Combining Ability for Disease Resistance, Yield, and Horticultural Traits of Cacao (*Theobroma cacao* L.) Clones

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ABSTRACT. Knowledge of genetic differences among commonly cultivated cacao clones, as well as the type of gene action involved for disease resistance, yield, quality, and horticultural traits, are essential for cacao breeders to select parental clones efficiently and effectively. This information is also critical for quantitative geneticists in designing and improving quantitative trait loci (QTL) localization strategies using breeding populations, whether they involve analysis of multiple populations crossed to one common parent or association genetic analysis. The objectives of this research were to 1) verify the genetic identity of parental cacao clones used to produce hybrids for field evaluation at the Centro Agrónomico Tropical de Investigación y Enseñanza (CATIE), Turrialba, Costa Rica, using molecular marker analysis, and 2) estimate general and specific combining ability (GCA and SCA) of the parental clones for resistance to frosty pod (*Moniliophthora roreri* Cif. and Par.) and black pod [*Phytophthora palmivora* (Butl.) Butl.] diseases, total number of pods, vigor (as measured by trunk diameter), and measures of maturity (months to first flowering and pod production). Misidentification of cacao clones was found at three levels. Molecular marker analysis revealed that six parental clones differed in identity to supposedly identical accessions from other germplasm collections. Trees of the parental clone UF 273 consisted of two clearly different genotypes, resulting in two types of progeny, requiring separate designation for correct statistical analysis. Out-crossed progeny, presumably from foreign pollen, and selfed progeny were also found. Two of the traits measured, percent healthy pods and percent pods with frosty pod, showed predominantly additive gene action, while the traits total number of pods and trunk diameter, demonstrated regulation by both additive and nonadditive gene action. Number of months to first flowering and first fruit both showed evidence of predominant regulation by nonadditive gene effects. Crosses of two parental clones, UF 712 and UF 273 Type I, were identified as potential candidates for QTL analysis as breeding populations, given their favorable GCA estimates for frosty pod resistance and total pod production, respectively.

Cacao is a perennial species widely cultivated in America, Asia, and Africa, whose beans are the basic component for cocoa and chocolate. The genus *Theobroma* L. is composed of 22 species with some economic value, of which four species are cultivated: *T. cacao*, *T. grandiflorum* (Willd. ex Spreng.) Schum., *T. bicolor* Humb. and Bonpl., and *T. angustifolium* Moçônio and Sessé. Only *T. cacao* is used for chocolate production (Cuatrecasas, 1964; Silva et al., 2004). *Theobroma grandiflorum*, known as cupuassu (or cupuaçu), is cultivated for production of sweet beverages, ice cream, confections, and a product similar to cocoa known as “cupulate” obtained from fermented seeds (Alves and Figueira, 2002).

Three main genetic types of cacao have been traditionally recognized: Criollo, Forastero, and Trinitario. The Criollo type

is known for the high-quality, nutty-flavored chocolate that it produces. Contrary to Criollo, Forastero cacao is generally recognized to have high vigor and prolificacy, and is traditionally sub-classified into Upper and Lower Amazonian groups. Forastero cacao constitutes an important genetic type for commercial production; its presence as an ancestor was recognized in ≈80% of the world plantations (Cheesman, 1944). The Trinitario genetic group is genetically considered to be a hybrid of Criollo cacaos and lower Amazonian Forastero “Amelonado,” obtained by repeated introductions of the last to the island of Trinidad over the larger part of a century (Motamayor et al., 2003; Pound, 1938).

Fingerprints from molecular markers currently provide the most definitive data for identifying cacao clones, for determining the most likely progenitors of a clone, and for studying interrelationships among clones and clonal types (Laurent et al., 1994; Lerceteau et al., 1997). It has become clear in recent years that mislabeling and contamination of artificial crosses is a very serious problem in cacao (Takrama et al., 2005). In fact, estimates of mislabeling range from 15% to 44% throughout all world cacao collections (Motilal and Butler, 2003; Turnbull et al., 2004). Mo-

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lecular markers also provide valuable information for calculating measures of genetic variability and diversity of genetic types, and of germplasm collections, enabling more efficient use of genetic resources. The variation among natural populations and cultivated types has been compared and contrasted (Laurent et al., 1994; Motamayor et al., 2002, 2003). The incorporation of molecular markers associated with QTL into breeding schemes (Brown et al., 2005; Clement et al., 2003a, 2003b) has the potential to greatly shorten breeding cycles, thereby increasing efficiency. Schnell et al. (2005) showed that association analysis can be applied to cultivated cacao populations, thereby identifying molecular markers likely to be in linkage disequilibrium (LD) with QTL for productivity, fruit quality, and vigor. A method proposed by Cervantes-Martinez and Brown (2004) uses F_1 populations of outcrossing species to enable QTL mapping in existing breeding populations. This approach also has the potential to increase the power of QTL detection by utilizing crosses made to one common parent in a partial full-sib mating design.

Crosses among selected clones were made in the partial full-sib mating design described above, and subsequently evaluated in a field experiment over a 5-year period beginning 2 years after planting at CATIE in Turrialba, Costa Rica, as part of the CATIE cacao breeding program for yield and disease resistance. The main objective of this project was to perform plant selection for clonal release. However, this set of crosses is also well designed for additional analyses; 1) the estimation of general and specific combining ability (GCA and SCA), as described in this project, and 2) potential QTL localization using haplotype-based analysis described above, pooling half-sib populations by common parents.

The knowledge of genetic differences among commonly cultivated cacao clones for disease resistance, yield, and quality and horticultural traits, as well as the type of gene action involved, is essential for cacao breeders to effectively select parental clones. Quantitative criteria such as GCA and SCA also constitute extremely useful parameters enabling breeders to make more efficient parental choices, providing information about the potential parental value in crosses, as well as describing gene action. Such information can also be critical for quantitative geneticists in designing and improving QTL localization strategies, whether they involve haplotype-based analysis (Cervantes-Martinez and Brown, 2004) and partial full-sib crossing schemes or association genetic analyses (Flint-Garcia et al., 2003; Schnell et al., 2005). Thus, the objectives of this research were to 1) verify the genetic identity of the parental cacao clones used in the hybrid field evaluation at CATIE using molecular markers; and 2) estimate the GCA and SCA of the parental clones for frosty pod and black pod resistances, number of pods, vigor (as measured by trunk diameter), and maturity traits. Discussion of the genetic and statistical implications for plant breeding and QTL analysis is presented.

Materials and Methods

Germplasm

Combining abilities were estimated from bi-parental crosses between two sets of cacao clones, widely used in the CATIE breeding program and in certain other international breeding projects, especially where frosty pod exists. Selection of these sources of resistance was performed at CATIE by artificially inoculating nearly 600 clones from the international germplasm

collection over a period of 10 years. The crosses were designed to select for enhanced resistance to the major pathogens in Central America and for productivity. The clones 'UF 273', 'UF 712', and 'ICS 95' formed parental Set 1, and the clones 'CC 137', 'ICS 6', 'CATIE 1000', 'Tree 81', 'CCN 51', 'SCA 6', 'ICS 44', 'CC 252', 'EET 75', and 'Pound 7' constituted parental Set 2. These clones exist in several international cacao germplasm collections (Wadsworth and Harwood, 2000). Crosses between clones of both sets were performed in a partial full-sib mating design, using from one to eight trees of each parental clone. Twenty-five of the possible 30 crosses between parents of Set 1 and 2 were made; those excluded were 'UF 273' x 'CC 252', 'UF 712' x 'ICS 44', 'UF 712' x 'Pound 7', 'ICS 95' x 'SCA 6', and 'ICS 95' x 'EET 75'. In each cross, at least one parent had demonstrated resistance to frosty pod, and the other parent had one or more complementary traits: high pod number, resistance to black pod or witches' broom [*Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora] (Aime and Phillips-Mora, 2005), and self-compatibility (Table 1).

Field evaluation

The 25 hybrids were planted in a field experiment at the experimental farm "La Lola" (lat. 10°06'N, long. 83°23'W, 40 m above sea level) on the Atlantic coast of Matina, Costa Rica, by personnel of CATIE in May 1997, using a randomized complete-block design with four replications. The experiment was grown on a loamy sand soil. The site has an average monthly temperature of 25 °C, with a minimum of 20 °C and a maximum of 30 °C, monthly precipitation of 295.2 mm, and average relative humidity of 91%. Each plot consisted of eight trees planted in double rows 12 m in length, with 3 m of spacing between both plants and plots. Trees failing to survive from the first planting were replanted on one of seven dates (Dec. 1997, June 1998, Oct. 1998, June 1999, Mar. 2000, June 2000, and May 2001). Eighty-nine percent of the seedlings were planted on the first date, 5% on the second, and the remaining 6% on one of the latter four dates. A small number of existing trees on the site were left for shade, and 'Gross Michel' banana (*Musa acuminata* Colla) was first planted for temporary shade, while sapote trees [*Manilkara zapota* (L.) Royen] were planted for permanent shade, at a spacing of 6 x 6 m, and 18 x 18 m. An initial fertilization with granular 10N-13.1P-8.3K was done at planting, and subsequent granular nutrient (18N-2.2P-12.5K-3.6Mg-0.3B-7.4S) was applied three times yearly (150 g/plant) in April, August, and December. Foliar fertilizer (Bayfolan; Bayer CropScience AG, Monheim, Germany) was also applied once yearly (300 mL/100 L). Weeds were controlled chemically three times yearly concurrent with fertilization (April, August, and December), supplemented with manual weeding. Formation pruning was made after the first jorquette production, followed by maintenance pruning in January and July. Ants were controlled chemically by direct application of pesticide to the anthill.

Phenotypic data was recorded on a single tree basis. The number of mature pods was taken monthly over a 5-year period. Each pod was scored as either healthy or diseased, and diseased pods were then noted as to whether they were infected by frosty pod and/or black pod. The total number of pods harvested per year was evaluated as one of the main yield components (Soria, 1978), and disease resistance was calculated as the percentage of healthy pods based on total pod production. Disease susceptibility was scored for frosty pod and black pod as the percentage of pods

Table 1. Donor characteristics of the cacao parents from the partial full-sib mating design.

Parental clone	Genetic type	Characteristics
UF 273	Forastero ²	Moderate pod susceptibility to <i>Phytophthora palmivora</i> ² Pod resistance to <i>Moniliophthora roreri</i> ² Moderate pod productivity ³ Self compatible ³
UF 712	Trinitario ²	Pod susceptibility to <i>P. palmivora</i> ² Pod resistance to <i>M. roreri</i> ² Low to moderate pod productivity ³ Self incompatible ³
ICS 95	Trinitario and Criollo ²	Pod resistance to <i>Moniliophthora perniciosa</i> ² Moderate pod resistant to <i>P. palmivora</i> ² Moderate pod resistance to <i>M. roreri</i> ² Moderate to high pod productivity ³ Self compatible ³
CC 137	Trinitario ² , Open pollination of UF-12 ³	Moderate pod susceptibility to <i>P. palmivora</i> ² Moderate pod susceptibility to <i>M. roreri</i> ² Moderate to high pod productivity ³ Self compatible ³
ICS 6	Trinitario ²	Moderate pod resistance to <i>M. perniciosa</i> ² Moderate to high plant resistance to <i>M. perniciosa</i> ² Fruit susceptibility to <i>P. palmivora</i> ² Pod susceptibility to <i>M. roreri</i> ² Moderate to high pod productivity ³ Self compatible ³
CATIE 1000	Forastero ²	Moderate pod resistant to <i>P. palmivora</i> ² Plant susceptibility to <i>M. roreri</i> ² Moderate to high pod productivity ³ Self compatible ³
Tree 81	Forastero, Derived from Pound 12 x Catongo ³	Moderate pod resistance to <i>P. palmivora</i> ² High pod productivity ³ Self compatible ³
CCN 51	Derived from CCN 1 ²	Plant resistance to <i>M. perniciosa</i> ² Fruit susceptibility to <i>P. palmivora</i> ² Pod susceptibility to <i>M. roreri</i> ² High pod productivity ³ Self compatible ³
SCA 6	Forastero ²	Moderate to high resistance to <i>M. perniciosa</i> ² Moderate pod resistant to <i>P. palmivora</i> ² Plant susceptibility to <i>M. roreri</i> ² Self incompatible ²
ICS 44	Trinitario ²	Intermediate pod resistance to <i>M. perniciosa</i> ² Plant resistance to <i>M. perniciosa</i> ² Moderate pod resistant to <i>P. palmivora</i> ² Pod susceptibility to <i>M. roreri</i> ² Self compatible ²
CC 252	Forastero ²	Moderate pod susceptibility to <i>P. palmivora</i> ² Moderate plant resistance to <i>M. roreri</i> ² Self incompatible ³
EET 75	Trinitario ²	Pod susceptibility to <i>P. palmivora</i> ² Pod resistance to <i>M. roreri</i> ² Low pod productivity ³ Self incompatible ³
Pound 7	Forastero ²	Intermediate seedling resistance to <i>M. perniciosa</i> ² Fruit resistant to <i>P. palmivora</i> ² Pod susceptibility to <i>M. roreri</i> ² High pod productivity ³ Self incompatible ³

²CocoaGen DB (Centre de Coopération Internationale en Recherches Agronomiques pour le Développement et al., 2005).

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with the respective disease. Vigor was evaluated by measuring the trunk diameter at 30 cm above ground every 6 months. Harvest efficiency was calculated as the ratio between yield and the increment in sectional area of the trunk at the evaluated period (Daymond et al., 2002). Maturity was evaluated in three ways; number of months to production of the first jorquette, and number of months each to the first flowering and fruiting.

Simple sequence repeat (SSR) marker analysis

Molecular marker analyses were performed by the horticulture genetics group of the USDA-ARS Subtropical Horticulture

Research Station, Miami, to verify the identity of parental trees of all clonal types. Sixteen additional cacao clones from other germplasm collections (sources given in Fig. 1) were also analyzed for comparison of genetic identity of clones used at CATIE. Leaf samples were collected from all trees possible used as parents at CATIE, and from one tree from each clone from other germplasm collections. DNA extraction of the leaf material, polymerase chain reaction (PCR) and capillary electrophoresis (CE) were performed as described in Schnell et al. (2005). A set of 12 polymorphic SSRs (mTc-CIR) developed at the Centre de Coopération Internationale en Recherches Agronomiques pour le Développement (CIRAD) in Montpellier, France (Lanaud et al., 1999; Pugh et al., 2004; Risterucci et al., 2000), were analyzed to determine the genetic similarity among trees of each parental clone from CATIE, and these SSRs were also used for comparison of clones from other germplasm collections (Fig. 1). Off-type trees of clones from CATIE were determined and verified with a second group of 12 mTcCIR markers, which had been chosen based on their ability to discriminate off-types and progeny from crosses made with off-type trees. All progeny from crosses made with parental clones containing off-types were also genotyped with the second group of SSRs, as described by Takrama et al. (2005).

Statistical analysis

MOLECULAR DATA. A Principal Coordinate Analysis (PCA) (Gower, 1966) was performed on parental trees and controls to verify the genetic type. SSR data was used to calculate modified Rogers' genetic distance (Wright, 1978), and the PCA analysis was performed on the genetic distance matrix. Progeny from crosses made from parental clones with off-types were separated according to parental type using the SSR data (Takrama et al., 2005). The percentage of self-pollination was estimated from the crosses for which offspring was genotyped to detecting off-types.

PHENOTYPIC DATA. Descriptive analyses, including the Shapiro-Wilk and Kolmogorov-Smirnov goodness-of-fit methods to test

normality, were conducted to verify analysis of variance assumptions for yield, disease resistance, vigor, and maturity measures. Natural logarithm transformation on harvest index, conditional on positive values, and square root transformations for three traits, percentage of healthy pods, number of pods with frosty pod, and number of pods with *P. palmivora* were determined to achieve a sufficiently normal distribution. The following mixed linear model was fit for yield, disease resistance and vigor:

$$y_{ijklr} = \mu + \delta_i + t_{ij} + g_{S1k} + g_{S2l} + \gamma_{S1S2kl} + v_{S1k} + v_{S2l} + v_{S1S2kl} + v_{ijkl} + \varepsilon_{ijklr}, \text{ for } i = 1, 2, 3, 4, 5; j = 1, 2, 3, 4; k = 1, 2, \dots, K; l = 1, 2, \dots, L; \text{ and } r = 1, 2, \dots, 8. \quad [1]$$

Fig. 1. Principal coordinate analysis (PCA) of the cacao trees of all clones used as parents of the factorial design (Tables 1 and 2). The clones used as controls were: 'ICS 95' from Colombia (C1), Trinidad (C2), Ecuador (C3), Peru (C4), and Ivory Coast (C5); 'CATIE 1000', 'CCN 51', and 'SCA 6' from Reading (C1); 'CC 137' and 'ICS 44' from CATIE (C1); 'UF 273' from Venezuela (C1, C2, C3, C4); and 'Pound 7' from Trinidad (C1). The PCA analysis was based on fingerprint data from 12 SSR markers.

Here, y_{ijklr} is the response trait, μ is the overall mean, δ_i is the effect of year i , ι_{ij} is the effect of replication j in year i , g_{S1k} , g_{S2l} , and γ_{S1S2kl} are the effects of parent k in Set 1, parent l in Set 2, and their interaction; v_{S1ik} , v_{S2il} , and $v_{S1S2ikl}$ are the interaction effects of years with the parent k in Set 1, parent l in Set 2, and both parents, respectively; v_{ijkl} is the pooled interaction effect of replications nested in years with each parent and pair-wise parental interactions; ϵ_{ijklr} is the residual term resulting from the effect of tree r , replication j , and the cross of parents k and l evaluated in year i . Years, replications within years, and their interactions with genotypes were considered as random factors, and the parental effects and their interactions were considered as fixed factors.

A simplified linear model was used for months to flowering, fruiting and first jorquette:

$$y_{ijklr} = \mu + \iota_{ij} + g_{S1k} + g_{S2l} + \gamma_{S1S2kl} + \epsilon_{ijklr} \text{ for } j = 1, 2, 3, 4; k = 1, 2, \dots, K; l = 1, 2, \dots, L; \text{ and } r = 1, 2, \dots, 8. \quad [2]$$

The specifics of the terms in [2] are the same as the corresponding terms in [1]. The following restrictions were defined for [1] and [2]: $E[y_{ijklr}] = \mu + g_{S1k} + g_{S2l} + \gamma_{S1S2kl}$;

$\sum_{k=1}^K g_{S1k} = \sum_{l=1}^L g_{S2l} = \sum_{k=1}^K \gamma_{S1S2kl} = \sum_{l=1}^L \gamma_{S1S2kl} = 0$, with $\gamma_{S1S2kl} = 0$ for any missing cross (Searle, 1971); δ_i , ι_{ij} , v_{S1ik} , v_{S2il} , $v_{S1S2ikl}$, and v_{ijkl} in [1] and ϵ_{ijklr} in [1] and [2] are normally distributed with expected value equal to zero, $\text{Var}[y_{ijklr}] = \sigma_\delta^2 + \sigma_\iota^2 + \sigma_{v_{S1}}^2 + \sigma_{v_{S2}}^2 + \sigma_{v_{S1S2}}^2 + \sigma_v^2 + \sigma_\epsilon^2$ in [1], and $\text{Var}[y_{ijklr}] = \sigma_\epsilon^2$ in [2]. The $\text{Cov}[y_{ijklr}, y_{i'j'k'l'r'}] = \sigma_\delta^2 \rho^{|i-i'|} + \sigma_\iota^2 \rho^{|j-j'|} + \sigma_{v_{S1}}^2 \rho^{|k-k'|} + \sigma_{v_{S2}}^2 \rho^{|l-l'|} + \sigma_{v_{S1S2}}^2 \rho^{|(k,l)-(k',l')|} + \sigma_v^2 \rho^{|r-r'|}$ if $k = k'$, $l = l'$, and $r = r'$, and 0 otherwise, for the specific case of trunk diameter in [1]. This structure corresponds to an autoregressive covariance model of the first order (Apollaza and Garrick, 2001).

Estimation of variance components was performed by restricted maximum likelihood (REML) using a ridge-stabilized Newton-Raphson algorithm. Standard errors of the variance and covariance estimates were obtained from the inverse matrix of

the empirical Fisher information matrix. The best linear unbiased estimators (BLUEs) of parental effects and interaction effects were obtained by solving the mixed model equations. Significance of effects and interactions was tested by the t test using the Satterthwaite approximation to the numerator df (Satterthwaite, 1946). GCA estimates were approximated as parental effects, and SCA estimates as parental interaction effects (Hallauer and Miranda, 1988). Several preliminary analyses were performed including progressive data corresponding to the different planting dates, from the earliest to the latest. The adequacy of the model was determined in all cases by the -2 REML log likelihood, Akaike, and Bayes Information Criteria (Littell et al., 1996). The results presented in this research correspond only to data of the first and second planting dates ($\approx 94\%$ of entire data set), as this model gave the best statistical fit.

All calculations were performed with SAS (version 9.1 for Windows; SAS Institute, Cary, N.C.) using a dual Intel Itanium 2 (Intel Corp., Santa Clara, Calif.) based IBM 64-bit application server with 32 GB of RAM (IBM Corp., White Plains, N.Y.). The descriptive analysis was performed with the UNIVARIATE procedure. The components of variance and fixed effects were estimated with the MIXED procedure (Littell et al., 1996).

Results

Analysis of parental clone identity and offspring accuracy

The three first principal coordinates of the PCA explained $\approx 61.5\%$ of the total variation (Fig. 1). The clone 'CATIE 1000' used as a parent was genetically identical to the check clone from Reading, U.K., and as a consequence these two clones overlap in the PCA plot. The parental clones 'SCA 6' and 'CC 137' had only one allele of a heterozygous locus that differed

from the checks from Reading and CATIE, respectively, and were probably different. The clone 'Pound 7' had two alleles different from its respective Trinidadian check clone, 'ICS 44' differed for three alleles from its respective check from CATIE, and 'CCN 51' differed by 11 alleles out of a total of 24 alleles (12 SSR markers) from its respective check tree in Reading. The clone 'ICS 95' differed by one allele from checks in Trinidad and Ivory Coast, by two alleles from the Colombian check, by three alleles from the Ecuadorian check, and by 18 alleles with the Peruvian check. The large difference between 'ICS 95' and the Peruvian check is reflected as the largest geometric distance in the PCA plot (Fig. 1).

One off-type out of five 'UF 273' trees and one out of six 'CC 137' trees were found in the 12 SSRs analyzed. The off-type 'UF 273' tree differed by one allele at each of two SSR markers, while the 'CC 137' off-type tree differed by only one allele at one homozygous SSR marker. Previous molecular analysis showed the existence of two types among the 'UF 273' parental trees in the CATIE collection, labeled as 'UF 273 Type I', and 'UF 273 Type II'. 'UF 273 Type I' has been known to have desirable horticultural characteristics for cacao breeding, while the 'UF 273 Type II' has been considered as an off-type of the 'UF 273 Type I'. Twelve partially informative SSR markers from CIRAD (mTeCIR) differentiating the two types had also been previously identified. Progeny of crosses from 'UF 273' were analyzed for off-types with the second group of 12 SSR markers. Fingerprint analysis of progeny showed that 'UF 273 Type I' had been crossed with the clones 'ICS 44', 'Tree 81', and 'Pound 7'; and 'UF 273 Type II' had been crossed with the clones, 'ICS 6', 'CCN 51', 'SCA 6', and 'EET 75'. Both 'UF 273' types were crossed with 'CC 137' and 'CATIE 1000', producing an approximately equal number of progeny. None of the six parental trees of the clone 'CC 137' showed allelic differences when analyzed with the 12 SSRs second group. The final number of trees considered in the analysis are shown in Table 2.

The percentage of self-pollination was estimated using the progeny of crosses analyzed for off-types (crosses with 'UF 273') only, using six markers of the second group. Three crosses out of nine did not have self-pollinated tress, three crosses had as many as three self-pollinated trees, two crosses had no more than five self-pollinated trees, and only one cross had 17 self-pollinated trees, giving an average rate of self-pollination over crosses of 11.8% (95% confidence). A fully informative marker or at least three independent, partially informative markers are required to identify self-pollinated trees with a 99% confidence. Since only data from progeny of the nine crosses with 'UF 273' as a parent

was available for analysis of pedigree accuracy, no correction for self or cross pollination was made in the statistical analysis. Otherwise, correcting by self-pollinated trees in only some crosses might bias the inference when comparing the GCA of parents and SCA of crosses. Thus, as progeny from self-pollination may exist in other crosses, the existence of self-pollinated progeny should be borne in mind when interpreting these results. This fact, however, does not detract from the value of these results compared to any past results, as the same condition likely has existed in most cacao research done prior to the use of molecular markers.

GCA and SCA analysis

The overall F test in the analysis of variance showed significant GCA effects for clones corresponding to Set 2 for all traits evaluated in this study (Table 3). The GCA effects of clones in Set 1 were not statistically significant for percent of pods with black pod and number of months to production of the first jorquette, but were significant for all other traits. The overall F test for SCA was significant for almost all traits, except months to production of the first jorquette.

Among parents of Set 1, the 'UF 273 Type I' clone had a significant positive GCA effect for the number of pods and harvest efficiency (Table 4). The 'ICS 95' clone had significant negative GCA effects for trunk diameter and harvest efficiency, and the 'UF 712' clone had a significant ($P = 0.06$) negative GCA effect for percent of pods with frosty pod (Table 4). The 'UF 273 Type II' clone had no desirable significant GCA effects for the evaluated traits. Among clones of Set 2, a significant positive GCA effect for 'CC 137' for total number of pods was found and a significant negative GCA effect was found for 'ICS 44', 'CC 252', and 'EET 75' for trunk diameter. The clones 'CC 137', 'CATIE 1000', and 'EET 75' had significant positive GCA effects for percentage of healthy pods, corresponding to a significant negative GCA for percentage of pods with frosty pod. None of the clones of Set 2 had desirable significant GCA effects for the maturity traits evaluated in this study.

Trunk diameter, months to fruiting, total number of pods, and months to flowering were the traits, for which the largest number of crosses had significant SCA effects, with 11, seven, six, and five crosses showing significant SCA effects out of 27 total crosses, respectively (Table 5). Only four crosses had significant SCA effects (or estimates that were consistent) for percentage of healthy pods, and pods with frosty pod. However, a larger ratio between the number of parents with GCA effects and the number of crosses with SCA effects was observed for these two

Table 2. Total number of cacao trees^a per cross considered in the partial full-sib design at La Lola farm, Matina, Costa Rica.

Set 2	Set 1			
	UF 273 TI	UF 273 TII	UF 712	ICS 95
CC 137	16	15	31	32
ICS 6	0	31	31	30
CATIE 1000	18	13	32	32
Tree 81	29	0	30	21
CCN 51	0	32	26	32
SCA 6	0	31	31	0
ICS 44	31	0	0	32
CC 252	0	0	32	24
EET 75	0	29	30	0
Pound 7	21	0	0	31

^aOnly includes trees established in the first and second planting dates.

Table 3. Numerator and denominator degrees of freedom, and F-statistic (F_c) with significance level of fixed effects for number of total pods (TP), trunk diameter (TRD), harvest efficiency (HE), percentage of healthy pods (HP), pods with frosty pod (PM), pods with phytophthora (PP), months to flowering (MFL), fruiting (MFUT), and first jorquette (MJOR) of cacao crosses evaluated in a field experiment at La Lola farm, Matina, Costa Rica.

		Trait																		
		TP		TRD		HE		HP		PM		PP		MFL		MFUT		MJOR		
Source		df _N ^z	df _D ^y	F _c	df _D	F _c	df _D	F _c	df _D	F _c	df _D	F _c	df _D	F _c	df _D	F _c	df _D	F _c	df _D	F _c
GCA ^x	Set 1 ^w	3	35.4	26.9**	396	28.6**	275	33.02**	25.1	9.9**	388	6.1**	419	1.6	63.6	21.5**	61.6	47.0**	67.3	0.8
	Set 2 ^v	9	435	4.7**	335	11.0**	34.5	2.15 ^t	376	15.9**	402	21.4**	56.4	3.5**	63.6	8.1**	61.8	8.3**	66.9	3.0**
SCA ^u		14	489	3.9**	699	3.6**	311	3.41**	415	2.2**	443	1.78	479	1.9*	72.6	4.9**	70.5	8.9**	76.2	1.5

^xNumerator degrees of freedom.

^ySatterthwaite approximation of denominator degrees of freedom (Satterthwaite, 1946).

^zGeneral combining ability.

^wSet consisted of 'UF 273 TI', 'UF 273 TII', 'UF 712', and 'ICS 95'.

^vSet consisted of 'CC 137', 'ICS 6', CATIE 1000', Tree 81', 'CCN 51', 'SCA 6', 'ICS 44', 'CC 252', 'EET 75', and 'Pound 7'.

^uSpecific combining ability.

^{*}Significant at 0.06.

^{**}Significant at 0.05 and 0.01, respectively.

Table 4. General combining ability (GCA) and standard error (in parentheses) for number of total pods (TP), trunk diameter (TRD), harvest efficiency (HE), percentage of healthy pods (HP), pods with monilia (PM), and pods with phytophthora (PP), months to first flowering (MFL), first fruiting (MFUT), and first jorquette (MJOR) of cacao crosses evaluated in a field experiment at La Lola farm, Matina, Costa Rica, over a 5-year period.

Set	Parental clone	Trait								
		TP	TRD	HE	HP	PM	PP	MFL	MFUT	MJOR
1	UF 273 TI	7.25*	0.04	0.65*	0.84	-0.63	-0.18	-0.71	-2.90	12.51
		(2.84)	(0.34)	(0.30)	(0.46)	(0.23)	(0.23)	(2.38)	(2.63)	(9.22)
	UF 273 TII	0.21	0.83*	0.13	-0.51	1.13*	0.00	-3.84	-5.05	-9.51
		(2.94)	(0.35)	(0.31)	(0.49)	(0.51)	(0.24)	(2.48)	(2.73)	(9.60)
	UF 712	-1.88	0.02	-0.19	0.21	-0.49 ^z	0.19	-0.70	0.12	-7.36
2	ICS 95	(1.66)	(0.19)	(0.17)	(0.26)	(0.26)	(0.12)	(1.34)	(1.48)	(5.21)
		-5.56**	-0.90**	-0.59*	-0.54*	0.02	-0.01	5.25**	7.83**	4.37
		(1.47)	(0.17)	(0.15)	(0.24)	(0.24)	(0.11)	(1.18)	(1.31)	(4.59)
	CC 137	2.74*	-0.20	0.32	1.33**	-1.46**	-0.08	0.75	0.15	2.42
		(1.32)	(0.17)	(0.21)	(0.22)	(0.23)	(0.13)	(1.13)	(1.25)	(4.36)
3	ICS 6	-0.26	0.08	-0.19	-0.41	0.32	-0.26	2.70	3.14	7.43
		(2.00)	(0.25)	(0.26)	(0.34)	(0.35)	(0.18)	(1.71)	(1.89)	(6.61)
	CATIE 1000	0.36	0.53**	-0.02	0.69**	-0.60*	-0.10	0.21	0.56	13.20**
		(1.42)	(0.18)	(0.21)	(0.23)	(0.24)	(0.16)	(1.22)	(1.35)	(4.73)
	Tree 81	1.30	-0.11	0.12	0.16	-0.03	-0.10	-0.75	-2.32*	-3.45
4		(1.07)	(0.14)	(0.16)	(0.18)	(0.19)	(0.11)	(0.92)	(1.03)	(3.57)
	CCN 51	1.84	-0.27	0.08	0.18	-0.32	0.46**	-2.32	-2.92	-3.93
		(2.01)	(0.25)	(0.26)	(0.32)	(0.34)	(0.18)	(1.71)	(1.89)	(6.63)
	SCA 6	4.38	-0.04	0.46	-0.19	0.81	-0.39	-2.16	-3.05	5.76
		(2.66)	(0.32)	(0.33)	(0.42)	(0.44)	(0.22)	(2.25)	(2.49)	(8.75)
5	ICS 44	-2.14	-0.43*	-0.44	-1.07**	0.90**	0.21	5.96**	6.74**	-8.85
		(1.70)	(0.21)	(0.23)	(0.30)	(0.32)	(0.17)	(1.46)	(1.62)	(5.61)
	CC 252	-2.90 ^z	-0.47*	0.20	-0.55 ^z	0.26	0.04	-2.10	-2.09	-7.73
		(1.54)	(0.19)	(0.22)	(0.29)	(0.30)	(0.16)	(1.33)	(1.49)	(5.14)
	EET 75	-4.84	-1.47**	-0.44	2.05**	-3.03**	-0.10	4.89	4.49	7.29
6		(3.03)	(0.36)	(0.33)	(0.51)	(0.53)	(0.26)	(2.57)	(2.84)	(9.99)
	Pound 7	-0.49	2.38*	-0.08	-2.18	3.15 ^z	0.31	-7.20	-4.40	-12.15
		(9.72)	(1.18)	(1.08)	(1.58)	(1.66)	(0.73)	(8.28)	(9.18)	(32.20)

^zSignificant at 0.06.

^{*}Significant at 0.05 and 0.01, respectively.

traits, with ratios of 6:4 and 7:4 for percentage of healthy pods and pods with frosty pod, respectively. The corresponding ratios for trunk diameter, number of months to fruiting, total number of pods, number of months to flowering, and harvest efficiency were 7:11, 3:7, 4:6, 2:5, and 2:2, respectively. The clone 'CC 137' had significant SCA effects with the four clones of Set 1 for total number of pods, while 'ICS 6' and 'CATIE 1000' had significant SCA effects with only one clone of Set 1. In contrast, 'Tree 81', 'CCN 51', 'SCA 6', 'ICS 44', and 'EET 75' had significant SCA effects with two clones of Set 1 for trunk diameter, while 'CC 137' had significant SCA effect with one parent of Set 1. The clone 'CC 137' was the only parent of Set 2 to show two signifi-

cant SCA effects for harvest efficiency with two parents of Set 1. Regarding the maturity traits, 'CC 137', 'ICS 6', 'CCN 51', and 'ICS 44' had between one and two significant SCA effects with the parents of Set 1.

Some particularly interesting F₁ populations were from the crosses of 'UF 273 Type I' x 'Tree 81', 'UF 273 Type I' x 'ICS 44', and 'UF 273 Type I' x 'Pound 7'. The positive GCA effect of 'UF 273 Type I' for number of pods, and its low SCA effect with 'Tree 81', 'ICS 44', and 'Pound 7' would permit selection of higher yielding segregates from these crosses for initiation of a recurrent selection program, and also potential release of clonal cultivars. Similarly, crosses with 'UF 712' are of interest

Table 5. Specific combining ability (SCA) and standard error (in parentheses) for number of total pods (TP), trunk diameter (TRD), harvest efficiency (HE), percentage of healthy pods (HP), pods with monilia (PM), and pods with phytophthora (PP), months to first flowering (MFL), first fruiting (MFUT), and first jorquette (MJOR) of cacao crosses evaluated in a field experiment at La Lola farm, Matina, Costa Rica, over a 5-year period.

Set 2	Trait	Set 1			
		UF 273 TI	UF 273 TII	UF 712	ICS 95
CC 137	TP	-6.22* (3.06)	7.07* (3.24)	5.12* (2.25)	-5.97* (1.96)
	TRD	0.50 (0.39)	-0.91* (0.42)	0.42 (0.27)	-0.01 (0.24)
	HE	-0.58 (0.36)	0.58 (0.42)	1.05* (0.29)	-1.05* (0.25)
	HP	-0.74 (0.52)	0.72 (0.56)	0.44 (0.35)	-0.42 (0.35)
	PM	0.73 (0.54)	-0.78 (0.59)	-0.30 (0.37)	0.35 (0.37)
	PP	0.020 (0.26)	-0.02 (0.28)	0.10 (0.18)	-0.28 (0.18)
	MFL	0.30 (2.68)	1.79 (2.84)	-4.12* (1.91)	2.03 (1.67)
	MFUT	0.90 (3.01)	1.52 (3.20)	-7.09** (2.11)	4.67* (1.87)
	MJOR	-10.17 (10.43)	9.54 (11.15)	3.69 (7.40)	-3.06 (6.49)
ICS 6	TP		0.5 (2.47)	4.28* (1.74)	-3.77 (2.57)
	TRD		-0.47 (0.30)	0.62** (0.21)	-0.15 (0.31)
	HE	NA ^z	0.05 (0.27)	0.21 (0.22)	-0.26 (0.29)
	HP		-0.01 (0.42)	0.38 (0.29)	-0.39 (0.46)
	PM		-0.42 (0.44)	0.18 (0.31)	0.24 (0.48)
	PP		-0.02 (0.21)	-0.13 (0.15)	0.15 (0.23)
	MFL		1.93 (2.11)	-4.83** (1.48)	2.90 (2.20)
	MFUT		0.63 (2.33)	-8.19** (1.64)	7.56** (2.44)
	MJOR		7.04 (8.18)	-4.15 (5.75)	-2.89 (8.52)
CATIE 1000	TP	-6.39* (2.59)	4.04 (3.61)	-22.20 (15.46)	24.55 (14.45)
	TRD	0.18 (0.35)	-0.68 (0.47)	1.88 (1.88)	-1.37 (1.76)
	HE	-0.07 (0.33)	-0.17 (0.46)	-1.99 (1.74)	2.24 (1.62)
	HP	-0.61 (0.46)	0.41 (0.62)	-4.17 (2.51)	4.38 (2.36)
	PM	0.55 (0.48)	-0.54 (0.65)	3.60 (2.64)	-3.61 (2.47)
	PP	0.09 (0.22)	-0.24 (0.30)	0.86 (1.25)	-0.7 (1.17)
	MFL	1.13 (2.30)	2.73 (3.15)	-0.80 (13.19)	-3.06 (12.33)
	MFUT	1.97 (2.61)	2.62 (3.56)	7.41 (14.64)	-12.00 (13.68)
	MJOR	-8.83 (9.05)	14.56 (12.42)	-54.41 (51.33)	48.68 (47.97)
Tree 81	TP	1.43 (2.29)		0.02 (2.70)	-1.45 (1.60)
	TRD	0.63* (0.27)		-0.14 (0.32)	-0.50** (0.18)
	HE	-0.25 (0.24)	NA	0.23 (0.28)	0.02 (0.16)
	HP	-0.69 ^y (0.36)		1.09* (0.43)	-0.40 (0.27)
	PM	0.67 (0.38)		-0.73 (0.45)	0.06 (0.29)
	PP	0.23 (0.18)		-0.39 (0.21)	0.16 (0.14)
	MFL	-0.43 (1.95)		-0.26 (2.29)	0.69 (1.39)
	MFUT	0.98 (2.16)		0.92 (2.54)	-1.90 (1.56)
	MJOR	2.56 (7.57)		1.06 (8.91)	-3.63 (5.36)
CCN 51	TP		1.99 (2.47)	-0.54 (1.77)	-1.45 (2.57)
	TRD		-0.60* (0.30)	-0.14 (0.21)	0.74* (0.31)
	HE	NA	0.09 (0.27)	-0.24 (0.22)	0.15 (0.28)
	HP		0.22 (0.40)	-0.31 (0.28)	0.086 (0.41)
	PM		-0.61 (0.42)	0.64* (0.29)	-0.03 (0.43)
	PP		0.20 (0.20)	-0.01 (0.14)	-0.19 (0.21)
	MFL		4.55* (2.11)	-0.88 (1.52)	-3.67 (2.77)
	MFUT		5.78* (2.33)	-1.91 (1.68)	-3.87 (2.42)
	MJOR		-0.46 (8.18)	6.01 (5.89)	-5.55 (8.49)
SCA 6	TP		-1.84 (3.41)	1.84 (3.42)	
	TRD		-1.34** (0.41)	1.34** (0.41)	
	HE	NA	-0.21 (0.39)	0.21 (0.39)	NA
	HP		0.01 (0.55)	-0.01 (0.55)	
	PM		-1.14 ^y (0.58)	1.14 ^y (0.58)	
	PP		0.21 (0.28)	-0.21 (0.28)	

continued next page

for selecting genotypes with resistance to frosty pod, given the negative GCA effect for percentage of pods with frosty pod damage. Additional interesting crosses for clonal selection are ‘CC 137’ x ‘UF 273 Type II’, ‘CC 137’ x ‘UF 712’, and ‘ICS 6’ x ‘UF 712’, characterized by their positive SCA effects for number of pods. Additionally, the negative SCA effects of ‘CC 137’ x ‘UF

273 Type II’ for trunk diameter, and negative SCA effects of ‘CC 137’ x ‘UF 712’, and ‘ICS 6’ x ‘UF 712’ for months to flowering and fruiting provide an opportunity for selection of yield, low vigor and earliness. Superior offspring could be used as parents in further breeding programs, or also be released as clonal cultivars after extensive field evaluation.

Table 5. Continued.

Set 2	Trait	Set 1			
		UF 273 TI	UF 273 TII	UF 712	ICS 95
ICS 44	MFL		2.42 (2.91)	-2.42 (2.90)	
	MFUT		2.03 (3.20)	-2.03 (3.20)	
	MJOR		4.94 (11.28)	-4.94 (11.28)	
	TP	2.13 (1.36)			-2.13 (1.36)
	TRD	0.45** (0.16)			-0.45** (0.16)
	HE	0.28 (0.15)	NA	NA	-0.28 (0.15)
	HP	0.09 (0.25)			-0.09 (0.25)
	PM	0.28 (0.26)			-0.28 (0.26)
	PP	-0.11 (0.12)			0.11 (0.12)
	MFL	-5.51** (1.17)			5.51** (1.17)
CC 252	MFUT	-6.20** (1.31)			6.20** (1.31)
	MJOR	-1.43 (4.49)			1.43 (4.49)
	TP			0.73 (1.90)	-0.73 (1.90)
	TRD			0.02 (0.22)	0.02 (0.22)
	HE	NA	NA	0.19 (0.22)	-0.19 (0.22)
	HP			1.21** (0.35)	-1.21** (0.35)
	PM			-1.03** (0.36)	1.03** (0.36)
	PP			-0.34 (0.17)	0.34* (0.17)
	MFL			-0.11 (1.64)	0.11 (1.64)
	MFUT			-1.70 (1.84)	1.70 (1.84)
EET 75	MJOR			17.11** (6.35)	-17.11** (6.35)
	TP		-10.75 (12.86)	10.75 (12.86)	
	TRD		4.00* (1.57)	-4.00* (1.57)	
	HE	NA	-0.33 (1.45)	0.33 (1.45)	NA
	HP		-1.38 (2.16)	1.38 (2.16)	
	PM		3.49 (2.27)	-3.49 (2.27)	
	PP		-0.12 (1.07)	0.12 (1.07)	
	MFL		-13.42 (11.03)	13.42 (11.03)	
	MFUT		-12.58 (12.21)	12.58 (12.21)	
	MJOR		-35.62 (42.86)	35.62 (42.86)	
Pound 7	TP	9.05 (7.82)			-9.05 (7.82)
	TRD	-1.76 (0.95)			1.76 (0.95)
	HE	0.62 (0.88)	NA	NA	-0.62 (0.88)
	HP	1.95 (1.28)			-1.95 (1.28)
	PM	-2.23 (1.35)			2.23 (1.35)
	PP	-0.41 (0.64)			0.41 (0.63)
	MFL	4.52 (6.69)			-4.52 (6.69)
	MFUT	2.35 (7.43)			-2.35 (7.43)
	MJOR	17.87 (26.04)			-17.87 (26.03)

[‡]Not applicable.

[‡]Significant at 0.06.

^{***}Significant at 0.05 and 0.01, respectively.

Components of variance-covariance

The component of variance due to years of evaluation was a considerable source of variation for trunk diameter, harvest efficiency, percentage of healthy pods, and percentage of pods with frosty pod (Table 6), explaining 82%, 32%, 12%, and 15% of the total phenotypic variance from random effects, respectively. The interaction of year with combining ability did not constitute an important source of variance for five out of the six traits, indicating stable differences of combining abilities among genotypes over the 5-year period of this study.

In the present experiment, 43 parameters (13 GCA, 25 SCA, and 5 components of variance) would be estimated when no correction by off-type trees is made. The number of parameters to be estimated increases to 46 (14 GCA, 27 SCA, and 5 components of variance), when the correct classification of the off-type offspring is incorporated. This obviates the use of more complex

variance-covariance matrix structure, such as in repeated measures, thereby avoiding over-parameterization of the model, and allowing complex analyses of such largely unbalanced designs. The restricted maximum likelihood estimate (REML) of the average correlation between observations in consecutive years of single trees was 0.85 for trunk diameter. We estimated the correlation of successive years for trunk diameter, only as it is known to be a fundamental component of the covariance structure for this morphological trait. Correlations between successive measurements can only be considered to be equivalent to the repeatability coefficient if the change in genetic correlation over time is negligible. Also, this relation can be seen as the upper bound of broad sense heritability on a single tree basis if measurement error is relatively constant (Apiolaza and Garrick, 2001; Littell et al., 1996). Dias and Kageyama (1998) estimated the repeatability of the total number of pods and healthy pods, and the percentage of diseased pods over a period of 5 years, starting with trees of

Table 6. REML^z estimates of components of variance and standard error (in parentheses) for sources of variance from the model analyzing the following dependant variables: number of total pods (TP), trunk diameter (TRD), harvest efficiency (HE), percentage of healthy pods (HP), pods with monilia (PM), and pods with phytophthora (PP) of cacao crosses evaluated in a field experiment at La Lola farm, Matina, Costa Rica, over a 5-year period.

Source	Trait					
	TP	TRD	HE	HP	PM	PP
Years ^y	3.72 (6.86)	7.51 (4.79)	0.83 (0.71)	0.91 (0.67)	1.34 (0.95)	0.03 (0.03)
Years × Set 1 GCA ^x	1.62 (1.67)	0.01 (0.005)	0 (0.04)	0.03 (0.05)	0	0
Years × Set 2 GCA ^w	0	0.02 (0.01)	0.07 (0.04)	0	0	0.03 (0.01)
Years × SCA ^v	0	0.1 (.01)	0	0	0	0
Within crosses ^u	145.36 (3.71)	1.59 (0.07)	1.73 (0.08)	6.93 (0.21)	7.36 (0.22)	1.56 (0.05)

^zRestricted maximum likelihood estimators (Searle et al., 1992).

^yYears of evaluation.

^{x,w}Interaction of years with the general combining abilities of parents of Set 1 and 2, respectively.

^vInteraction of years with the specific combining abilities.

^uTrees within crosses and replications.

11 years of age. In the present experiment, the phenotypic data was taken for a period of 5 years starting the second year after planting, in which the trees were still in continuous growth.

Discussion

Misidentification in germplasm collections has been a major concern among cacao geneticists, due to its implications on the correct assessment of genetic diversity and in the production of controlled crosses. Incorrect identity of clones in germplasm collections leads to serious consequences when cacao accessions are incorporated into breeding programs (Figueira, 1998; Motilal and Butler, 2003). The presence of misidentification was observed at three levels in this research, by comparing SSR loci of parental trees with corresponding accessions from different germplasm collections, by comparing molecular data obtained from supposedly identical trees from the same clone (Fig. 1), and by assessing progeny for the consequences of incorrect pollination.

Six clones: 'SCA 6', 'CC 137', 'Pound 7', 'ICS 44', 'ICS 95', and 'CCN 51' differed in identity to supposedly identical accessions from other collections. The parental clone 'CCN 51' had the most uncertain identity, followed by the clones 'ICS 44' and 'ICS 95', which are known for their productivity (Table 1). Additionally, the presence of two types of trees among the parental clone 'UF 273' resulted also in two types of progeny descending from this clone, requiring their identification by SSR markers, and designation for correct statistical analysis.

Combining ability estimates of parents having discrepancies at the DNA level with accessions used as checks should be considered as estimates only for these specific genotypes, and not for all clones named as such. The existence of self-pollinated progeny, as discussed above, should also be cause for considering some possible future deviation from these results. However given the generally low amount of self-pollination found in the progeny of 'UF273' crosses, we feel that there is no substantial reason to doubt these results, especially when compared to past results. Calculations of combining abilities of parental clones using models (1) and (2) and progeny free of off-types result in the best linear unbiased estimators (BLUE) of the genetic

parameters (Searle et al., 1992). Having to code off-type progeny differently in the statistical analysis, results in estimates with lower precision, and with lower power for detection of significant effects, as the sample size is smaller. Schnell et al. (2004) identified misclassified parental trees and pollen contamination at the time of crossing as the main sources producing off-type progeny in one cacao cross, 'SCA 6' × 'ICS 1'. Obviously, verification of the genetic identity of parental trees before crossing and discarding off-types before field evaluation contribute substantially to more accurate and more precise parameter estimates in cacao breeding, and is essential for successful recurrent selection of any type.

Although combining ability effects were generally significant overall, their relative importance differed across traits considered in the study. Using the interpretation of GCA and SCA given by Sprague and Tatum (1942), percentage of healthy pods and percentage of pods with frosty pod damage were the two traits showing predominantly additive gene action, in that 43% and 50% of the clones had significant GCA effects, respectively, and only 15% of the crosses had significant SCA effects. Little information is available regarding the gene action regulating frosty pod resistance, in fact the only previously reported result states that its inheritance is polygenic (Phillips-Mora and Castillo, 1999). Genetic effects controlling the percent diseased fruit were studied by Dias and Kageyama (1995) using a diallel mating design in southern Bahia, Brazil, finding more important additive genetic effects; however, there is no evidence of frosty pod in that region. The findings of our research are among the first giving any insight on the genetics of frosty pod resistance in cacao, and this information is useful in planning for resistance breeding.

Twenty-nine percent and 50% of the clones had significant GCA estimates for total number of pods and trunk diameter, respectively, while 22% and 41% of the crosses had significant SCA estimates for these traits, indicating regulation by both additive and nonadditive gene action. There was no clear correlation among clones showing significant GCA effects for the two traits. Pearson's correlation coefficients between the two traits varied from -0.09 to 0.44 when calculated by cross, and the correlation over all crosses was significant but very low (0.18), which seems to limit, if not negate, the potential of a causal effect of one trait on the other. This weak association between productivity and vigor (trunk diameter) was also reflected in the combining ability estimate for harvest index, with only 14% of the parents showing significant GCA estimates and 7% with significant SCA estimates. This result was expected giving that the Pearson's correlation coefficient between the sectional area 30 cm above ground accumulated by year, and the total number of pods varied from -0.11 to 0.27 among crosses, and with a significant value of 0.10 over all crosses.

Several authors have reported the additive component to be the most important component of gene action for fruit and seed production traits (Ojo, 1982; Ramirez and Enriquez, 1988; Soria et al., 1974; Tan, 1990). Combining ability of pod production was also studied by Dias and Kageyama (1995), who found an important nonadditive component for the number of collected fruits, in agreement with our results. Number of months to flowering and fruiting showed evidence of being regulated somewhat more by nonadditive gene effects, given that 18% and 26% of

the crosses had significant SCA effects, respectively, with only 14% and 21% of clones showing significant GCA effects. No evidence for this type of gene action was found for response to black pod, nor for number of months to first jorquette. Ndoumbé et al. (2001) studied the genetics of the resistance to black pod disease caused by *Phytophthora megakarya* Brassier and Griffin in a complete diallel of six parents, finding significant low broad sense and narrow sense heritabilities. The genotypes evaluated in this study may not have shown their full potential for *P. palmivora* resistance due to both low field incidence of this disease during the 5-year experiment, and that the effects of *P. palmivora* might have been minimized by the presence of frosty pod in two ways: *P. palmivora* is less virulent and attacks only mature pods, and conversely, *M. roreri* is more aggressive and attacks young pods leaving only few available to be infected by *P. palmivora*.

Among the parents of Set 2, the GCA effects of the clones 'EET 75', and 'Pound 7' for frosty pod resistance, the GCA effect of the clone 'CC 137' for total number of pods, and the GCA effect of the clone 'Tree 81' for maturity stand out with significance. Interestingly, the clone 'CCN 51' has been generally considered to be successful donor of productivity in current breeding programs in South America. The GCA effect for yield of the 'CCN 51' clone used as a parent in this study was not significant, and it differed genetically from the genotype being used in other countries, such as Ecuador and Brazil (Fig. 1). This specific case points out even more importantly the danger of clonal misclassification in breeding programs, in that a great deal of time, effort, and resources used to develop segregating populations from misidentified clones are wasted.

In addition to the original breeding objectives and those of our research, parents of Set 1 have potential utility for quantitative trait locus analysis given their overall progeny size. In particular, the clone 'UF 712' showed combining ability for frosty pod resistance, indicated by its negative, significant ($P = 0.06$) GCA effect for the percent pods with frosty pod disease. The clone 'UF 273 Type I' demonstrated evidence of important additive gene effects for productivity, given by significant GCA effects for total number of pods. Considering the predominantly additive gene action, the progeny size, and the larger number of crosses with common parents, the 'UF 712' clone meets many of the assumptions to combine related F_1 populations for QTL mapping of frosty pod resistance (Cervantes-Martinez and Brown, 2004). However, the level of heterozygosity of the clone is relatively low ($\approx 27\%$, unpublished), also an important criterion that may lead to a sparse map for mapping frosty pod. Likewise, the clone 'UF 273 Type I' could be a useful common parent for mapping of productivity QTL.

Cacao breeders require quantitative information based on genetic designs for more systematic and effective parental selection in future breeding programs. The research presented here provides precise information on the combining abilities of an important set of cacao clones used worldwide, and insight into the gene action of productivity, disease response, vigor, and maturity traits. Our results demonstrate clearly the negative effect of including mislabeled germplasm in any breeding project, and the importance of incorporating molecular marker analysis for verifying identity. Two potential parental clones, 'UF 712' and 'UF 273 Type I', are identified as candidates for combined QTL analysis using breeding populations for frosty pod resistance and productivity. Future molecular analysis of individual trees having one of those two clones as a common parent would not only provide useful information for breeders and geneticists on genomic regions of

interest, but could also provide data for testing the efficiency of the haplotype-based QTL analysis method.

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