

Conventional propagation methods

Budding

The average final success rate for brown budding on low-vigour rootstocks (selfed seeds of Catongo or EET 55) was better than that obtained on vigorous rootstocks (IMC 67 x IFC 1 or IMC 67 x Catongo) with success rates of 37% and 53% in Côte d'Ivoire, and 30% and 53% in Ecuador, respectively. It is possible that the outgrowth of the scion suffered more competition with the more vigorous rootstock than with the less vigorous rootstock plants.

Cuttings

The success rate with cuttings was low both in Côte d'Ivoire and Ecuador. It varied from 11 to 67% depending on the genotype in Côte d'Ivoire, and from 20 to 58% in Ecuador. It depended on the quality of the wood taken from the mother trees, but also on the moisture content of the air and the substrate in the nursery.

These two methods were labour-intensive and time-consuming as budwood availability on the mother trees was often limited.

The success rates with cutting, budding and somatic embryogenesis were different. The rates for the different methods were not correlated with genotype (Figure 3); environmental factors appeared to be more important than genetic effects.

Plant performance in the field

In Ecuador, the mortality rate after one year for *in vitro* plantlets reached 31%, which was mainly due to the high mortality of the SCA 6 and IMC 67 plants, which were very small when planted in the field. The rate was comparable to that for cuttings, but was higher than for seedlings or budded plants (Table 1). In Côte d'Ivoire, *in vitro* plantlets survived better in the field (27% mortality) than cuttings and seedlings, but less well than budded plants.

Table 1: Average mortality rate (%) for the different clonal forms and seedlings (one-year-old)

	SE	S	C	B	Bv
Ecuador	31	3	23	8	3
Côte d'Ivoire	27	33	36	19	19
Mean	29	18	29	13	11

SE: *in vitro* plantlets; S: seedlings; C: cuttings; B: budding on low-vigour rootstock; Bv: budding on vigorous rootstock

A comparison of jorquette heights and the trunk diameter of the main axis more than a year after planting out in Ecuador revealed a highly significant difference between *in vitro* plantlets and the other clonal forms, and between genotypes, along with a significant interaction between genotype and clonal form (Table 2).

Table 2: Analysis of variance: Fisher F values for the different clonal forms with five genotypes for three architecture variables, measured one year after planting

Effect	H	D	C *
Clonal form	31 **	44.4 **	2 ns
Genotype	4.3 **	4.1 **	4.3 **
Interaction	3.6 **	4.2 **	0.1 ns

H: jorquette height, D: trunk diameter, C: number of leaf scars/m of trunk
* measured on 5 genotypes
** significant difference ($p < 0.001$)

The jorquette height and trunk diameter of *in vitro* plantlets were significantly greater than those for seedlings and other clonal forms (Table 3). A comparative study of the architecture of *in vitro* plantlets and seedlings, involving the measure of leaf scar density on the orthotropic axis, showed that the phyllotaxy had not been modified. The leaf scar density was similar for *in vitro* plantlets and seedlings, but varied between clones.

Table 3: Comparison of the average values for architecture variables of the different clonal forms and seedlings

	SE	S	C	B	Bv
Jorquette height (cm)	85	66	49	54	61
Trunk diameter (cm)	1.4	1.2	0.9	0.9	1.1
Number of leaf scars /m*	58	62			

SE: *in vitro* plantlets; S: seedlings; C: cuttings; B: budding on low-vigour rootstock; Bv: budding on vigorous rootstock
* measured on 5 genotypes

Conclusion and perspectives

This study showed that it is possible to produce plantlets by somatic embryogenesis for a large number of genotypes. Although the process is long and requires substantial investment, this technique offers the advantage of allowing the multiplication of genotypes in large numbers to produce plants with the same architecture as seedlings, whereas this is not feasible with conventional horticultural production methods. However, the yield from this method is limited by the primary embryogenesis phase, which is not very efficient, by the production of a significant number of abnormal plantlets and by a difficult acclimatization phase that can lead to the loss of a large number of plantlets, especially when combined with stress during shipment and excessive watering in the nursery, as occurred in Côte d'Ivoire.

The first results, obtained on viability and field growth in Ecuador and Côte d'Ivoire confirm the merits of producing *in vitro* plantlets: their architecture is identical to that of seedlings and the

faster development over the first months of growth means that they may start bearing earlier. Similar results on conformity and vigour in the field were obtained by Masseret with EET clones (Masseret *et al*. 2005). In the continuation of the study, we hope to assess production capacity, susceptibility to pests and diseases and plant management requirements. It will show whether this material offers long-term comparative advantages over seedlings and other clonal forms.

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Figure 1: Average of embryogenic primary and secondary explants for some genotypes from Côte d'Ivoire

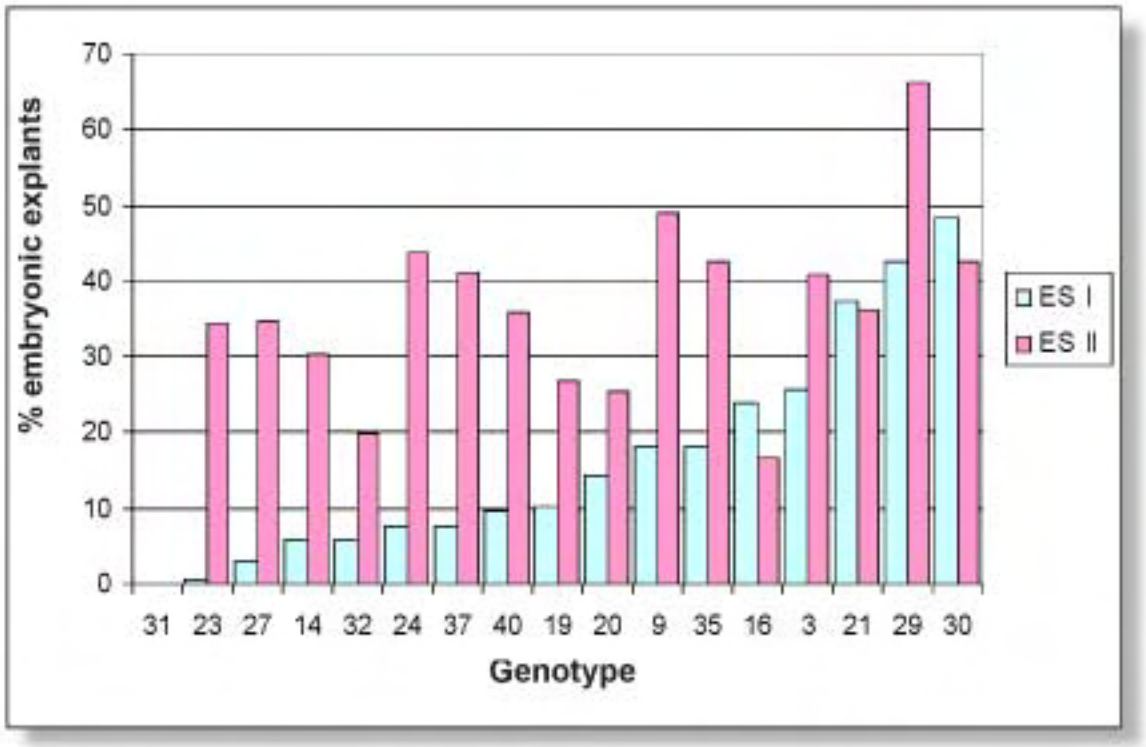


Figure 2: Percentage acclimatization success of plants obtained per genotype in Pichilingue, INAP

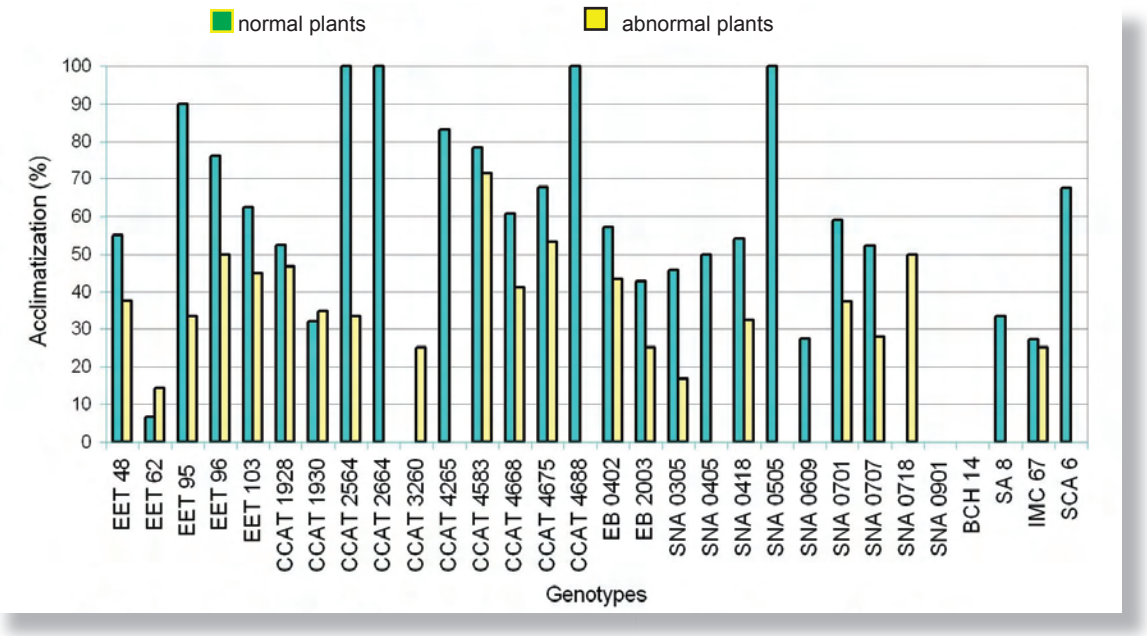
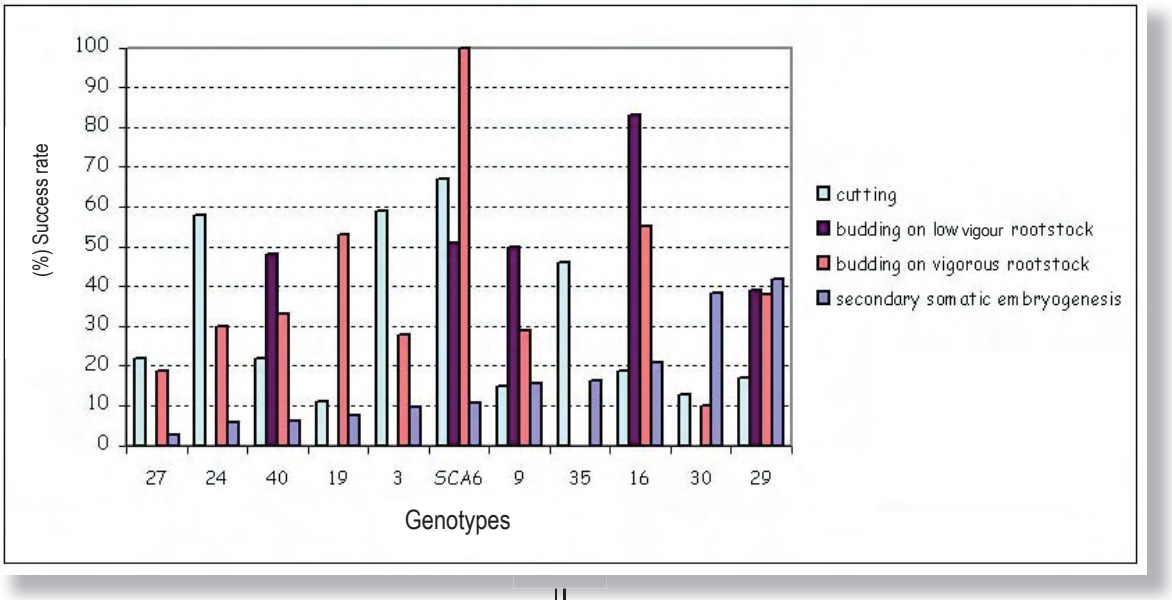


Figure 3: Success rate for secondary somatic embryogenesis and conventional propagation methods with some genotypes in Côte d'Ivoire



Comparison of Methods to Obtain Twin Seedlings by Splitting of Cocoa Seeds

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Scope

The CNRA in Côte d'Ivoire is involved in studies to detect QTL's for Black Pod resistance and other important traits. A double-cross progeny was created in 2003 and 2004 {(N 38xGU 133/1)x(IMC 57xSCA 6)} in the context of the CFC/ICCO/Bioversity International project. This population is expected to segregate for three sources of resistance to *Phytophthora* pod rot, for high yield and tolerance to cocoa mirids. The objective is to compare the stability of QTLs for these traits between Ghana (CRIG, Tafo) and Côte d'Ivoire (CNRA, Divo) growing environments. In order to use exactly the same population in both environments, attempts were made to obtain a large number of identical twin seedlings by splitting of freshly harvested seeds.

The production of twin seedlings has been attempted elsewhere with largely variable results. Bertrand and Cilas (1990) reported a highest success (45% germinated twin seedlings) in an experiment carried out in Togo with peeled split seeds sown directly in soil, as is done with normal seeds. The authors supposed that improvements in the method may help to increase success rates. The split seed method, if successful, has several other possible applications in cocoa breeding, for example in the more efficient selection of individual genotypes in segregating populations (by increasing the genetic variance) and for genetic studies in cocoa trials (Betrand and Cilas 1990).

Experiments were carried at the Divo Research Centre of the Centre National de Recherche Agronomique (CNRA) to identify the best conditions for the germination of peeled split seeds. The following treatments were applied: dorsal and lateral cutting of the cotyledons and four germination substrates: soil, sand, saw-dust and cotton wool. Hereafter, we describe the main results and provide details of the method with cotton wool, now adopted by the CNRA as a routine method for germination of twin seeds. The latter method has given over 90% of twin seedlings established successfully in the Divo nursery after one month in large-scale experiments in 2003 and in 2004.

Methods

The soil substrate (a mixture of 70% top soil and 30% red soil placed in polybags) was tested directly in the cocoa nursery. The other substrates (sand, saw dust and cotton wool) were tested in cocoa propagators, such as those used for making cuttings. In the soil, sand and saw dust, the half seeds were placed at one cm depth with the cut surface upwards and the substrates were wetted as normally practised for seed germination.

The cotton wool was placed in polystyrene cups and was wet daily (see description below). After removing the mucilage with saw dust, the testa was removed from all seeds used for the experiment. In total, 140 split seeds and 70 normal seeds were sown in the soil, sand and saw dust substrates. These numbers were 124 and 62 for the cotton wool substrate, respectively. Fifty percent of the seeds were cut laterally (one cotyledon and a half embryo for each half seed) and 50% dorsally (two half cotyledons and one half embryo for each half seed). Observations were made at 5, 10, 15, 20 and 25 days after sowing and germination was determined as the average % of twin seedlings that both germinated for the five observation dates as well as by the maximum germination percentages obtained. After 25 days, the seeds germinated in the propagator (in sand, saw dust and cotton wool) were transplanted into the soil substrate in polybags placed in the nursery. The height of the seedlings was measured three months after sowing.

Results and Discussion

There was no significant difference in the average germination percentage for the laterally or dorsally cut seeds, except in the case of the sand treatment for which there was a lower maximum germination of the dorsally cut seeds (37%) than for the laterally cut seeds (63%).

The effect of the substrate was highly significant (Table 1). The average germination percentage over the 25-day period was lowest for sand (35%), intermediate for saw dust (65%) and soil (64%), and highest for cotton wool (89%). The maximum germination percentages at 15 days were similarly high for the saw dust, soil and cotton wool treatments (approximately 90%) and relatively low for the sand treatment (approximately 50%).

The germination of the half seeds was faster than that of the control seeds (intact peeled seeds). For example, at 10 days after sowing, germination was 92% for the half seeds on cotton wool and only 73% for the uncut seeds on the same substrate. However, maximum germination of the control seeds after 15 days was slightly higher than that of the twin seeds, reaching 100% for the soil, sand and saw dust treatments and 75% for the cotton wool treatment

The height after three months of the control seedlings germinated in soil, sand, saw dust and cotton wool was 33, 36, 28 and 22 cm, respectively. As expected, the corresponding height of the twin seedlings was lower (29, 25, 25 and 18 cm, respectively). The relatively low height of the twin seedlings germinated on the cotton wool is attributed to the late transplanting of the seedlings to the soil (25 days growing on sterile cotton wool). In fact, in later experiments where this method was used, the growth of the twin seedlings was satisfactory when transplanting was done between 10 and 15 days after sowing.

Table 1: Germination rate, expressed as average germination percentage, observed over the first 25 days after sowing, of twin cocoa seeds (both half-seeds germinating) in four substrates

Substrate	Germination (%)	Significance (1)
Cotton	88.5	a
Saw dust	64.6	ab
Soil	64.0	ab
Sand	34.7	b

Conclusions

1. Germination of both of the peeled half seeds was equally successful with the use of soil (70% top soil and 30% red soil), saw dust and cotton wool substrates. However, the sand substrate produced the least favourable results.
2. Germination rate was highest with the cotton wool treatment. This may be partly due to the effect of the wetting of the radicle on the day of sowing (see Figure 1), which has been shown to enhance germination (radicle growth).
3. The maximum percentage of germinated twin seedlings in our study in the soil substrate (approximately 90%) was two times higher than reported by Bertrand and Cilas (1990), who also used peeled twin seeds and a soil substrate (45%). The difference might be due to the quality of the half seeds (precision of the cut), of the soil mixture (we used a rich and light soil mixture) or of the irrigation (too much humidity will favour fungal growth).
4. Although the soil and saw dust also provided good results, the CNRA has chosen the cotton wool method for routine application of the split seed method. Due to its originality and the need for technical precision, we attach a description of the details of this method hereunder.

Reference

Bertrand, B. and C. Cilas. 1990. L'utilisation de vrais jumeaux dans l'eperimentation cacaoyère. *Café Cacao Thé* **34**: 295-298.

Description of the Cotton Wool Method for Obtaining Twin Cocoa Seedlings

Preparations

- Place about 10 g of cotton wool loosely on the bottom of 100-150 ml plastic or polystyrene cups of 8-10 cm height. The cotton fills the cup up to about 2-3 cm height.
- Pour approximately 3 ml of water on the cotton.
- Align the cups in a cocoa propagator (such as used for placing cocoa grafted plants or cuttings, which can be a concrete box covered with a plastic or glass lid or a plastic tunnel placed in a well-shaded nursery). The soil of the propagator may be slightly humid, but should not be too wet. If such a propagator is not available, a large covered tray placed on a table in a well-shaded nursery may be considered.
- Use only seeds from freshly harvested pods.
- Break the ripe pods and use sawdust to remove the mucilage.

Splitting of the seed

- Remove the seed testa (integument) with a scalpel and identify where the embryo is located (often visible as a white point at the plumper end of the seed).
- Split each seed laterally into two halves by using a scalpel, starting from the plump end (where the embryo is located) to the narrower end. Try to ensure that each half seed contains half of the embryo and one entire cotyledon.

After splitting

- Place the two halves of the seed slightly inclined with the cut face upwards and the unwounded side in contact with the wet cotton, with the half embryos facing each other (Figure 1).
- Place a small droplet of distilled water on the embryos to stimulate growth of the root of the embryo (radicle).
- Cover the half seeds with dry cotton. Use just enough cotton to cover the wounded part of each of the half seeds (2-3 g of cotton for each half seed).
- Leave the cups open and close the lid of the propagator so that the relative humidity will be high and make sure that the shade is sufficient so that the temperature inside the propagator does not increase too much.

- After 24 hours, when the split seeds have hardened off, open the lid of the propagator and cover the emerging radicle with the same cotton that was used to cover the half seed. Wet the cotton on top of the half seeds slightly (0.5 ml of distilled water for each half seed).
- Afterwards, if necessary, wet the cotton on top of the seeds with 5-10 drops every day until at least ten days after sowing.

Transplanting

- Leaf expansion of the split seedlings begins in the propagator between the 10th and 15th day.
- The plantlets should best be transplanted into polythene bags containing high quality humid organic soil placed in the same propagator 10 days after the start of germination.
- The polybags should be lined up in double rows and labelled to identify the twin seedlings.
- The lids of the propagator (or plastic covering the tunnel) should then best stay partly opened. However, it is important to make sure that the seedlings are protected from heavy rain or insect damage.
- Four to six weeks after planting, the polybags with the plants can be taken out of the propagator and placed into a normal nursery bed.

Final considerations

⊙ This method when applied on a large number of seeds showed high success rates and uniformity in vigour between twin seedlings. However, twin-seedlings are less vigorous than normal seedlings.

Figure 1: Laterally cut, peeled cocoa seed one day after placing on wetted cotton wool. The germination has been stimulated by placing a droplet of water on the radicles on the day of sowing, and the half seeds are then covered with cotton wool.

The differences between twin and normal seedlings tend to decrease with time in the nursery.

⊙ Important factors for success seem to be the use of a sterile substrate (cotton) for germination with strict control of humidity (not too high or too low). The droplet placed on the radicle stimulates the germination (outgrowth of the root). The importance of eliminating the seed coat before splitting the seed is yet to be studied.

Is Genetic Variation for Sensory Traits of Cocoa Pulp Related to Fine Flavour Cocoa Traits?

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Summary

A first attempt is made to link some of the known fine flavour attributes found in cocoa liquor or in dark chocolate (excluding basic cocoa flavour) with the sensory traits of the pulp of ripe pods of cocoa varieties representing different genetic cocoa types. Overall pulp preference expressed by the sensory panel appeared related to the known fine flavour potential of the cocoa varieties tested, and specific pulp flavour attributes appeared related to specific types of fine flavour cocoa types. EET 62 (Nacional x Trinitario type) displayed a characteristic odour when the fruit was opened and had sweet pulp with an intense flavour identified as floral and fruity. This flavour may possibly be related to the typical Arriba flavour. The ICS 1 clone, known for its typical Trinitario fresh fruit flavour, showed a combination of relatively high acidity and sweetness of its pulp and also an intense fruity flavour. The pulp of the CCN 51 clone and of two Huallaga clones was rated as astringent and acid, with low flavour quality. This could be related to the low potential for fine flavour cocoa of many Forastero types (which may otherwise however have a potential for strong cocoa flavour). The two Ucayali clones tested displayed sweet pulp with a medium intensity of fruity flavour that resembles the pulp of the SCA 6 clone known to produce cocoa with a dry fruit or floral flavour. The pulp of the Pandora 1 clone from Colombia was characterised by a typical flavour associated with that of soursop (guanabana) and may therefore represent a new type of fine flavour cocoa. The results suggest that a significant part of fine flavour attributes of cocoa products (excluding basic cocoa flavour) can be related to sensory traits in the pulp of ripe cocoa pods. More research is required to further verify and possibly explain this relationship. However, the results suggest that rapid screening of germplasm

and of breeding populations for pulp characteristics may be useful for selecting cocoa varieties with known as well as with new fine flavour traits. Such would be of great value to producers and other stakeholders in the rapidly growing and increasingly more diverse "gourmet" cocoa and chocolate market. Methods for rapid pulp flavour assessments to meet different needs in cocoa breeding for high cocoa quality are proposed.

Introduction

Objectives of cocoa breeding broadly include high yield, resistance to diseases and pests and good quality. Quality of fine flavour cocoas is known to be affected by genetic and geographic origin, and thus breeding programmes have aimed at maintaining the cocoa flavour associated with the country of origin (Lockwood and Eskes 1996). Research on cocoa flavour has been receiving increasing attention over the last 15 years and has broadened the spectrum of recognised flavour traits (*e.g.* Clapperton *et al*. 1994; Eskes 2006; Sukha and Butler 2005). Besides variation in the intensity of basic cocoa flavour, presence of specific aromas such as "fruity" (*e.g.* as found in Trinitarios), different types of "floral" (*e.g.* as Nacional and Scavina types), and nutty and caramel flavours (*e.g.* in Criollo types) have been demonstrated (Sukha and Butler 2005). The market for fine flavour cocoa (mainly as dark chocolate with high cocoa content) is rapidly expanding and diversifying, providing new opportunities for the producers to obtain premium prices for high quality cocoa beans. Besides selecting for traditionally recognised fine flavour cocoa types, the challenge for breeders is also to look for new fine flavour types that may be of interest for the cocoa market. Cocoa breeding is complex, as many traits need to be selected for simultaneously. Selection for special flavour attributes is a laborious and time-consuming process that requires collaboration between breeders, specialists in post-harvest technologies, sensory specialists and chocolate manufacturers. Most cocoa selection programmes do not have the capacity to carry out routine sensory evaluations of genotypes in their collections or in the breeding populations. These factors reduce the efficiency and speed of progress in the selection of fine flavour cocoa types, especially if this trait is to be combined with good yield and resistance to diseases. Therefore, the use of a quick method to identify special flavour traits of cocoa genotypes would be very useful in cocoa breeding.Regular visitors to cocoa plantations are familiar with the broad variation that exists in the taste of cocoa pulp. The most conspicuous variations that can be observed are related to the level of acidity and sweetness. This is especially noticeable when pulp of cocoa pods of different genetic origins is tasted. For example, it is well known that Criollo and Scavina genotypes have very sweet pulp whereas the pulp of Trinitario and Forastero clones are generally more acid. Sensory traits of the pulp and of the beans (degree and type of bitterness) have been used over the last 10 years in Ecuador as criteria for pre-selection of trees that might produce the "Arriba" flavour profile associated with the Nacional cocoa variety (Gilles Roche, CIRAD, personal communication), but no published studies exist on the possible relationship between pulp traits and fine flavour cocoa.

The objective of the current study is to assess if variation in sensory traits of the pulp in ripe cocoa pods from different genetic origins can possibly be related to the known variation of fine flavour traits of cocoa beans.

Materials and methods

In September 2007, an experiment on flavour attributes of ripe pulp of different cocoa types from the collection of the Universidad Nacional Agraria de la Selva in Tingo Maria, Peru, was carried out. The varieties chosen included genotypes that are known to vary for fine flavour attributes (Table 1). EET 62 is associated with the Arriba floral flavour and ICS1 with the typical Trinitario fruity flavour. The U and H clones represent sub-spontaneous and cultivated origins collected in the Huallaga and Ucayali river basins, respectively (Garcia Carrion 2000; Dapeng Zhang *et al.* 2006). The Huallaga accessions appear to be mainly true Forasteros while some are of hybrid origin.

Two ripe pods of each of nine clones were collected in the morning, numbered 1 to 9, and placed in the laboratory for sensory evaluation in the afternoon. The panel consisted of six persons, who scored the following flavour attributes on a 0 to 5 point scale: intensity of volatile aroma (odour) when opening the pod, pulp acidity, pulp sweetness, pulp astringency, bean bitterness, presence of a special flavour in the pulp after tasting it, the type of flavour of the pulp and overall preference of the panellists for the taste of the pulp.

Classical statistical analyses using linear models (ANOVA, linear correlation) were performed to calculate the treatment effects, and Principal Component Analysis (PCA, using the XLSTAT 2007 programme version 8.01) was done to visualise the associations between traits and treatments.

Results

Tables 2 and 3 show the results obtained for the sensory evaluation of the pulp and beans of the nine varieties (clones) tested. Differences between varieties were significant for all traits at p=0.05. Discrimination between varieties was highest for pulp astringency (F=13.8) and lowest for pulp sweetness (F=3.5). Differences between panellists were not significant for overall preference, pulp acidity and pulp sweetness suggesting that these traits were evaluated in a uniform manner by the panellists. For ease of reading, the varieties in Tables 2 and 3 have been ordered according to the mean overall preference scores for the pulp.

The panel expressed highest overall preference for EET 62 and lowest preference for CCN 51 and H 56 (Table 2). The odour on opening the pods and the flavour intensity in the pulp were also perceived strongest for EET 62. The flavour intensity was intermediate for ICS 1, PAN 1, U 43 and U 45 and lowest for U 53, H 60, H 56 and CCN 51. The pulp acidity was highest for ICS 1, H 60, H 56 and CCN 51, and lowest for the three U clones and for EET 62 (Table 3). Pulp sweetness was high for six clones (EET 62, the three Ucayali clones, PAN 1, and ICS 1), intermediate for the two Huallaga clones and low for CCN 51. Pulp astringency was high for CCN 51, intermediate for the two Huallaga clones, low for PAN 1 and ICS 1, and very low for EET 62 and the three Ucayali clones. The bitterness of the beans was high for CCN 51 and average for the other varieties.

The predominant type of flavour identified in the pulp was "fruity" (including mainly fresh fruit flavour). The pulp of EET 62 was also considered to be "floral", whereas the PAN 1 variety displayed a very characteristic flavour identified as that of soursop (*guanabana* in Spanish and *graviola* in Portuguese). The intense flavour of ICS 1 was considered to be a fresh fruit flavour. The pulp of the CCN 51 variety did not contain a specific flavour according to three of the panellists whereas two panellists identified a low intensity of fruity flavour.

Linear correlation coefficients are presented in Table 4. The odour, flavour intensity and sweetness of the pulp were all positively correlated with overall preference, and pulp flavour intensity was positively correlated with odour and pulp sweetness. Pulp acidity, astringency and bean bitterness were positively correlated with each other and negatively correlated with pulp sweetness. Pulp acidity, pulp astringency and bean bitterness were not significantly correlated with pulp preference, odour and pulp flavour.

The associations between the genotypes and sensory attributes, as analysed in the PCA, are depicted in Figure 1 and described in Table 5. The most contrasting varieties are EET 62 (sweet pulp with strong flavour and odour), Ucayali clones (sweet pulp with low acidity and low astringency), CCN 51 (high pulp acidity and astringency, and very bitter beans) and the Huallaga clones (acid and quite astringent pulp).

Discussion

The analyses show strong effects of genotype on the sensory traits of ripe pulp. The most preferred pulps (EET 62, PAN 1, Ucayali clones and ICS 1) were sweet and rich in flavour. The EET 62 and ICS 1 clones are well-known for their floral and fresh-fruit, fine flavour cocoa traits, respectively, which might be related to the "fruity-floral/sweet" and "fruity/acid/sweet" flavours in the pulp, respectively (Table 3, Figure 4). The sweet and fruity pulp of the Ucayali clones resembles the taste of the well-known pulp of SCA 6, and might be related to the special cocoa flavour associated with the SCA 6 clone. The two Huallaga clones had acid pulp and did not show any special pulp flavour. These pulps are similar to that of many known Forastero types (such as IMC 67), which may have a good potential for strong cocoa flavour, but do often not have special flavour attributes. Interestingly, the H 60 had an odour on opening the pod that was not observed for H 56. This might be due to the likely hybrid origin of this clone (Table 1), including possibly SCA 6 as one of the parents (Dapeng Zhang, personal information). The least preferred pulp (CCN 51) was acid, very astringent and presented little or no special pulp flavour. This might be related to the reported presence of acidity and astringency in cocoa liquors made of CCN 51 beans that have not undergone special fermentation treatments to remove these unfavourable traits.

Interestingly, while the sweet and acid traits were generally opposed traits, the pulp of ICS 1 appeared to have considerable levels of both acidity and sweetness. This is why this clone is located in the middle of Figure 1. The combination of sweet and acid pulp could well be related to the origin of ICS 1 (a Trinitario clone), descending from hybridisation between Criollo (sweet pulp) and Forastero (acid pulp). The not well known PAN 1 also has a similar acid/sweet pulp, but it has a very typical soursop flavour. Therefore, the cocoa made with beans of this clone may also display a soursop related flavour that could be a new flavour to the current spectrum of fine flavour cocoa traits.

The relationship between cocoa pulp flavour traits and traits of cocoa products (liquor or dark chocolate) could possibly be due to the presence of aromatic substances or aroma precursors in the pulp that may migrate into the cocoa beans during the fermentation process. As indicated by Rohsius *et al.* (2006), the mycropyle of the cocoa bean becomes permeable during the fermentation process allowing for the entry of acetic acid and other soluble compounds into the cotyledon. It may, however, also be that some of the aromatic substances that are present in the beans are also present in the pulp. Our findings do not suggest that the strength of the basic cocoa flavour in cocoa products is related to any characteristic of the pulp.

More research is certainly required to further verify and possibly explain the relationship between pulp flavour traits and flavour of cocoa products. These studies could involve comparisons between pulp flavours and flavours of cocoa liquor of diverse genotypes, and fermentation of de-pulped beans with low fine flavour potential in fermentation boxes that contain a pulp of rich flavour, and vice-versa.

Although the potential for fine flavour cocoa is largely determined by the cocoa genotype, it is recognised that the environment, post-harvest handling and chocolate preparation techniques may equally affect the flavour of cocoa products. Our results suggest, however, that rapid screening of germplasm and of breeding populations for pulp characteristics can be attempted to select cocoa varieties that display good potential for known as well as for new fine flavour traits.

Possible applications in screening and evaluation of fine flavour cocoa genotypes

Rapid selection of fine flavour cocoa genotypes in diverse populations

According to our results, the genotypes with most preferred cocoa pulps appear to be of direct interest for pre-selection of fine flavour cocoa. Preferred pulps tend to be sweet and show quite intense, diverse types of floral and/or fruity flavours. If the interest is to carry out screening of fine flavour genotypes in diverse populations (germplasm collections, double crosses, farm populations, *etc.*) a start can be made by rapid screening of the genotypes for pulp preference. Field screening can be done by one or two persons opening at least two ripe pods of each genotype in the field and by subjective scoring of preference, indicating presence of special flavours. The most preferred genotypes can subsequently be evaluated by a more detailed method (see below).

Rapid selection of known fine flavour cocoa genotypes in segregating populations

Complex crosses are required in cocoa breeding to try to select for genotypes with known fine flavour attributes (fruity, floral), high yield and resistance to diseases or pests. Pre-selection for the specific quality traits can be carried out by field screening for pulp flavour attributes of individual trees (as above). However, the observers need to have a good idea of the flavour attributes of the type of quality which is being selected. Therefore, the flavour attributes of the control varieties that have the required quality traits need to be well known to the observer. For example, based on the results of our study, if the objective is to select for the typical Trinitario "fresh fruit" flavour, the pulp that one would select for should have medium to high acidity and sweetness, associated with the fresh-fruit flavour of ICS 1 pulp.

Detailed evaluation of pulp flavour traits

Detailed evaluation of pulp flavour traits may be necessary for special studies, such as to relate different pulp flavour traits with fine flavour attributes in fermented and dried cocoa beans and in roasted cocoa products. The method could be similar to the one used in our study, with possible adaptations as required for specific objectives. In general, it will be necessary to have a trained panel of four to six members and to apply two to four replicates depending on the importance of the study. The flavour traits are scored on a 1 to 5 point scale, where 1 = absence of the trait, 3 = medium intensity of the trait and 5 is high intensity of the trait. Each replicate would include the following steps:

1. Collection of ripe pods of the test genotypes.
2. Simultaneous collection of ripe pods of control genotypes, which can be representatives of known fine-flavour types (*e.g.* ICS 1, SCA 6, Nacional) and of commercial varieties grown locally with or without fine flavour traits (Amelonado, IMC 67, CCN 51, *etc.*).
3. Opening of the pods in the laboratory and placing of the pulp in numbered plastic boxes that are closed to contain the pulp odour. The pulp is best left for one or two hours to adapt to the environment and to attain uniform temperatures.
4. Observation of the intensity and type of odour of the pulp is done alter opening the lid of the plastic box by each member of the panel.
5. Tasting of the pulp of two or three beans is followed by scoring of the degree of sweetness, acidity, astringency and intensity and type of flavour (different types of fruity and floral, using the control varieties as standards for known flavours).
6. The overall preference of the pulp based on the above traits is scored afterwards.
7. Alter peeling of two beans, the cotyledons are scored for the intensity of bitterness.

Data analysis may involve linear statistics as applied in our study (ANOVA, correlation studies, PCA) to establish associations between traits and between control and test genotypes.

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Table 1: Cocoa varieties from the UNAS collection that were tested for sensory traits of pulp and beans

Variety	Country of Origin	Type of clone	Genetic origin
EET 62	Ecuador	Commercial clone	Nacional x Trinitario
CCN 51	Ecuador	Commercial clone	Three-way cross
Pandora 1 (PAN1)	Colombia	Luker farm	Unknown
ICS 1	Trinidad	Commercial clone	Trinitario
U 43, U 45, U 53	Peru (Ucayali river basin)	Sub-spontaneous cocoa types	Forastero
H 56	Peru (Huallaga river basin)	Farm selection	Forastero (1)
H 60	Peru (Huallaga river basin)	Farm selection	Forastero x Scavina (1)

(1) According to ancestry inference analysis of molecular data (Dapeng Zhang, personal communication)

Table 2: Overall preference and aroma perception of cocoa pulp from nine cocoa varieties

Variety	Overall preference	Odour on opening the pod	Pulp flavour intensity	Predominant pulp flavours (2)
EET 62	4.3 a (1)	3.9 a	4.0 a	Fruity,floral
PAN 1	3.2 b	1.7 d	2.5 bc	Soursop
U 53	2.8 bc	1.9 cd	1.8 cd	Fruity
U 43	2.8 bc	2.6 bc	2.3 bc	Fruity
ICS 1	2.7 bcd	2.3 bcd	3.0 b	Fruity
H 60	2.3 bcd	3.0 b	1.8 cd	Fruity
U 45	2.3 bcd	2.2 bcd	2.2 bcd	Fruity
CCN 51	2.0 cd	1.5 d	1.3 d	No aroma, fruity
H 56	1.8 d	1.5 d	1.7 cd	Fruity
Mean	2.7	2.3	2.3	
F-value panelists	2.07 ns	2.6*	4.7*	

(1) Different letters identify significant differences between means according to Duncan's test at 5% probability
(2) Identified by at least two out of the six panellists

Table 3: Perception of acidity, sweetness and astringency of cocoa pulp and bitterness of the beans (cotyledons) from nine cocoa varieties

Variety	Pulp acidity		Pulp sweetness		Pulp astringency		Bean bitterness	
EET 62	1.5	cd (1)	3.2	a	0.7	d	2.2	bc
PAN 1	2.3	bc	2.8	ab	1.3	cd	2.8	b
U 53	1.2	d	3.0	ab	0.7	d	1.8	c
U 43	1.2	d	3.0	ab	1.2	d	2.5	bc
ICS 1	2.8	ab	2.8	ab	1.3	cd	2.5	bc
H 60	2.8	ab	2.0	bcd	2.2	b	2.3	bc
U 45	1.0	d	2.7	abc	0.7	d	2.0	bc
CCN 51	3.5	a	1.5	d	3.3	a	4.2	a
H 56	3.0	ab	1.7	cd	2.0	b	2.5	bc
Mean	**2.2**		**2.5**		**1.5**		**2.5**	
F-value panelists	**2.4**	**ns**	**1.7**	**ns**	**5.8****		**8.5 ****	

(1) Different letters identify significant differences between means according to Duncan's test at 5% probability

Table 4: Coefficients of linear correlation between pulp and bean flavour attributes of nine cocoa varieties

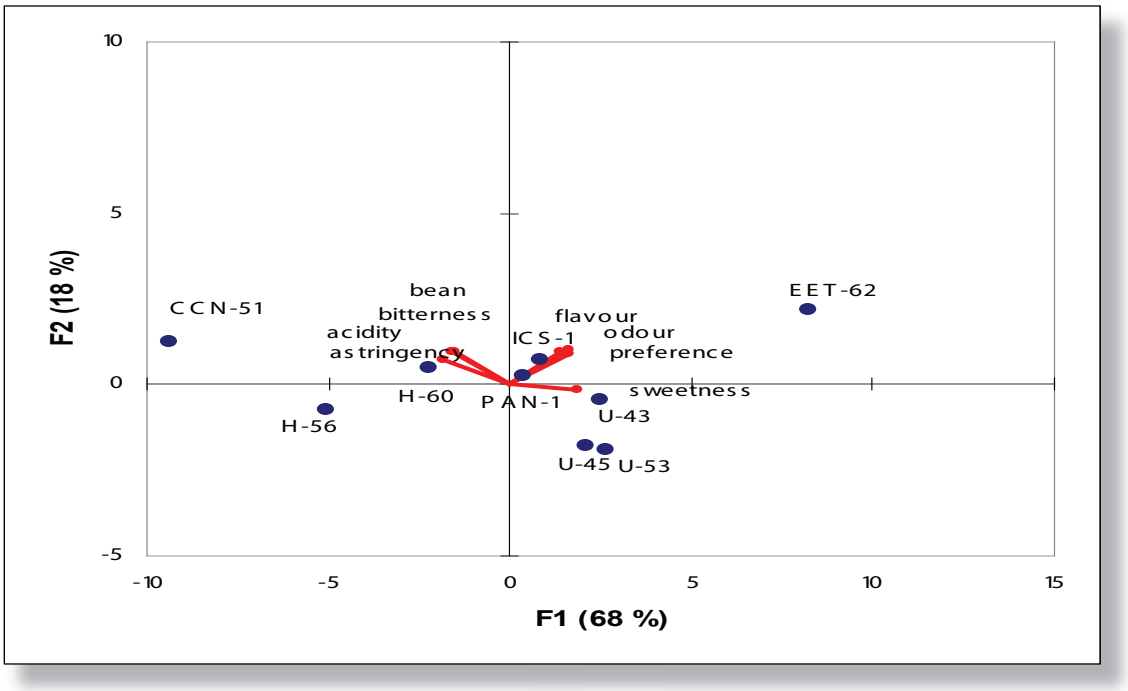
	Odour at opening the pod	Pulp flavour intensity	Pulp acidity	Pulp sweetness	Pulp astringency	Bean Bitterness
Overall preference	0.70 * (1)	0.88 **-	0.48	0.78 *	- 0.61	- 0.32
Odour at opening the pod		0.73 *	- 0.38	0.50	- 0.40	- 0.42
Pulp flavour intensity			- 0.36	0.72 *	- 0.62	- 0.36
Pulp acidity				- 0.81 **	0.87 **	0.71 *
Pulp sweetness					- 0.91 **	- 0.63
Pulp astringency						0.84 **

*(1) The level of significance is indicated by *(=0.05) and by ** (=0.01)*

Table 5: Description of typical pulp and bean flavour attributes of nine cocoa varieties

Variety	Description
EET 62	Intensive odour and fruity and floral sweet pulp
CCN 51	Astringent and acid low flavour pulp, very bitter beans
Pandora 1 (PAN1)	Sweet pulp with characteristic soursop flavour
ICS 1	Acid and sweet, intensive fresh fruit flavour
U 43, U 45, U 53	Sweet pulp with medium flavour intensity
H 56, H 60	Acid, medium astringent, low sweetness and low fruity flavour

Figure 1: Principal Component Analysis plot for five sensory traits of the pulp and for bean bitterness of nine cocoa varieties. The percentages between brackets indicate the percentage of variation explained by axis 1 (F1) and axis 2 (F2)



Assessment of Resistance of Nacional Cocoa Accessions to *Ceratocystis fimbriata* in Ecuador

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Introduction

'Nacional' cocoa is a unique type of cocoa that is native to Ecuador, and has a distinctive fine flavour. Efforts to preserve this valuable trait have been carried out by INIAP. From one of these initiatives originated a new germplasm collection selected for fine flavour traits, the "Sabor Nacional Arriba (SNA)" collection established at the end of the 1990s. In addition to the fine flavour, 'Nacional' cocoa has some other valuable traits such as resistance against major fungal diseases in Ecuador. One of them, "Ceratocystis wilt" or "Mal del Machete", caused by the fungus *Ceratocystis* fimbriata Ellis & Halsted, has caused the death of thousands of cocoa trees in the past. Just on one farm, the loss of 35 thousand trees of the 'Trinitario' type ICS 1 clone was recorded (Wallenius 1958). Resistance to this disease has been observed among Nacional cocoa germplasm (Rorer 1918; Miño 1994). The purpose of this research was to assess the resistance against *Ceratocystis* wilt in the SNA collection, maintained at the Pichilingue Research Station of INIAP.

Materials and Methods

Eighty-six clones of the SNA germplasm collection were evaluated. IMC 67 and ICS 1 were used as resistant and susceptible controls, respectively. Additionally, the Forastero clone, CCN 51, was included in the test.

Cocoa twigs of 1.5 cm diameter and 4 cm length, divided longitudinally, were placed in a wooden box covered by polyethylene, which served as a humid chamber (Figure 1). Over the surface of each twig, a suspension containing 3×10^4 c.f.u./ml of *C. fimbriata* was applied (Delgado & Echandi 1965; Soria & Salazar 1965, Miño 1994). The isolate, LH3, from the collection of the National Department of Plant Protection of the 'Pichilingue' Experimental Station of INIAP, was used (Delgado & Suárez 2003). The inoculum was prepared from 8-day-old colonies grown on potato-dextrose-agar (PDA) plus Thiamine HCl (0.01%).

Six pieces of twigs per clone were inoculated. For each of the controls and the CCN 51 clone, eight pieces were inoculated. Evaluation was carried out four days after inoculation under a stereo microscope (10X). The following scales were used for rating mycelium and perithecium development of *C. fimbriata* on cocoa twigs; for mycelial growth: 0= no growth, 1= scarce, 2= little, 3= abundant, 4= twig section totally covered, and for perithecium formation: 0= no perithecium formation, 1= scarce (1 to 5 perithecium), 2= little (6-15), 3= abundant (more than 16), 4= twig totally covered with perithecia (Soria & Salazar 1965). According to the average values recorded, the clones were classified by their reaction against *C. fimbriata* according to the following categories: resistant (R)= 0-1.0; moderately resistant (MR)= 1.1-2.0; susceptible (S)= 2.1-3.0; and highly susceptible (HS)= 3.1-4.0 (Delgado & Echandi 1965).

Results and Discussion

Most of the accessions were observed to be susceptible (S) and highly susceptible (HS) in relation to *C. fimbriata* mycelium development. However, the clone SNA 0106 was even more resistant than the resistant control, IMC 67. Although CCN 51 has IMC 67 in its pedigree, it appeared to be susceptible (Table 1).

For perithecium formation, the clones SNA 0106, SNA 0905, SNA 0430, SNA 0101, IMC 67, CCN 51 and SNA 0205 were observed to be resistant. The SNA 0106 and SNA 0905 clones formed no perithecium at all (Table 2). This latter condition is also meaningful for disease epidemics, since all fungal parts are infective, even mycelium.

Conclusions

The Nacional cocoa clone, SNA 0106, constitutes a new source of resistance against *Ceratocystis* wilt due to its capacity to inhibit the growth of mycelium and peritecia formation of *C. fimbriata.* This clone should be included in the breeding programme of Ecuadorian cacao. The relative resistance against *C. fimbriata* of IMC 67 was confirmed. Attention must be paid to the susceptibility of the CCN 51 clone, which has been widely planted throughout Ecuadorian cocoa growing areas, in order to prevent epidemics of *C. fimbriata.*

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Figure 1: Sections of cocoa twigs four days after inoculation

Table 1: Mycelium development of *C. fimbriata* in nacional cocoa clones of the "Sabor Nacional Arriba" germplasm collection

Reaction	Accession
R	SNA 0106
MR	IMC 67
S	SNA 0905, SNA 0437, SNA 0205, SNA 0301, SNA 0425, SNA 0430, CCN 51, SNA 0101, SNA 0407, SNA 0107, SNA 0302, SNA 1009
HS	SNA 0203, SNA 0428, SNA 0503, SNA 0604, SNA 0907, SNA 0410, SNA 0607, SNA 0614, SNA 0105, SNA 0204, SNA 0405, SNA 0432, SNA 0433, SNA 0436, SNA 0602, SNA 0720, SNA 0903, SNA 1005, SNA 16, SA 15, SNA 0103, SNA 0201, SNA 0412, SNA 0438, SNA 0708, SNA 0709, SNA 0719, SNA 0808, SNA 1006, SNA 0112, SNA 0202, SNA 0406, SNA 0418, SNA 0421, SNA 0422, SNA 0505, SNA 0603, SNA 0611, SNA 0701, SNA 0704, SNA 0707, SNA 0711, SNA 0902, SNA 0904, ICS 1, SNA 0102, SNA 0104, SNA 011, SNA 0303, SNA 0305, SNA 0402, SNA 0403, SNA 0408, SNA 0409, SNA 0415, SNA 0417, SNA 0419, SNA 0420, SNA 0423, SNA 0424, SNA 0501, SNA 0504, SNA 0512, SNA 0608, SNA 0609, SNA 0610, SNA 0612, SNA 0613, SNA 0703, SNA 0718, SNA 0901, SNA 0906, SNA 1001, SNA 1002, SNA 1003

Table 2: Perithecium formation of *C. fimbriata* in Nacional cocoa clones of "Sabor Nacional Arriba" germplasm

Reaction	Accession
R	SNA 0106, SNA 0905, SNA 0430, SNA 0101, IMC 67, CCN 51, SNA 0205
MR	SNA 0301, SNA 0604, SNA 0607, SNA 0437, SNA 0302, SNA 0611, SNA 1009, SNA 0425, SNA 0436, SNA 0503, SNA 0808, SNA 0902, SNA 0203, SNA 0432, SNA 0720, SNA 0907, SNA 0407, SNA 0406, SNA 0428, SNA 0433, SNA 0701
S	SNA 0107, SNA 0201, SNA 0602, SNA 0707, SNA 1005, SNA 1006, SNA 0105, SNA 0204, SNA 0410, SNA 0412, SNA 0420, SNA 0409, SNA 0415, SNA 0501, SNA 0612, SNA 0613, SNA 0614, SNA 0708, SNA 0709, SNA 0711, SNA 0103, SNA 0112, SNA 0305, SNA 0418, SNA 0421, SNA 0423, SNA 0424, SNA 0438, SNA 0505, SNA 0704, SNA 0719, SNA 0104, SNA 0202, SNA 0303, SNA 0402, SNA 0408, SNA 0417, SNA 0419, SNA 0422, SNA 0603, SNA 0609, SNA 0610, SNA 0703, SNA 0901, SNA 0903, SNA 0906, SNA 1001, SNA 1002, SNA 1003, SNA 16
HS	ICS 1, SNA 011, SNA 0405, SNA 0504, SNA 0512, SNA 0608, SNA 0403, SNA 0718, SNA 0904, SNA 0102

"P" Means Pound, Peru or Progeny?

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The 32 clones that Pound (1943) collected in Peru and took to Trinidad via Barbados have long been referred to as the "Pound" clones although A.F. Posnette (personal communication) maintained that such a modest man would not have used his own name. Perhaps "P" represented "Peru"?

An entirely different explanation is given in "First Report on the Selection of Cacao trees for Resistance to Witches' Broom Disease", an undated Report by F.J. Pound.

"The introduction of budded progeny of disease-free cacao trees from the Upper Amazon in 1943 added to the material under trial, clones which can be placed in the final trials without passing through preliminary trials.

These form a series waiting to be taken into groups 25 at a time for the last testing and have been given the serial numbers P.1 to P.32. To them have now been added four more local selections which have passed their preliminary trials P.33 to P.36. As soon as other clones pass their preliminary trials they will also be added to this list."

Evidently Pound used "P" to mean the clonal off-spring of a selected tree.

P for Pound is established usage in cocoa, and a fitting tribute to a man, who contributed so much to today's cocoa economy. There is no reason to change the names of these famous clones.

Reference

Pound, F.J. 1943. Cacao and Witches' Broom Disease (*Marasmius perniciosus*). Report on a recent visit to the Amazon territory of Peru September 1942 – February 1943. Government Printer, Trinidad and Tobago.

Update on the INGENIC Asia-Pacific Working Group (formerly known as the Regional Asia-Pacific Cocoa Breeding Group)

K. Lamin, MCB (Chair of the WG)
S. Lambert, Mars Inc. (Secretary of the WG)

The Regional Asia-Pacific Cocoa Breeding Group was informally established at a meeting that was held in June 2004 in Singapore. The collaborating institutions from regional cocoa producing countries are: the Indonesian Coffee and Cocoa Research Institute, Malaysian Cocoa Board, Papua New Guinea (PNG) Cocoa and Coconut Institute, University of Southern Mindanao, Philippines, Central Plantation Crops Research Institute, Vittal, India and Nong Lam University, Vietnam. The group was recently formalised as the INGENIC Asia-Pacific Working Group.

The priority of this group is breeding of cocoa clones that will have high yields in the presence of cocoa pod borer (CPB), black pod and vascular streak dieback diseases (VSD). Two regional projects started in 2005/6 and are ongoing:

1-Distribution of hand-held penetrometers. Hand-held penetrometers were distributed to all participating institutions to measure pod hardness for identification of cocoa clones with a hard sclerotic layer that is an important parameter in CPB resistance. This was sponsored by Mars Inc. All participating institutions have now the same penetrometer, and a regional ring test to calibrate the equipment is being organised. Basic protocol for the utilisation of penetrometer was also agreed on so that the best method is used in all institutions. This will facilitate regional exchange of information on good planting material with a hard sclerotic layer to be used in regional breeding for CPB resistance.

2-Regional exchange of superior bi-parental crosses. It was agreed that each collaborating institution (six sites) will prepare three crosses (minimum 200 seeds of each cross for each collaborating institution) using as parents the clones with characteristics that could be the most useful to be tested by all other sites. The ultimate aim of this activity is to transfer within the region the best genes for cocoa pod borer resistance, high yield and other good characteristics. High yield in the presence of CPB should be the priority selection criterion. However, some sites that do not have CPB will use the best parents they can offer with good establishment ability and high yields in the presence of *Phytophthora* and VSD in their crosses.

This activity is well advanced with more than 3,000 hybrid seeds having been exchanged. Exchange of seeds from PNG was partly postponed due to cocoa pod borer intrusion since the hybrid cocoa seeds almost ready for regional distribution had to be destroyed. New sets of pollinations were performed, and part of the planned exchange from PNG to regional cocoa

research institutions has already been completed. Preparation of the necessary documents (quarantine formalities) hampered the process of exchange, and, at times, slow clearance from quarantine caused low germination rates. All participating institutions are still preparing more hybrid seeds to be exchanged to achieve the planned number of crosses and seeds.

Hybrid seeds from first distribution round were germinated and are, in the majority of cases, already planted in the field. As evaluation of individual trees will be performed, differences in the ages of seedlings are not critical. Due to the excellent parents used in the production of these hybrids, there is a high probability of obtaining some very good clones with high yield, good bean quality and tolerance to common pests and diseases (CPB, VSD, Black Pod, *etc*).

Currently, funding of the field trials is a critical factor that may affect implementation and maintenance of the trials by the collaborating institutions. The majority of collaborating institutions has no available specific funding for these field trials. The cocoa breeders of the collaborating institutions are making efforts to plant these valuable cocoa hybrid seedlings in the field so as not to lose them. However, dedicated funding is badly needed for good establishment, maintenance and precise evaluation of these hybrid seedlings. Without this support, the tremendous value of these regional seed exchange and hybrid trials could be seriously affected. A request for support has been submitted to the World Cocoa Foundation.

INGENIC Survey on the Development of CacaoNet Conservation Strategy Summary of Replies of the Opinions Received 1st June 2007

Following CacaoNet's launch at the 15th International Cocoa Research Conference, Costa Rica, October 2006, INGENIC held an open meeting to discuss the development of a CacaoNet cocoa conservation strategy. At this meeting, it was agreed that INGENIC should establish a way to allow individuals to contribute their views in more detail. On 25th April 2007, INGENIC sent an email to its mailing list asking for opinions on a series of questions relating to the development of the CacaoNet conservation strategy. The following is a Summary of replies of the opinions received by 1st June, including the responses received after a preliminary report was compiled for the CacaoNet Steering Committee meeting, which took place on 22nd May. The responses received will be made available to the INGENIC mailing list, and through the INGENIC website archive, together with this initial Summary of replies.

1. What type of material should be included in a Global Cocoa Collection?

1.1 Should a distinction be made between a collection for immediate or mid-term use (active or working collection) and a base collection aimed at the conservation of the totality of genetic diversity in as small a collection as possible?

Summary of replies

Opinions differ on the need to distinguish types of collection and there were some different interpretations of the terms depending on the perspective of the contributor, for example from breeders and genebank curators. Some see no need for such distinctions, emphasizing instead that the important point is that the collections are actually used. However, several respondents consider that there is a clear distinction between the active/working collections used by breeders to create new varieties with the characteristics needed by the farmers in their regions/countries today, and the base/extended core collections comprising the widest possible range of genetic diversity for future needs.

While only minor changes over time are expected for the base/extended core collection, the evolution of a breeder's working collection will be more dynamic as new needs and interests appear.

However, some genebank curators used the term "working/active" to refer to their main collection since this is the material they are currently characterizing/evaluating, and used the term "base collection" to refer to a back-up collection, perhaps conserved as in vitro samples.

One respondent considered that the only "working collections" that should be associated with CacaoNet are intermediate quarantine collections, such as the ICQC at Reading. Such collections provide disease-free material to the global cocoa community, which cannot be done from the base collections. However, the ICQC,R collection should be considered distinct from any core collection since it is a dynamic collection, and the prioritization of its accessions will have been based on different criteria from conservation and/or duplication (though some accessions may be common to both the quarantine collection and core collections).

One respondent noted that the high cost of maintaining large working germplasm collections is often overlooked, and commented that it could be argued that pressure from the Genetic Resources Movement to use the germplasm is hampering development of better varieties for farmers, at least in some situations, because it is taking resources away from breeding programmes.

Full evaluation and documentation of material in working collections, and the value of pre-breeding were raised as priorities to ensure better utilization of the germplasm.

Whilst one respondent appeared to favour a single centre, several others consider that the base collection could consist of accessions from a network of sites, rather than one all-embracing base collection. These sites could be selected for their suitability for the cultivation of different types of cocoa as well as where material happens to be (Brazil, for example). The base collection could include (or consist of) stored DNA, provided that intact plants can be recovered to order without irreversible genetic change.

One proposal would be to consider the currently available international accessions (held mainly in CATIE, CRU, ICQC,Reading) as the Global Public Domain Base Collection. Further research and evaluation of the material in this Base Collection would allow the identification of a Global Core Collection, representing the widest range of genetic diversity within the fewest accessions, which would be prioritized for long-term conservation with international support. The suggestion was made that for cost-efficiency and breeders' needs, this Core Collection might contain approximately 800 fully evaluated, documented and duplicated accessions.

Whatever terminology is used, it is clear that these types of collection have different users, require different conservation strategies and could be of interest for support by different donor groups/ institutions. CacaoNet needs to establish clear definitions of these types of collection, so that future discussions on a conservation strategy can move forward from a common understanding of the terms used.

1.2 What are the main criteria for the choice of accessions based on an "ideal" situation, based on breeders' needs, curators' views, genetic diversity, *etc*.

Summary of replies

Several respondents consider that the choice of accessions for long-term conservation should be largely based on genetic diversity. Information revealed by molecular markers should play a major role in the selection criteria. One respondent commented that recent research suggests that 11 or 12 distinct genetic groups exist among the germplasm currently in collections. He proposed that since many of the accessions currently maintained are sibs or closely related Trinitario types, the new classification developed by Motamayor et al. 2007 (in press) could be used to reduce redundancy in the collections whilst ensuring that the widest range of genetic diversity is conserved. Another respondent commented that the range of genetic diversity available in the public domain should, if possible, be expanded through additional exploration missions in the centre of origin/diversity of cacao. One respondent commented that the collection should be comprehensive and that if/when cheap long-term storage becomes a reality, any question of redundancy becomes much less important.

For breeders' working collections, the choice of accessions will vary according to local needs and is likely to change with time, possibly with the reduction of the size of the collection as the breeding programmes mature. The working collections might take into account the preservation and utilization of accessions of strategic importance and immediate relevance for farmers in terms of pest/disease resistance, industrial properties, agronomic characters, etc. with genetic diversity as a secondary criterion.

In all types of collections, information on the characteristics and origins of the accessions would be very valuable in the selection of the accessions.

2. How best to conserve a Global Cocoa Collection?

2.1 What are the advantages/disadvantages of conserving material as clonal accessions, seedling populations, pollen, cryopreserved tissues/somatic embryos, DNA?

Summary of replies

Conservation of accessions as clones has several advantages:

- *They can be characterised and evaluated (in various environments if appropriate), used as parents, and propagated conventionally (including distribution via quarantine), and all the information obtained can be shared.*
- *risk of genetic change is minimized and for selected materials, genetic gain is preserved.*
- *Material is immediately available, without the regeneration stage needed for cryopreserved collections.*

One respondent noted that the elimination of the use of rootstocks (e.g. by propagation by rooted cuttings, or plantlets from tissue culture) can improve genebank management since it eliminates the possibility of mislabelling occurring as a result of outgrowth of the rootstock.

However, one respondent considered that seedlings are sometimes easier to manage, and could be used for certain poorly represented accessions. Another commented that seedling populations might have an application if a large collection of limited genetic origin were made as seed - but if that were the case the collection could be rationalized using molecular markers. He added that maintaining populations as seedlings would not generate genes that are not in the foundation trees, and is arguably more difficult than maintaining clones. Another respondent considered that seedling populations should not be recommended without a full programme of screening and characterisation to define what use they could have. This wastes a great deal of effort, since many would need to be discarded.

Living tree collections, whether as seedlings or clones, require substantial land and other resources. There are several new biotechnological techniques, which have potential as a means of providing a safety or "back-up" collection, though these are largely as yet unproven. Cryopreservation of somatic embryoids appears to have potential and be cost-effective, though further research is needed.

If pollen is conserved, the pollen compatibility reaction should be taken into account.

Further research would also be useful to investigate the potential of the movement of genes (through seeds or pollen, rather than genotypes) in accelerating the safe and efficient transfer of materials from the global collections.

2.2 How can the security of collections be assured? What are the requirements for safety duplication?

Summary of replies

Long-term funding is essential to assure the security of the collections. The security and suitability of each site (environmental, political, etc.) should also be considered. Whilst some commented that accessions should be replicated in two or three different locations, ideally geographically distant (field collections, quarantine centres, centres in West Africa/South Asia), others were concerned that planning for duplicate living collections would be a mistake when funding for the existing collections is not yet assured. Others emphasized the importance of fingerprinting technology to avoid misidentification problems during duplication.

Cryopreservation of tissues/somatic embryos would provide a useful back-up and an offer has been made by USDA-ARC to maintain the cryopreserved somatic embryos at the Center for Genetic Resource Conservation (the old National Seed Storage Lab) in Ft. Collins, CO.

3. Where to conserve a Global Cocoa Collection?

3.1 How can we make best use of the existing International collections at ICG,T and CATIE, and the ICQC,R quarantine collection at Reading University?

Summary of replies

Several respondents referred to the existing expertise, field collections and facilities at these international collections, though reference was also made to the possible need for further investment to ensure that these facilities could accommodate and facilitate any future increase in demand for germplasm.

Several respondents commented that the efficient utilization of germplasm held in the international genebanks has been hindered by the lack of information for many of the accessions including passport data, characterization/evaluation (particularly agronomic character) data and information on their genetic diversity. Mislabelling and redundancy in the collections have also reduced their efficiency. Molecular data are becoming available which will help curators resolve mislabelling and reduce redundancy, and identify genotypes, which could be added to the international collections to increase their genetic diversity. Additional resources are needed to enable accessions to be fully characterized/evaluated especially for valuable agronomic and disease resistance attributes.

Although the principal role of the international collections is conservation, they may also play an important role in the development of materials useful in precautionary breeding for diseases not present in other cocoa growing regions. An example would be the development of QTLs and identification of accessions with field resistance to Frosty Pod disease in the CATIE collection.

The importance of an effective quarantine system in ensuring the safe transfer of material between collections/institutions in regions affected by different pests and diseases was highlighted. One respondent commented that the quarantine facility at Reading functions well, though it is essential that the material passed through quarantine is prioritized. The Reading group emphasized that the ICQC,R should be considered as a working collection for distributing material rather than as a part of the conservation framework. Another respondent suggested that it would be useful to establish the feasibility of applying specific treatments to permit the safe transfer of germplasm between countries within a region, or overseas.

3.2 How could genetically distinct material at other collections be conserved and made publicly available?

Summary of replies

One respondent considers that conservation and making germplasm available are quite separate issues that should not be linked; though we may have a duty to conserve, the owners of germplasm do not have a duty to share it.

Another respondent considers that although there is undoubted value in a number of accessions that are not publicly available, there should not be any funding/direct collaboration by CacaoNet to support those accessions while that material remains unavailable. Nevertheless, it is likely that support provided through CacaoNet to a collection holding a mixture of national and public domain accessions would enable capacity building, which would benefit the whole collection.

Others note that there are governmental regulations in some countries such as Brazil, which prevent the distribution of national germplasm and until this situation changes these resources will not be available to the cacao research community. Others suggest that attempts to negotiate the exchange and public availability of accessions based on disease resistance traits may be more successful than when based on productivity or specific quality traits.

One respondent understands the focus of CacaoNet on public domain accessions, but wonders to what extent CacaoNet could also serve as reposi-

tory for genebank and in situ information on national germplasm, such as the genotypes believed to hold potential for the production of fine-flavoured cocoa in Latin America (Central America, Andean countries), but that is not yet well-represented in the international collections. Whilst there may be reluctance on the part of national authorities to put their materials in the international public domain, it may be that they are willing to participate in a global system of information sharing on cacao genetic resources. It may be that projects such as the Bioversity project on native germplasm in Nicaragua might look to CacaoNet as a potential repository of georeferenced information.

One respondent commented on the need to develop in situ conservation strategies.

Others see CacaoNet as the international framework that will lead to cooperation and the economic and technical support necessary to support the conservation efforts at the collections and establishment of germplasm exchange agreements based on mutual benefits. They envisage that CacaoNet would also help to reduce or eliminate duplication among national institutions, and provide a cost-effective instrument for information exchange and institution building.

3.3 What would be the advantages/disadvantages of establishing a "virtual" collection, with accessions distributed in collections across the world, as opposed to a centralized collection?

Summary of replies

Several respondents commented that lack of funding would preclude the complete duplication of the accessions in each of the existing international collections, which would in any case contain a mixture of important accessions and other clones included for other reasons. Moreover a virtual collection is cheaper, easier and faster to establish than a new centralized collection since it builds on the existing framework and will provide access to a complete spectrum of genetic diversity to the cacao research community.

One respondent commented that there are few advantages to creating a large central collection and doing so will remove a large incentive to donate material from collections that might receive funding and support if part of the virtual collection.

A virtual collection would allow strategic germplasm to be easily distributed within a given geographic region in accordance with local/regional needs and quarantine measures. A virtual collection might also encompass some of the farmer varieties and landraces in Central and South American countries, which are often not included in the centralized collections.

However, without duplication, there is a high risk of losing unique genotypes. Therefore valuable genotypes should be held in least at two centralized collections. The centralization of these important genotypes will facilitate the characterization and evaluation of the genotypes under uniform and standardized conditions.

4. Rationalization of current base collections containing public domain germplasm

4.1 Is there a need to rationalize current international collections? What type of activities should the rationalization process consist of?

4.2 What criteria can be applied to reduce redundancy in current collections? How can aspects such as unique genetic background, genetically similarity/hybrid origin of accessions, agronomic value and resistance traits be taken into account?

Summary of replies: There were some different interpretations of "rationalization" and the extent to which it could be undertaken in the collections. Rationalization focused on the eradication of mislabelled and duplicate accessions was seen as an important step by many respondents. One respondent commented that from molecular fingerprinting studies, it appeared that the rate or non-congruency of nomenclature between collections ranges from 35-40 percent. It was suggested that the rationalisation process should consist of a molecular and/or morpho-physiological characterization of the existing genotypes, the verification of the true types, and a comparison of all genotypes with these true types, finally leading to the elimination of duplicates and very closely related materials. The USDA-ARS team in partnership with the international collections has now generated DNA fingerprints for most of the accessions, though one respondent cautioned that the number of microsatellite markers used may not be sufficient in all cases and others warned that it would be foolish to eliminate accessions due to their similarity to others until the relationships between valuable traits and molecular characteristics are more fully understood. A new research project at CATIE supported by Bioversity (Vavilov-Frankel Fellowship Award) and in collaboration with USDA-ARS will give genebank curators the confidence they need to rationalize the collections based on a comparison among molecular profiles and phenotypic traits of apparent identical clones.

Diversity analysis and gap identification was suggested as another step to rationalize the collections. CacaoNet should consider ways to capture and conserve ex situ more of the genetic diversity that exists in situ.

Several respondents commented that some traits, such as disease resistance and perhaps organoleptic/quality traits, are more important than others. It might be appropriate therefore to prioritize traits, and then identify those clones known to possess the characteristics of highest priority, perhaps using a similar approach to the ranking tool being developed at ICQC,R. However, it was noted that we do not fully understand the inheritance of these traits and that important material could be missed because it has not been properly tested. Others warned that there could be new pests and diseases of which we are currently unaware, and

material with resistance characteristics to these could easily be missed in the prioritisation process.

Another respondent suggested that where material is of limited genetic origin, for example much of Pound's material, if genetic similarity can be demonstrated and inferior individuals can be identified for (presumably highly heritable) traits like bean weight or cocoa butter content they would be strong candidates for preservation in vitro. He considered that agronomic value would be a difficult criterion to take into account given GxE in the usual sense of locations and the specific one of planting density. Pest and disease resistance criteria might be even more difficult to assess since the field reaction may differ widely between different locations given the variation in strains, environmental conditions and disease avoidance mechanisms. In the Summary of replies, there is a need to rationalize collections, but material should not be removed until it has been preserved, and only once there is consensus that the technology is sufficiently reliable.

One respondent proposed that, for a long-term public domain collection of c.800 accessions, approximately 600 of these would be selected based on genetic diversity considerations and the remaining 200 would be selected on the basis of their agronomic characteristics. The main criteria used to select the 600 accessions might be: genetic uniqueness (mainly based on molecular data), genetic origin (with priority to spontaneous or sub-spontaneous materials, and avoiding accessions of hybrid origin such as Refractario types that are probably better represented by parental types) and agronomic value (accessions with similar molecular profile need to be evaluated and the accession with highest known agronomic value is maintained). The main criterion for selecting the 200 accessions would be agronomic value (best genotypes for yield, quality, disease/stress resistance, plant size, etc.) with genetic diversity considerations as a secondary priority. This group would probably include many accessions of hybrid origin (e.g. selected clones in the public domain and selections from public domain hybrid populations, such as Refractario and Trinitario types).

One respondent suggested that collections could be rationalized in terms of reducing the number of trees representing each accession, if the safety of the accessions could be assured through the use of well managed nurseries and/or if the material was replicated in other collections.

This is an area which is likely to stimulate much further discussion and require detailed input from molecular biologists, geneticists, curators and breeders. It was suggested that CacaoNet consider creating an appropriate body, such as a working group or standing technical committee, to further consider this issue.

4.3 Is any further research required to allow collections to be rationalized without the loss of valuable genetic diversity?

Summary of replies

Several respondents agreed that thorough characterization and evaluation will be required to be able to take the right decisions when rationalizing the Global collection. Phenotypic characterization and field evaluation of the collections is often inadequate and requires further resources.

It will be important to determine segregation of relevant traits within redundant groups through the use of molecular tools.

Genetic structures within each collection, as well as the global structure in the American genepool need to be analyzed. The analysis will include genetic distances among accessions, populations, and geographical groups. Permutation tests need to be used to determine the optimum sample size and sampling strategies that will maximize the probability of maintaining genetically distinct accessions in each collection. The geographic distribution of cocoa diversity needs to be mapped using a GIS tool. Maps of the demographical distribution showing diversity richness and areas showing the level of complementary diversity need to be developed.
The safe movement of vegetative material within regions should be given further consideration.

Discussion Paper on Germplasm to be Considered in Setting up a Long-Term Global Cacao Conservation Strategy by CacaoNet

As recommended in the INGENIC Survey on the Development of CacaoNet Conservation Strategy (above), the CacaoNet Steering Committee agreed at its May 2007 meeting that a Working Group be created to address the complex issues involved. The newly formed Conservation Strategy Technical Working Group (CSTWG) will oversee the process of preparing the draft global conservation strategy of cacao genetic resources for consideration by the CacaoNet Steering Committee (SC). Jan Engels has agreed to act as Convener for the CSTWG and Michelle End will act as the Coordinator.

We would be pleased to receive any comments on this paper, which will be collated for the CSTWG discussions. This paper is an attempt at clarifying the concepts handled in the INGENIC survey (see separate note in this Newsletter), as a basis for further discussions in the CSTWG, Please send your responses to Michelle End (michelle.end@bccca.org.uk) and Jan Engels (j.engels@cgiar.org.)

Cacao genetic resources covers all material from the wild plants growing in the forests of South and Central America, to the accessions held in national and International genebanks (both as *in vitro* or in field genebank collections), the material in breeders' trials and the trees growing in farmers' fields. CacaoNet intends to develop a strategy, which will ensure that the total range of this diversity is conserved efficiently and rationally and that the effective utilization of these cocoa genetic resources is promoted.

In situ resources: This includes the wild, semi-wild and cultivated material that exists in natural habitats or in farmers' fields, including traditional varieties and landraces. A priority for CacaoNet will be to identify sources of genetic diversity, which is currently under-represented in *ex situ* genebanks, and to facilitate the collecting of material from such sites, particularly where it is threatened.

Ex situ genebanks: This comprises all cocoa germplasm currently maintained in field genebanks or in *in vitro* collections wordwide. Funding for these collections is currently provided by national/international private and/or public institutions, but is often insufficient for effective conservation. Most of the accessions are clonally propagated. Many of the accessions have not yet been fully characterized, either morphologically or genetically, and have not been fully evaluated. A priority for CacaoNet will be to review existing information, and to facilitate where necessary the collecting of new characterization/evaluation data for these collections in order to identify priorities for long-term conservation and utilisation.

1. Cacao Germplasm Accessions considered as internationally available.

Germplasm that is freely available to any *bona fide* user is an important asset for the global cocoa community and the world cocoa economy. It acts as a genetic reservoir of the characteristics, many of which are complementary to those in existing national collections, which are vital in developing the new varieties so important for more sustainable production. Once material has been assigned public domain status, it can be exchanged legally and fairly, through the use of Material Transfer Agreements.

For these reasons, many international donors will only provide support for the conservation and utilization of germplasm that is in the public domain such as the material that is part of the multilateral system of the International Treaty (IT), *i.e.* all species/crops that are included in Annex I of the Treaty. Similarly, through Article 15 of the IT, the germplasm maintained by the CGIAR system is also in the public domain. Unfortunately, cacao is not a crop included in Annex I, but it is planned that through the collaboration of CacaoNet with the FAO/IT[1] system and by following the same rules and conditions that apply to Annex I species, that cacao eventually will be treated as an "associated crop", thus opening possibilities for long-term support for instance through the Global Crop Diversity Trust.

Many cacao producing countries have ratified the IT and thus recognize the authority of the Governing Body and the conditions under which germplasm included in the multilateral system will be exchanged and benefits shared. The International Cocoa Genebank, Trinidad (ICG,T) and the cacao collection at CATIE, Costa Rica have established formal agreements with the Governing Body of the IT so that their collections of germplasm, built up as an international resource and already freely available to all *bona fide* users, can formally be placed in the public domain. Efforts are underway to confirm the legal status of the accessions incorporated into these international collections after December 1993, when the Convention on Biological Diversity (CBD) came into force.

[1] International Treaty of Plant Genetic Resources for Food and Agriculture (IT)

Countries might also consider placing some of their accessions from their national collections in the public domain, with a view to their inclusion in the CacaoNet Global Strategic Cacao Collection (described below), once it has been established that they represent a unique genetic resource and adequate Access and Benefit-Sharing (ABS) arrangements have been developed preferably similar to those of the IT.

2. "CacaoNet Global Strategic Cacao Collection" (CacaoNet Collection)
This is envisaged as a collection of public domain accessions that can be considered as strategic for long-term conservation and use of cacao germplasm. It will consist mainly of accessions of *Theobroma cacao*, though accessions of related *Theobroma* and *Herrania* species might also be included. Its identification, maintenance and management should be considered as one of CacaoNet's main priorities for which stable long-term funding needs to be sought. This collection will result from a coordinated evaluation and rationalization effort of available cacao genetic resources, through application of agreed criteria (mainly genetic diversity/uniqueness and agronomic value) to identify priority accessions which can be added to the CacaoNet Collection once they have been placed in the public domain.

The absolute size of the collection will inevitably be partially determined by the financial resources available and the methodology, or methodologies, used to conserve and safeguard the collection. For example, it may be that new *in vitro* technologies may offer a relatively inexpensive way to conserve large numbers of samples, though information resulting from a thorough characterization/evaluation of these would need to be made easily available if they are ever to be utilized. A collection of DNA samples may also be potentially useful, if the samples could be used to rapidly screen, for example, the presence of newly developed molecular probes, though again, the information system and means to regenerate matching cryopreserved samples (if this was the method of conservation selected for those accessions) would have to be in place for the utilization of this germplasm. Field genebank collections of trees, especially of priority accessions frequently requested due to their genetic/agronomic characters of highest value at the current time, always have the advantage that they can be re-propagated and distributed quickly as well as evaluated on an on-going basis as new needs arise.

The collection may be conserved as a "centralized" collection (maintained at one or two sites only) or preferably as a "virtual" collection (based on a collaborative network among cocoa collections worldwide). In either case, CacaoNet will develop the information system necessary to efficiently manage information from the collection(s) and make it freely available to stakeholders, so that it is easy for any potential users of the germplasm to establish what material is held where, and in what form, so that they can decide on the most appropriate way to acquire it for their own collection.

The two international genebanks at ICG,T and CATIE already hold genetically diverse collections of internationally available cacao germplasm and will form the backbone of the CacaoNet Global Strategic Collection. Many of the accessions held at ICG,T and CATIE have been at least partially characterized and evaluated, and these data can be used to establish which accessions should be prioritized for long-term conservation in the CacaoNet collection, and in what form they should be conserved.

Existing data already indicate that some accessions, currently only held in national collections, are likely to be of high priority for inclusion in the CacaoNet collection and it is hoped that appropriate access and benefit-sharing agreements can be established to allow this material to be fully evaluated and included in the CacaoNet collection as public domain material.

3. "Working Collections"

Although many respondents to the INGENIC enquiry recognized a difference between collections set up for long-term conservation and "working collections" there seems to be some different interpretations of terms.

In its widest connotation "working collection" was considered to include any material that was currently being evaluated/studied, including material in the international and national collections. It was also used to refer to transient collections such as the International Cocoa Quarantine Centre, Reading and national breeders' collections, which do not have a role in long-term conservation and are expected to change composition with time. A proposal was also made to establish a "Global Breeders' Working Collection, a transient collection of a limited number of publicly available accessions, which had been chosen as priorities to meet current breeding needs.

For clarification, it might be helpful to describe the three types of "working collection" that CacaoNet could be involved with, in one way or another:

International and National Cocoa Genebanks: CacaoNet may have a role in facilitating the evaluation and characterization of accessions and in using the data obtained to prioritise accessions for inclusion in the CacaoNet Global Strategic Collection for long-term conservation and/or the CacaoNet Global Breeders' Working Collection (described below).

CacaoNet Global Breeders' Working Collection: This is a group of global public-domain accessions considered of direct value for breeders in different parts of the world. The collection is of limited size and its composition will vary over time according to the current needs. The accessions that are part of it are to be identified based on criteria set by CacaoNet. These materials are the priority accessions to be made available through intermediate quarantine (ICQC,R) to all *bona-fide* end-users. Many of the accessions identified as being part of this collection are also part of the CacaoNet Global Strategic Collection. Such accessions, could be marked with a special flag in CacaoNet's information management system. Additionally, the Global Breeders Working Collection might contain accessions, which have low value for long-term conservation, but which are still very important for the utilization of cocoa germplasm in the more immediate future. Such materials might include accessions from pre-bred and/or hybrid populations, which can accelerate progress in breeding since they represent specific combinations of characteristics, but which would not be appropriate to include in the CacaoNet Global Strategic Collection if their genetic diversity was already conserved in the collection through the inclusion of their parents.

CacaoNet Quarantine "Collection": CacaoNet may have a role in coordinating and prioritizing accessions to pass through quarantine facilities to ensure the safe distribution of germplasm, whether that should be for conservation or utilization purposes. Although some have referred to the material held in the International Cocoa Quarantine Centre at the University of Reading (ICQC,R) as a "working collection", it will not form a CacaoNet collection as such since it is simply a subset of the CacaoNet Global Strategic Collection and CacaoNet Global Breeders' Working Collection that is held in a particular place (*i.e.* a quarantine facility) for a finite period of time. Such material could be identified by a special flag in the CacaoNet information management system, possibly with a link to indicate quarantine status and availability.

Michelle End

Jan Engels

Bertus Eskes

INGENIC GENERAL ASSEMBLY

Minutes of the Meeting held 16th October, 2006 in San José Costa Rica

Chairman: Bertus Eskes, Secretary:

Michelle End

1. The minutes of the previous INGENIC General Assembly (GA) held on 20th October 2003 in Accra, Ghana were read by INGENIC's Secretary, Michelle End, and were adopted.
2. The Chairman presented some of the main activities that INGENIC had been involved in over the past three years including the molecular biology working group, the INGENIC website, the development of regional breeding programmes and the development of the CacaoNet concept.
3. The Secretary/Treasurer presented a summary of the INGENIC accounts for 2003-2006 and her estimates for the next three years. INGENIC had sufficient funds to cover its current activities including the 5th Workshop, but more funds would be required to continue activities beyond 2006. The contributions from BCCCA and USDA towards INGENIC's running costs were gratefully acknowledged. INGENIC secured funding from CTA, USDA and Stiftung der Deutschen Kakao- und Schokoladenwirtschaft to enable ten scientists to attend the 15th ICRC and INGENIC workshop.
4. The Editor presented her report on the status of the Newsletter. She reminded participants that INGENIC had released the tenth issue of its Newslet-

ter in September 2005. To date, some 104 articles had been included in the newsletters reporting on research and activities carried out in over 17 institutions. Although the idea that the Newsletter could follow a defined structure had been proposed at the last GA, it had not been possible to adopt this due to the diversity of the articles received. As suggested at the last GA, Mrs. Bekele had contacted Mr. Lass regarding the Cocoa Growers' Bulletin as the journal of choice for the more detailed articles, but Mr. Lass had no immediate plans for a further edition due to his time constraints. The positive feedback received indicated that the Newsletter continued to have an important role in sharing information within the cocoa breeding community. A number of articles had been received for the next edition, and the Editor expressed the appreciation of the INGENIC Committee for this vital input, and for the encouragement of the readership. The financial and/or logistical support of BCCCA, CIRAD, CRIG, CRU, MCB, UESC and USDA was gratefully acknowledged.

5. The Chairman thanked Mrs. Bekele for all the hard work that she had put into producing the Newsletters. He then opened a discussion on the possible format of future Newsletters and/or discussion groups to cost-effectively facilitate an exchange of ideas within the research community. The following points and suggestions were raised:

- Although electronic distribution offers a very convenient and cost-effective way to distribute information to those, who have reliable access to the internet, it was likely that hard copies, or CD-ROM copies, would still be needed by those, who cannot receive it electronically.
- Detailed articles take considerable time to produce and there will be competition for such articles from peer-reviewed journals and other Newsletters, such as GRO-COCOA and the WCF Updates. Although a move to a peer-reviewed journal co-produced by the INGENIC/INCOPED/INAFORESTA would perhaps stimulate authors to submit their articles, it was unlikely that the IN- groups would have the resources, including editorial time, to launch such a publication. Accordingly, the Chairman stressed that the INGENIC newsletter's focus should remain on short, informal pieces to stimulate discussion.
- To improve timely access to such information, it was suggested that the articles could be published on the INGENIC website as soon as they were received and then collated to form an edition of the Newsletter with the objective of publishing annual issues.
- It was suggested that one or more internet discussion groups be set up to stimulate discussion on specific topics, following the model of the FAO discussion topics, which are launched each month. Outputs from these discussions would be summarized by the moderator into short articles for the Newsletter.
- Institutions and/or research groups might be asked if they could prepare short updates on their current activities. These requests could be staggered so that an update was received from at least one institute/group each month.
- It was suggested that it would be very useful to share more information with the other IN-research groups, perhaps by encouraging the submission of relevant articles from the other IN- groups and sharing notifications of recent publications.

  It was agreed that these suggestions should be taken forward with a view to encouraging more dynamic exchange of ideas within the research community.

6. Dr. Mark Guiltinan, Chairman of the Molecular Biology Group, gave a demonstration of the website that he had set up for INGENIC. The website included a communications page enabling INGENIC members to access and update email lists, as well as view archived email exchanges. The website contains pages with the contents of all INGENIC Newsletters and of all Proceedings of its International Workshops. Dr. Guiltinan invited members to contribute to the further development of the website, particularly through suggestions for new inclusions and updates to the "Links" and "Announcements" pages. Dr. Schnell commended Dr. Guiltinan for his work on the website, and suggested that it would be worth demonstrating it to the other IN- groups to see if they might be interested in developing similar and/or linked websites.

7. Dr. Yaw Adu-Ampomah, Vice-Chairman Africa region, gave an update on the Regional Breeding initiative for Africa. A group of interested researchers had met in Ibadan in 2005 and early 2006 to discuss how a collaborative approach could benefit breeding in the region. The group had considered the priorities for breeding work, carried out an analysis of strengths and weaknesses and discussed how best to seek funding for collaborative approaches. One of the most serious problems facing cocoa farmers in West Africa is CSSV, and COPAL had held a meeting to discuss the development of a proposal for a regional CSSV project for submission to the CFC. Dr. Eskes reported that a group of breeders from the Americas had met during the

CFC/ICCO/Bioversity project regional meeting in Venezuela, February 2006 to discuss ideas for regional breeding work and these discussions are summarized in the Proceedings of the meeting (a CD-Rom was distributed to all participants). The possibilities for collaborative work on Monilia coordinated by CATIE were discussed although it was not possible to develop a proposal for submission to CFC since Costa Rica is not an ICCO member. Dr. Eskes also reported that in South-East Asia there is an on-going effort to promote exchange of information and germplasm to tackle CPB.

8. The Chairman reported on the launch of CacaoNet, a global network for cacao genetic resources, which had taken place during the 15th ICRC, and the Discussion Meeting organized by INGENIC held later that same week to initiate discussions on cacao germplasm strategies and on how INGENIC could participate in the CacaoNet initiative. The meeting had provided an opportunity for participants to provide an update on the current status and prospects for their germplasm collections, and their initial thoughts on strategies for conservation, germplasm characterization and germplasm evaluation/utilization. The Secretary agreed to produce a note from the meeting, which would be circulated/made available on the INGENIC website in due course. There is much need for further discussion on this, and it was agreed that INGENIC should initiate discussions via the internet with a view to collating the various opinions expressed and contributing these to the CacaoNet Steering Committee and/or Standing Technical Committee that would be in charge of developing a long-term conservation strategy for cocoa.

9. Dr. Guiltinan reported that the Molecular Biology group, formed in 2003 following the previous INGENIC workshop, had held its second meeting the previous day. Some 30 researchers had participated, and twelve presentations had been given to provide updates on key areas in cocoa molecular biology including the genome mapping work at CIRAD, the genetic fingerprinting work coordinated by USDA and the formation of a microarray group.

10. The composition of the INGENIC Committee was brought up for debate. The Chairman informed the gathering that all of the Committee positions were up for election. Nominations for replacements for the existing Committee were invited, but none were forthcoming. It was accepted that the current members would retain their seats until the next GA.

11. The Chairman asked for suggestions for the theme for the next INGENIC workshop. He suggested that one possibility would be the current status of cocoa variety development and distribution. This suggestion was received with interest since it was acknowledged that there have been many developments since the topic was last discussed at a workshop meeting. It was agreed that a draft title be proposed as a subject for an internet discussion.

12. The INGENIC Committee was acknowledged for its work, and the Chairman expressed appreciation for this on behalf of the Committee.

In Memoriam

Dr. Aliyu Abdul Karimu

Died August 17, 2005 at age 49

Research Officer (Plant Breeder) of the Cocoa Research Institute of Ghana (CRIG)

by Dr. Boamah Adomako and Mrs. Victress Johnson

Dr. Karimu joined the Plant Breeding Division of the Cocoa Research Institute of Ghana (CRIG) as an Assistant Research Officer in October, 1981. In 1985, he was awarded a scholarship to pursue postgraduate studies at the University of Reading, U.K. and passed out with a PhD degree in Environmental Genetics in 1992. He returned to Ghana soon after his study to contribute his quota to CRIG and the Ghana cocoa industry.

In February 1992, he was upgraded to Research Officer and by virtue of satisfactory performance and conduct, he was promoted Senior Research Officer on 1st October, 2004.

Until his death, Dr. Karimu was a resourceful plant breeder, who made substantial contributions to the development of improved planting materials of the CRIG mandated crops (cocoa, coffee, kola, cashew, and shea). In particular, he made a significant contribution to the development and eventual release for propagation in the Cocoa Seed Gardens of the Ghana Cocoa Board three female parents, PA 7, PA l50, and Pound 7. These parents, which are high-yielding, are also resistant/tolerant to the black pod disease. He continued to search diligently in farmers' farms for more resistant/tolerant genotypes to the black pod disease. He also worked assiduously to obtain early bearing and high-yielding kola planting material for farmers.

He was the leader of the Kola Development Thrust of CRIG and a member of other thrusts of the Institute, including the Cocoa Improvement and Cocoa Fungal Diseases Management Thrusts. He also made significant contributions to the on-going CFC[1]/ICCO[2]/CRIG[3] Biodiversity International projects and other projects.

Dr. Karimu authoured or co-authoured many scholarly articles and publications and was an inspiring mentor to his colleagues. He treated every co-worker with respect and was a very good CRIG "citizen," deeply involved in the Plant Breeding Division affairs.

Dr. Karimu was very committed to his profession and to his chosen scientific field. He was always willing to share his knowledge and expertise with others. In addition to his activities at the Institute, Karimu was a member and active participant in professional organizations, most notably the International Group for the Genetic Improvement of Cocoa (INGENIC) and the Ghana Science Association (GSA).

Outside of work, Karimu enjoyed international travel and had a deep appreciation for history and other cultures. He loved listening to a wide array of music. He had a quick wit and liked to tell a good joke. Karimu was an excellent mix of humour and dignity. He left behind a beloved family and a multitude of friends and co-workers.

Dr. Aliyu Abdul Karimu was survived by a wife and seven children. He will always be remembered as a loving husband, a devoted father, a good friend, a dedicated scientist and as the phrase was aptly coined, "a gentleman and a scholar."

May his soul rest in perfect peace.

[1] Common Fund for Commodities
[2] International Cocoa Organization
[3] Cocoa Research Institute of Ghana

Global Approaches to Cocoa Germplasm Utilization and Conservation

Final report of the CFC/ICCO/IPGRI project on
"Cocoa Germplasm Utilization and Conservation:

a Global Approach" (1998-2004)

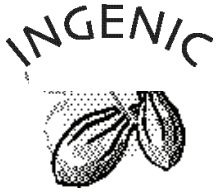
A.B. Eskes and Y. Efron, editors

Copies of these two documents, that were released in 2006 and 2005 respectively, are available from Dr. Albertus B. Eskes (B.ESKES@CGIAR.ORG):

1. The Final report for the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: a Global Approach (1998-2004)." Published in 2006 and edited by A.B. Eskes and Y. Efron. CFC Technical Paper 50. ISBN-13: 978-92-9043-734-5
   ISBN-10: 92-9043-734-0

2. The Proceedings of the International Workshop on "Cocoa Breeding for Improved Production Systems." October 19-21, 2003, Accra, Ghana.
   Edited by F. Bekele, M.J. End and A.B. Eskes. Published in 2005 by INGENIC and COCOBOD, Ghana.

INGENIC Committee



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