Backcross populations for genetic analysis of *Theobroma cacao*. Catongo X Tree 33 (Catongo X Pound 12)

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RESUMEN

Se estudió una población retrocruzada de 165 árboles de cacao (*Theobroma cacao*), originada del cruce intecIonal entre Catongo y Pound 12, en el Centro Agronómico Tropical de Investigación y Enseñanza (CATIE). Después de 27 meses de plantación en el campo (33 meses después de la germinación), se hicieron las siguientes observaciones: a) 116 árboles (70.3%) florecieron y 34 (20.6%) árboles tuvieron mazorcas; b) el árbol de mayor altura fue de 168 cm y el más corto de 24 cm, con un promedio de 95.6 cm; c) el diámetro más ancho de tallo, a 30 cm encima del suelo, fue de 6.5 cm y el más pequeño a 0.8 cm, con un promedio de 4.0 cm; d) un gen de color se expresó en los brotes de follaje, flores y semillas: 75 árboles mostraron brotes de color rojo y 59 (78.7%) estaban en proceso de florecer (con estambres rojos); 87 árboles tenían brotes de color verde pálido y 57 (65.5%) estaban en proceso de florecer (con estambres blancos). Esta segregación con una relación de 1:1 fue consistente con un gen simple, probablemente por vía de la antocianina biosintética, denotándose como homocigota recesivo en Catongo y homocigota dominante en Pound 12; e) los estudios de autocompatibilidad también indican un condicionamiento debido a un gen simple que se expresa en forma recesiva-dominante; y f) los datos sobre las mazorcas y las semillas correspondieron al primer cuatrimestre del tercer año.

SUMMARY

A backcross population of cacao (*Theobroma cacao*), now totalling 165 trees, originating from an intraclonal cross between Catongo and Pound 12, has been established at the Agricultural Research and Training Center (CATIE) in Turrialba, Costa Rica. Twenty-seven months after planting in the field (33 months after germination), the following observations have been made: a) 116 trees (70.3 %) are flowering, and 34 (20.6 %) are bearing pods; b) the tallest tree is 168 cm, the shortest is 24 cm, and the average height is 95.6 cm; c) the largest trunk diameter, 30 cm above the ground, is 6.5 cm, the smallest 0.8 cm and the average is 4.0 cm; d) a gene for color is expressed in flush leaves, flowers, and seeds. Seventy-five trees give red leaf flush and 59 (78.7%) of these trees are flowering (red staminodes). Eighty-seven of the trees give pale green leaf flush, of which 57 (65.5%) are flowering (white staminodes). This 1:1 segregation is consistent with a single gene, probably in the anthocyanin biosynthetic pathway, homozygous recessive in Catongo and homozygous dominant in Pound 12; e) autocompatibility studies also suggest conditioning by a single gene expressed in a dominant-recessive fashion; f) pod and seed data for the first quarter of the third year in the field are reported.

INTRODUCTION

Cacao, the source of chocolate flavor and cocoa butter, is a major cash crop for a large number of than a million people, worldwide. In contrast to many food crops that have benefitted from advances in plant breeding technology over the past 75 years, cacao breeding for the selection of high-yielding, disease- and insect-resistant trees has been slow and uncertain. Because advances in tree quality have lagged, much prime land that could be used for food crops is instead used to grow cacao. In Ghana and Cote d'Ivoire, two of the world's leading cacao-producing nations, over 3 million hectares are devoted to growing cacao. A major reason for slow progress in cacao breeding, as with other tree crops, is long generation times (3-5 years for cacao), but more importantly, lack of a genetic linkage map to aid breeders in the selection process.

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Now, at CATIE, a genetic linkage map for cacao is being developed, made possible by advances in molecular genetic technology, in particular the polymerase chain reaction (Saiki *et al.* 1988), and the randomly amplified polymorphic DNA(RAPD) technique (Williams *et al.* 1990; Welsh and McClelland 1990). The initial map will contain only DNA markers of defined nucleotide sequence and by itself will have value primarily as a framework for locating markers related to traits useful to cacao breeders. Thus, an important part of the linkage map studies is to relate phenotypic traits to molecular markers.

The vehicle for map construction is a genetically defined population of cacao trees initiated at CATIE in 1978 by crossing an upper Amazon clone (Pound 12) with a lower Amazon clone (Catongo). From this cross, some 120 hybrid trees were planted under two different types of shade tree and have been the source of extensive agroforestry studies (Alpízar *et al.* 1986; Heuveldop *et al.* 1988; Fassbender *et al.* 1988; Beer *et al.* 1990; von Platen 1991; Morera *et al.* 1993; Mora *et al.* 1993). In 1990, pollen from one of the hybrid trees was used to pollinate the Catongo parent in a backcross designed to produce a segregating population to be used for linkage map construction. Currently 168 of these backcross progeny are growing in the field at CATIE, and construction of the molecular marker linkage map is well underway. A preliminary, low-density amplification fragment length polymorphism (AFLP) linkage map using the backcross segregating population described in the present report has been described (Osei 1993; Osei *et al.* 1993).

As noted above, the DNA marker map is of value primarily as a framework for selection of traits important to plant breeders. When the map is complete, it should be possible to relate DNA markers, detectable at the seedling stage, to traits such as disease or insect resistance that may require mature plants for evaluation. Thus, as we proceed with construction of the marker map, we are beginning to gather information about phenotypic traits, sometimes called descriptors, in the backcross population. A list of descriptors currently being measured for future mapping includes: 1) flower, seed, and flush leaf color, 2) autocompatibility, 3) growth rates, 4) time of flowering, 5) time of pod bearing, 6) yield, 7) pod index, 8) seed index, 9) number of ovules per flower, and 10) number of seeds per pod. Most of these so-called quantitative traits are determined by more than one gene, but it may be possible to determine map positions of these several genes using current technology. Future descriptors to be measured and mapped include: 1) amount of cocoa butter per seed, 2) triglyceride composition of seeds, 3) chocolate flavor of seeds, 4) resistance to various fungi, and 5) resistance to various insects.

The present report documents phenotypic data for the backcross population for the period from May 1991, when the trees were put into the field, until the end of August 1993. Four other backcross populations are being developed at CATIE using Catongo X Pound 12 hybrid pollen and future reports will document the characteristics of these segregating populations as they become available.

MATERIALS AND METHODS

Catongo X Pound 12 interclonal cross

Progenies from this cross (over 120 trees) were established in the field at CATIE in 1977 as part of a long-term experiment to test the effect on yield of growing cocoa under two different types of shade trees, the timber tree *Cordia alliodora* (laurel) and the leguminous tree *Erythrina poeppigiana* (mountain inmortelle or poró). Several reports on various aspects of this experiment have been published (Alpízar *et al.* 1986; Heuveldop *et al.* 1988; Fassbender *et al.* 1988; Beer *et al.* 1990; von Platen 1991; Morera *et al.* 1993; Mora *et al.* 1993).

Catongo is a highly homozygous, autocompatible, white-seeded and white-flowered lower Amazon Forastero type, found near Urucuca, Bahia, Brazil in 1939 (Wood and Lass 1985). Catongo is classified as moderately susceptible to *Phytophthora palmivora* (Phillips and Galindo 1989). Pound 12 is one of the Nanay origin clones collected as budwood near the headwaters of the Amazon by Pound in 1943. Pound 12 is an autoincompatible, purple-seeded, purple-flowered, upper Amazon Forastero and is moderately resistant to *P. palmivora* (Phillips and Galindo 1989).

Catongo X (Catongo X Pound 12) backcross

In June 1990 at CATIE, pollen from one of the hybrid progeny (tree number 33) was used to pollinate the Catongo parent. In December, 1990, three pods from this backcross were taken to Penn State University, where 132 seeds (62 purple and 70 white) were germinated and planted in a greenhouse. After growing in the greenhouse for some 10 months, the seedlings were devastated by spider mites and none survived. Also in December 1990, 197 seeds (95 purple and 102 white) were planted in a nursery at CATIE, and in May 1991, 191 of these seedlings were established with 3 X 3 m spacing in the field. Currently, 165 of these seedlings have survived and these trees are the subject of this report. The trees are growing in a 0.25 ha field with drainage ditches and mixed shade trees including guava, poró, and banana.

Agronomic management includes chupon control and fertilization according to a yearly schedule as follows: August- 90 g per tree 18-5-15-3.7 N-P-K-Zn; December- 90 g per tree 18-5-15-3.7; April-80 g per tree 10-30-10. On April 27, 1993 the field was sprayed with 100 l of a 1000-fold dilution of Roundup• to control weeds. No insecticides have been applied despite some damage by leaf-cutting ants, aphids, and caterpillars.

RESULTS AND DISCUSSION

Hybrid pollen parent

In general, progeny of the Catongo X Pound 12 cross displayed the hybrid vigor or heterosis which has long been the basis for cocoa breeding programs. Tree 33, the one chosen as pollen parent for the backcross to Catongo, was above average in yield and pod index, as seen in Table 1. The actual yield data collected for the hybrid progeny were kilograms dry seeds/tree/year (Morera *et al.* 1993). Yields per hectare were calculated on the basis of 1111 trees per hectare. R-resistant; MR-moderately resistant; MS-moderately susceptible; S-susceptible; N.D.-not determined; Pod index-number of pods required to produce 1 kg dry seeds; Seed index-average weight of dry seeds; AC-autocompatible; AI-autoincompatible.

Resistance to *P. palmivora* was measured using the method developed at CATIE by Phillips and Galindo (1989), in which resistance is assessed by lesion size six days after pod inoculation with an aqueous suspension of *P. palmivora* spores. However, the resistance data shown in Table 1 are

Table 1. Genetic profile of Catongo and Pound-12 Clones and their hybrid progeny.

Genotype	(kg/ha)	Pod index	Seed index	Compatibility	*P. palmivora*
Catongo	400	30.8	0.9	AC	MS
Pound 12	400	27.9	0.8	AI	MR
CxP (Ave)	739	23.1	1.3	1:1 AC:AI	21% R 23% MR 19% MS 37% S
CxP tree 33	1000	21.2	1.1	AC	N.D.

based on lesion size 10 days after inoculation, which is now thought to give a more reliable estimate (Phillips, personal communication). Resistant clones show lesions of less than 2 cm diameter, moderately resistant from 2.1 to 4 cm, moderately susceptible from 4.1 to 6 cm, and susceptible above 6.1 centimeters.

The hybrid progeny clearly show evidence of enrichment for *P. palmivora* resistance genes when compared to data from 204 clones in the CATIE germ plasm collection where it was observed, using the six-day criteria, that only 9.3% were resistant, 45.6% were moderately resistant, 30% moderately susceptible, and 15% susceptible. Further evidence of enhanced *Phytophthora* resistance in these hybrids is the fact that CATIE 1000, the most resistant clone in the entire germ plasm collection, is derived from an earlier Catongo by Pound 12 cross (Phillips and Galindo 1989). The backcross population is too young to do a complete *P. palmivora* resistance study, but we can expect to find segregation of disease resistance genes that can be placed on the genetic linkage map once the trees are old enough to do the studies.

Backcross population
Quantitative traits

Precocity. Cocoa has a dimorphic growth pattern characterized by a change from orthotropicto plagiotropic growth at the transition from juvenile to adult. This event is marked by division of the single orthotropic meristem into as many as five meristematic plagiotropic shoots that grow more or less parallel to the ground forming the so-called fan branches of a growing cocoa tree.

The event is called jorquette formation and is followed, sometimes quickly, by the appearance of the first flowers. One of the seedlings in the Penn State greenhouse flowered after 9 months, and 3 after 11 months. Some of the trees at CATIE were flowering and beginning to bear pods after 15 months in the field. On the other hand, after 27 months in the field, about a third of the CATIE trees have yet to produce flowers, even though all but six have reached the adult stage as evidenced by jorquette formation. It should be noted that the Penn State greenhouse is at 41°N latitude where day lengths in June and July approach 16 hours of light, in contrast to CATIE at 10°N where approximately 12 hours of dark and light are experienced

Table 2. Precocity of Catongo x (Catongo x Pound 12) backcross population.

Period	Trees flowering	White flowers	Red flowers	Trees with pods
Sep-Nov'92	12	6	6	1
Dec'92-Feb'93	42	21	21	24
Mar-Sep'93	62	30	32	9
Total Sep'93	116	57	59	34

throughout the year. It will be important to learn if early flowering can be induced in tropical cocoa by artificially extending the day length experienced by developing seedlings.

The data in Table 2 summarize information about flower and pod production for the developing backcross population. Several of the trees appear to be exceptionally fertile as seen by multiple pod bearing and the ability to carry fruit to maturity. It should be noted that three trees only set fruit after hand pollination in connection with autocompatibility studies, so it is likely that more than 34 trees are capable of setting fruit. Of the 34 pod-bearing trees, 22 exhibit green leaf flush and white flowers.

Growth rates. Increasing height as well as trunk diameter 30 cm above the ground were measured at intervals after planting in order to assess growth rates. Standard biological growth curves were seen with the developing cocoa seedlings, slow growth or lag phase for about six months, followed by rapid growth or log phase from about 6 to 10 months, then slow growth continuing more or less indefinitely thereafter (Fig 1). Distribution of tree sizes after two years in the field at CATIE is shown in Fig. 2. It was possible to compare growth rates with field-grown saplings at CATIE to greenhouse-grown plants at Penn State.

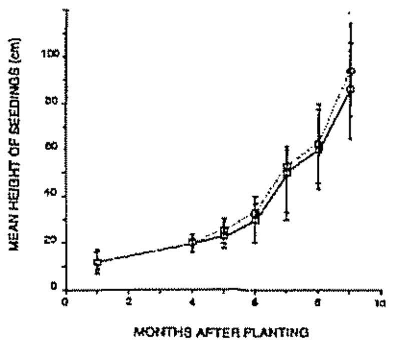

Fig 1. Monthly mean seedling height of progenies of Catongo X (Catongo X Pound 12) backcross population. Values are given as mean plus or minus standard deviation. Circles are seedlings originating from purple seeds, squares are from white seeds.

Although the general shapes of the growth curves were not different, there was much less variability in the greenhouse-grown trees, alerting us to the fact that much of the extreme variability seen in the field-grown trees may not be genetic in origin but due to environmental conditions such as differences in soil nutrients, shading, different soil chemicals produced by guava, poro, or banana trees, or other factors. This is an important consideration when attempting to find and map markers related to growth rates.

Pod data. Sixteen trees produced mature fruit in the months of June, July, and August 1993. Of these, six produced only one pod and seven produced as many as four, to a maximum of seven. The data, including tree number, are shown in Table 3.

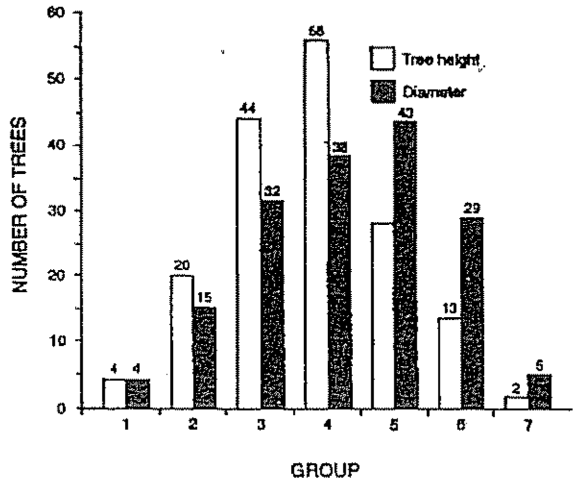

Fig. 2. Variation in jorquette height and trunk diameter 30 cm above the ground in progenies of Catongo X (Catongo X Pound 12) backcross population. Numbers above the bars are the total number of trees in the group. For tree height, group numbers includes trees within the following ranges: Group 1- 20-49 cm, Group 2- 50-69 cm, Group 3- 70-89 cm, Group 4- 90-109 cm, Group 5- 110-129 cm, Group 6- 130-149 cm, Group 7, 150-169 cm. For trunk diameter, group numbers have the following values: Group 1- 0-0.9 cm, Group 2- 1.0-1.9 cm, Group 3- 2.0-2.9 cm, Group 4-3.0-3.9 cm, Group 5- 4.0-4.9 cm, Group 6- 5.0-5.9 cm, Group 7- 6.0-6.9 cm.

Table 3. Backcross (Catongo x (Catongo x Pound 12) pod data summary June-August 1993.

Tree No.	Flush Color	Quantity	Weight (g)	Length (cm)	Diameter (cm)
1	Red	509 2	509	14 2	8 6
11	Red	6	303+/-86 7	12 2+/-1 88	7 3+/-0 86
14	Green	1	294	10	7 9
18	Red	4	403+/-35 9	14.4+/-0 8	7 6+/-0 49
19	Green	1	517	14 5	8 6
42	Green	5	594+/-84.4	15 9+/-0 85	8 98+/-0.41
84	Green	7	448+/-66 0	14 0+/-1 44	8.13+/-0 35
99	Green	4	386+/-83.4	13 6+/-0 82	7 82+/-0 64
125	Red	1	231	11 5	6.6
126	Green	1	456	15 0	8 4
139	Red	6	419+/-109	13 73+/-1 60	8.1+/-0 76
145	Green	1	520	16 0	8 2
147	Green	1	316	11 0	7.5
148	Green	3	702	16.6	9 2
170	Red	3	413	12 6	8 0
179	Green	3	461	13 1	8.7

Data for trees with more than three pods is expressed as mean, plus or minus standard deviations. For those trees with more than one but less than four pods, the numbers are simple averages.

Table 4. Backcross [Catongo x (Catongo x Pound 12)] Seed Data Summary, June-August 1993

Tree no.	Quantity	Fresh wt (g)	Dry wt (g)	White/Purp	Seed index	Pod index
1	39	74.0	53.4	24/54	1.37	18.8
11	37.5+-8.0	46.5+-8.9	30.2+-8.3	44/181	0.81+-.16	35.3+-10
14	19	30.5	19.9	19/0	1.04	50.2
18	37.8+-3.4	56.6+-9.5	39.9+-5.7	44/107	1.05+-.06	25.4+-4.1
19	37	59.6	43.8	27/10	1.18	22.8
42	43.6+-5.0	76.5+-8.9	53.9+-9.1	218/1	1.24+-.23	18.9+-3.0
84	42.7+-5.7	56.3+-9.1	38.1+-6.9	297/2	0.89+-.06	27.0+-5.1
99	42.2+-11	86.1+-13	45.3+-13	169/0	1.05+-.16	24.4+-10
125	31	28.8	16.1	1/30	0.52	62.1
126	22	39.0	26.6	22/0	1.21	37.6
139	42.2+-11	57.8+-7.1	41.3+-6.4	80/183	0.89+-.16	24.6+-3.8
145	30	50.3	34.1	30/0	1.14	29.3
147	12	18.1	12.3	12/0	1.03	81.3
148	49.3	79.3	55.7	131/17	1.13	18.1
170	25.7	37.5	24.5	5/72	0.89	55.9
179	41.0	75.2	43.4	111/12	1.06	22.9

Seed data. Seeds from the harvested pods (Table 3) we recorded. The wide variability seen in tree size is also seen in the seed data where major differences in number of seeds per pod, weight of seeds, and pod index were noted. It is also interesting to note that in those trees producing green leaf flush and white flowers, virtually all the seeds in these open-pollinated pods were white. White colored seeds can only come from a homozygous recessive condition, suggesting that these trees were likely to be self-pollinated and thus autocompatible.

Other seed data of interest to cocoa breeders and chocolate manufacturers include cocoa butter content and chocolate flavor. The seeds collected in this study will be used to assess these properties in the hope that segregation of these quantitative traits in the backcross population will allow correlations with molecular markers and ultimately identification of genes responsible for these traits. A study (Lockwood 1992) of the cocoa butter content of 126 cacao clones revealed that Pound 12 at 59.1% dry bean weight had the third highest cocoa butter content, while Catongo at 56.6% was much lower, suggesting that we can expect to see segregation for this trait in the backcross population.

Single gene traits

Color. Lack of color, as seen in pale green flush leaves, flowers and seeds, is the consequence of a homozygous recessive condition. Catongo has all these traits, in contrast to Pound 12, which has a red leaf flush, red flower staminodes, and purple seeds resulting from a homozygous dominant or heterozygous condition for this trait. The F1 hybrids all have varying shades of red leaf flush and red flower staminode color indicating a heterozygous condition, confirmed by the backcross of a heterozygote (tree 33) to a homozygous recessive (Catongo) to produce a 1:1 segregation of leaf flush and flower color in the backcross population (Table 2). A gene, most likely encoding an enzyme in the anthocyanin biosynthetic pathway, is probably responsible for this situation. The utility of using white seeds to confirm genetic crosses was first noted some 60 years ago (Wellensiek 1932).

Autocompatibility. Catongo is autocompatible and Pound 12 is autoincompatible, as noted in Table 1. Of 86 F1 hybrids tested, 41 were self-compatible and 45 were self-incompatible (Table 1; Morera *et al.* 1993). This 1:1 segregation can be explained by the presence of a single gene, homozygous recessive in one parent and heterozygous in the other. Presently we are not certain which of the parental trees is homozygous or heterozygous, though it seems probable that Catongo is heterozygous because tree 33, the F1 pollen parent for the back-cross, is also autocompatible and therefore must have the same allelic composition as Catongo. Thus it is predicted that the backcross population will segregate in a 3:1 ratio of autocompatible to autoincompatible. Preliminary results show that some of the backcross trees are autocompatible and some are not, but more determinations are needed to establish the ratios with confidence.

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