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Cacao breeding in Bahia, Brazil - strategies and results

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ABSTRACT - *Cacao was introduced in Bahia in 1756, becoming later the largest producer state in the country. In order to support the planting of cacao in the region, a breeding program was established by CEPEC at the beginning of the 1970s. For a long time, the program consisted in testing new hybrids (full-sibs) and releasing a mixture of the best ones to farmers. Lately, particularly after the witches´ broom arrival in the region, in 1989, recurrent breeding strategies were implemented, aiming mainly the development of clones. From 1993 to 2010, more than 500 progenies, accumulating 30 thousand trees, were developed by crossing many parents with resistance to witches´ broom, high yield and other traits. In this period, more than 500 clones were put in trials and 39 clones and 3 hybrids were released to farmers. In this paper the strategies and results achieved by the program are reviewed. Overall the program has good interface with pathology and genomic programs.*

Key words: Theobroma cacao, *breeding strategies, selection methods*.

INTRODUCTION

Cacao (*Theobroma cacao* L.) is a perennial crop, originally from the Amazon rain forest of South America. Its product, the cocoa beans or seeds, is the major ingredient of the chocolate manufacture and of its derivatives. The cocoa beans were already consumed by Mayas and Aztecs, and likely by Olmecs (1500-400BC), and today are an important component of the economy of many producer and processor countries.

Cacao is typically cultivated in the tropics, by small farmers (mostly less than 3 ha) in third world countries. Around 70 % of the cocoa beans produced in the world come from West Africa, mainly from Ivory Coast (40 %), Ghana (20 %), Nigeria (5 %) and Cameroon (5 %). Brazil, until the arrival of the witches' broom [*Moniliophthora* (ex-*Crinipellis*) *perniciosa*] in Bahia, its main producer region, was the second largest cocoa producer in the world, dropping to the fifth position thereafter.

Bahia is still the largest cocoa producer state in Brazil, with 64 % of the country's production, followed by Pará (25 %), Rondônia (8 %) and Espírito Santo (3 %). In Bahia, cacao was introduced in the Southeast as seeds from the lower Amazon in 1756. For more than two centuries, farmers planted seeds derived from these early introductions (*Cacau Comum*). In 1940s and 1950s the first selections were made in plantations derived from these early introductions by research institutions, namely, the ICB (Cacao Institute of Bahia), IPEAL(Agronomic Institute of East) and EEG (Experimental Station of Goytacazes) and open-pollinated seeds of those selections were released to farmers. Lately, with the creation of the Executive

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Commission of the Cacao Plan (CEPLAC), the focus turned to interclonal hybrids (full-sib families) between imported and local germplasms. More recently, with the introduction of the witches´ broom disease in Bahia and Espírito Santo, recurrent selection programs were implemented aiming to support the development of clones.

The present paper briefly reviews the botany and genetics of cacao and then the strategy currently adopted in the cacao breeding program at the Cacao Research Center (CEPEC/CEPLAC), in Bahia, and some results achieved from that program.

BOTANY

Theobroma cacao L. until recently was considered as belonging to the Sterculiaceae family, but was suggested to be part of the Malvaceae family (APG II 2003). The genus *Theobroma* comprises 22 species (Cuatrecasas 1964). Despite the several attempts, crosses with cacao have had reasonable success only with *T. grandiflorum* (see Martinson 1966).

The cacao plant produces up to 125 thousand flowers per year (Lachenaud and Mossu 1985) and each flower can produce up to 14 thousand pollen grains (Massaux et al. 1976) and up to 74 ovules (Bartley 2005).The flowers are hermaphrodite and mainly pollinated by insects of the genus *Forcipomyia* (Billes 1942). However, at the end of the period of receptivity (2-3 days after the flower opening), 50 to 75 % of the flowers have no pollen and drops of the tree (Parvais et al. 1977, Reffye et al.1978). As a consequence, usually not more than 2 % of the flowers produced in a plant result in mature fruits (Alvim 1984). Outcrossing rates in cacao ranges from 18 to 66 % (Posnette 1950, Vello 1971), but up to 100 % in self-incompatible plants (Voelker 1938). Usually pollination occurs among neighbor plants (Yamada and Guries 1998), but can reach up to 40-60 meters (Knaap 1955, Benton 1986,). Pollinations involving a single pollen plant donor are common in cacao, resulting in a reasonable proportion of full-sib families in natural crosses (24-70 %) (Yamada and Guries 1998).

The species has a strong sexual incompatibility system, controlled by the *S*-locus with modifiers (Knight and Rogers 1955, Cope 1962), which limits the production in farms and makes difficult some types of crosses by breeders. In breeding programs, incompatibility is usually overcome by mixing cacao pollen with that of a close genus (*Herrania*).

The cacao plant is very flexible in terms of propagation. Many seeds are produced per plant, the pollination process is quite easy and successful in compatible crosses. Besides that, cacao is easily propagated by grafting and budding (with more than 90 % of success) and by rooted cuttings. Tissue culture is also quite successful, particularly for specific clones (Maximova et al. 2002) and protocols for transgenics exist (Maximova et al. 2003).

GENETICS

The first genetic map in cacao was produced in 1995, based on 193 marker loci (Lanaud et al. 1995). Today, 1259 SSRs and SNPs loci are mapped in the 10 cacao chromosomes. For many agronomic traits of importance in cacao breeding, quantitative trait loci (QTLs) have been mapped (Clement et al. 2003, Faleiro et al. 2006, Brown et al. 2007, Araújo et al. 2008,). However, saturated maps on specific regions are still underway and the maps available are still based on a few crosses. Markers from specific expressed regions have also been developed, particularly associated to new sources of resistance to witches' broom (Lima et al. 2010).

The genome size of cacao is relatively small, with around 400 Mb, being only double of that of the model plant *Arabidopsis thaliana* (Figueira et al. 1992). In 2010 the whole genome of two cacao varieties were sequenced, one belonging to the *Criollo* population (Argout et al. 2010) and the other to the *Forastero* population (unpublished). This opens new perspectives in the identification of genes of interest to breeders and the development of markers more efficient in the identification of segregants in breeding populations, for marker assisted selection.

The structure of the genetic diversity in cacao is quite well studied, not only by using phenotypic traits (Bartley 2005), but also by molecular markers (Marita et al. 2001, Motamayor et al. 2008). Three major populations exist, the *Forastero* from the Amazon area, being very diverse and rich in genes of resistance to diseases, yield and at some extent quality; the *Criollos* from Central and North America (Mexico) is usually characterized by its good flavor; and the *Trinitario* which is a result of the natural cross of the two original populations. Further, within these major populations there are several subpopulations. Recently, based on molecular markers, Motamayor et al. (2008) suggested 10 populations instead the three cited.

Considerable genetic diversity has been observed for many traits in cacao (Bartley 2005). Overall, germplasms of the upper Amazon (*Forasteros*) tend to be more diverse than the other groups (Marita et al. 2001). However, quite high diversity has been found in most populations.

GERMPLASM

Quite large germplasm collections have been maintained in most producer countries, particularly those in America. The collections in Ecuador include around 13 thousand genotypes and that one in Belém, Brazil, around 20 thousand genotypes (2000 accessions). This last one has around 90 % of its accessions collected directly from wild. The two international germplasm collections, one in Costa Rica (CATIE) and the other in Trinidad (CRU/ University of West Indies), have 1150 and 2300 accessions, respectively. The collection at CEPEC has 1300 accessions, from more than 20 countries, and includes wild and improved germplasms. Small collections of other *Theobroma* spp also exist (Silva et al. 2004).

Despite the large collections by most producer countries, with few exceptions, most of them are poorly evaluated for agronomic traits. This fact can have a profound impact on the genetic gains of further steps of the breeding program, particularly considering the positive correlation between the performance of germplasms in collections and as parents in breeding populations observed by some authors.

Also, despite the availability of a large number of accessions in collections, most programs are based on a few germplasms. Most programs even today relies heavily on few selections made around 1940-50, including those of the series Scavina, Pound, Nanay, Parinari, UF (United Fruit), IMC (Iquitos Mixed Calabacillo) and ICS (Imperial College Selection).

BREEDING OBJECTIVES

By far, bean yield and disease resistance are the traits that receive most attention of cacao breeders, despite some emphasis be also put on sexual compatibility and bean quality (flavor, chemical composition). Yield is usually measured as the weight of the dry (often wet and converted to dry) beans produced per plant or area. The three components of yield are the number of pods per tree or area, the number of beans per pod and the weight of individual beans.

Some diseases of cacao, under high severity pressure can cause losses up to 100 %. Witches´ broom is the most important disease of cacao in Brazil, being responsible for large losses in Bahia and in the Amazon region. Ceratocystis wilt (*Ceratocystis cacaofunesta*) is another important disease in Brazil, being a very serious problem for some susceptible varieties (Theobahia). Black pod rot, caused by *Phythophthora* spp, is the most widely distributed and important disease of cacao in the world and also causes losses in some regions of Brazil. Moniliasis (*Moniliophthora roreri*) occurs in most producer countries in South America, except Brazil, being a constant threat to the country´s cacao plantations.

Sexual incompatibility is also an important limiting factor of cacao yield. Many germplasms useful to breeders, including most of those with resistance to diseases, are self-incompatible. Therefore, some programs have put a considerable effort in eliminating self-incompatible plants from their populations, particularly when the final objective is the development of clones.

More recently, with the growing interest of the industry for high quality chocolate, some breeding programs have dedicated time also to traits related to flavor. For example, the Ecuadorian breeding program has concentrated a lot of effort in breeding for the *Arriba* flavor in the *Nacional* population. More recently, the Brazilian breeding program has also involved many fine flavor germplasms, particularly aiming to explore the European market of fine chocolate.

BREEDING STRATEGY AND RESULTS

For a long time plantings in producer countries were made with open-pollinated seeds from trees selected (or not) in local populations. Lately, with the start of breeding programs, plants were selected in plantations for distribution as clones or open-pollinated families. With the discovery of heterosis in cacao (Montserin and Verteuil 1956), most breeding programs in the world passed to develop interclonal hybrids (full-sib families). Also, all these programs adopted a “single-generation breeding strategy”, in which the new hybrids formed did not come from selected segregants in a previous generation, instead, they were formed from other germplasms already in collections.

More recently, however, after some pioneers (Clement et al. 1994, Pires et al. 1996) many cacao breeding programs have adopted recurrent selection as part of their strategy of producing new varieties. In CEPEC’s recurrent selection program, two mating designs have been used to produce the base populations. In the first one, a factorial mating design is used and a reciprocal recurrent selection scheme is followed (Figure 1). In Figure 1, eight parents of each of two populations (A and B) are intercrossed following a factorial (North Carolina II) mating design, producing three types of progenies: X progenies resulting from crosses between parents selected at population A; Y progenies from crosses between parents selected in populations A and B; and Z progenies resulting from crosses between parents selected in population B. Usually members of population A are better for some attributes (e.g., disease resistance) and those from B for other attributes (e.g., yield), such way that, through the cycles of breeding, population A is improved for its attributes through the crosses of the type X and population B is improved for their attributes through the crosses of the type Z. In each generation, segregants are selected in the progenies of crosses of the type Y to be tested as clones. The crosses of the type Y is also used for identifying parents in populations A and B, which maximize the general and specific combining abilities, to advance the generations of reciprocal recurrent selection.

Parents	A1	A2	A3	A4	B1	B2	B3	B4
A5	X	X	X	X	Y	Y	Y	Y
A6	X	X	X	X	Y	Y	Y	Y
A7	X	X	X	X	Y	Y	Y	Y
A8	X	X	X	X	Y	Y	Y	Y
B5	Y	Y	Y	Y	Z	Z	Z	Z
B6	Y	Y	Y	Y	Z	Z	Z	Z
B7	Y	Y	Y	Y	Z	Z	Z	Z
B8	Y	Y	Y	Y	Z	Z	Z	Z

Figure 1. Factorial mating design involving eight parents of the population A and eight of the population B, in a recurrent selection scheme.

Another mating design used in CEPEC's program to form the base populations is the Single-Pair Mating (SPM) design. However, here the pairs are not picked at random as the design assumes. Instead, the parents in a pair are chosen to complement each other in terms of beneficial traits. In these populations, individuals are either selected by mass selection or, more recently, family and within-family combined selection.

The overall approach currently adopted in CEPEC´s program, as well as the products of the program are outlined in Figure 2. In summary, breeding populations are formed by using a factorial or a single-pair mating design and planted as a replicated trial or as unreplicated progeny blocks of 50-300 trees/progeny. At the beginning of this strategy, in 1993, farm populations were also used as base populations and a considerable effort was put in selecting trees in many plantations of the region. Since many of these plantations were formed by a quite large mixture of interclonal hybrids released by CEPEC, at different periods, planted in large numbers and most of them exposed to a high severity of witches´ broom, they provided a good opportunity for genetic gains for witches´ broom resistance and yield (Lopes et al. 2003).

From 1993 to 2010, around 500 full-sib progenies, summing up around 30 thousand trees, were established as base populations at the CEPEC's breeding program (Figure 2). When forming these base populations several factors were considered. First, in some of them, molecular diversity was considered when identifying the parents to be included in the crossing scheme, aiming to maximize the chance of success in combining different genes/alleles for the traits considered (see Pires et al. 1996). Second, resistance to witches´ broom was a strong component, being considered in the formation of all populations. In this respect, currently, emphasis has been put in increasing the diversity of the sources of resistance, aiming to reduce the chances of adaptation of the fungus to released varieties, as already observed for the main source of resistance – the Scavinas. Third, since the program focused mainly in the development of clones after the witches' broom introduction in the region, and considering that most sources of resistance were self-incompatible, a considerable effort was put in including sources of self-compatibility in the base populations and selecting for that trait. Forth, with the risks represented by the introduction of moniliasis in the country, some sources of resistance (ICS-95) were included in the formation of some base populations. Besides that, currently, large base populations using other sources of resistance to moniliasis recently introduced in the country are being formed as part of the preventive breeding strategy adopted by CEPLAC and a platform for marker assisted selection in the absence of the pathogen is being implemented.

The second step in CEPEC´s program is either the selection of individual trees or of full-sibs (or hybrids). Although the strategy of selecting hybrids is not discarded in the program, currently emphasis has been given to the development of clones. Within this strategy, single trees are selected either by mass selection or family-within-family combined selection and grafted on chupons (a basal shoot)

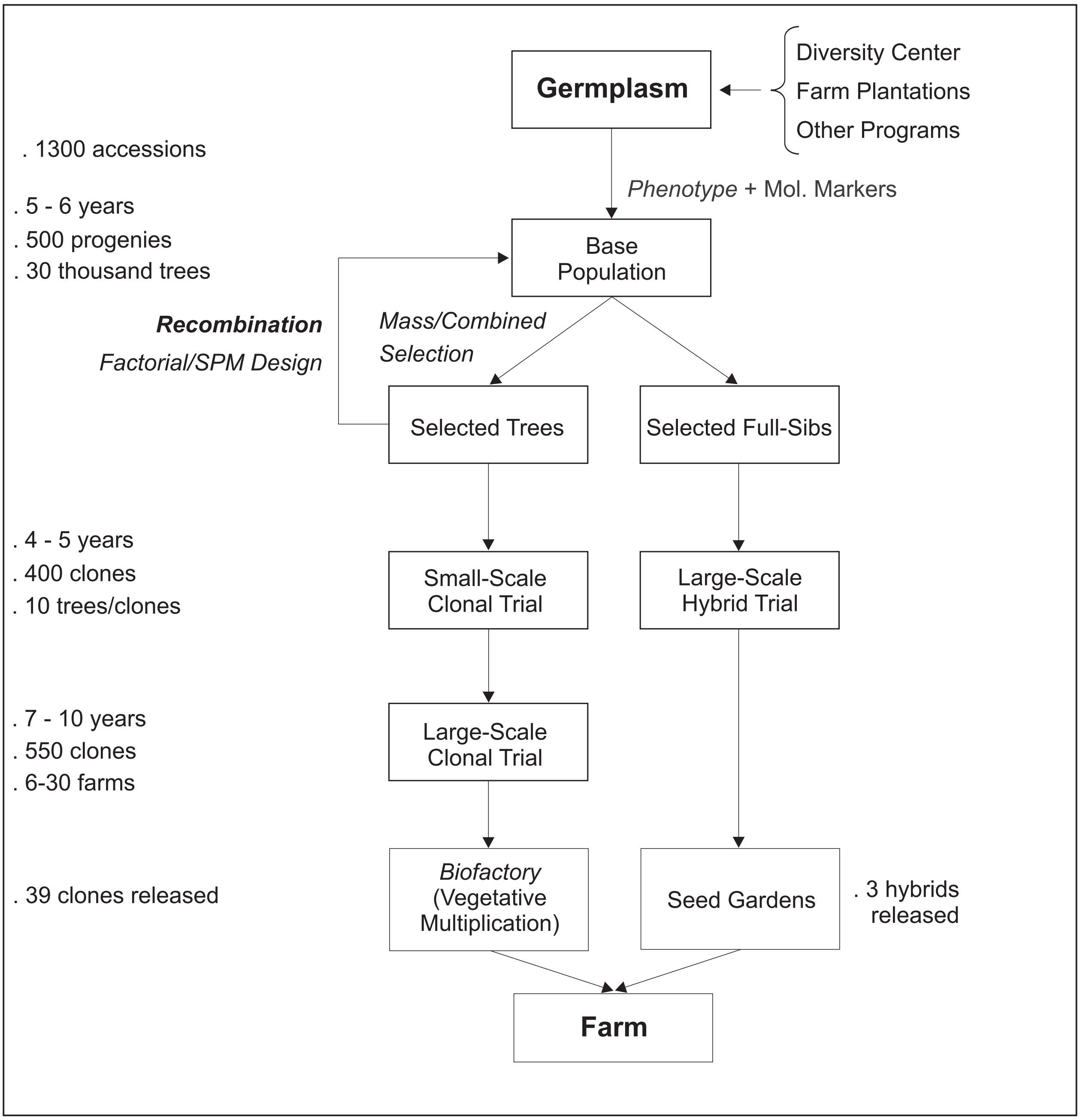

Figure 2. Cacao breeding program at the Cacao Research Center (CEPEC/CEPLAC) and populations/varieties produced from 1993 to 2010. See text for details.

of adult trees in small-scale clonal (SSC) trials. On these, 10 plants per clone are used, in one or two replicates, and established in CEPEC's experimental station. The main objective here is a better evaluation of the selected plants, under the same propagation system adopted by farmers, but also increasing the amount of propagation material for the large-scale (multilocation) clonal trials. Up to now, more than 400 clones have been put in SSC trials in CEPEC.

The third step is testing the selected clones under farm conditions, on replicated, multilocation, large-scale clonal (LSC) trials. Various experimental designs have been used, as for example: a) completely randomized design with single-tree plots, in 3-6 farms, with most clones established in all farms; b) randomized complete block design, with 2-3 replicates per farm, in 6 farms, with potentially better clones established in all farms and potentially intermediate-good (or less known) clones in smaller number of farms; c) completely randomized design, with 5 trees/farm, with a large number of clones put in large number of farms and considerable use of statistical tools to deal with the unbalanced nature of the design and different ages of the clones in the trials. Up to now more than 550 clones have been tested in this step. It should be pointed out that the number of clones in the LSC trial is bigger than that in SSC trial because: a) some clones were already being used by some farmers and claimed as good ones, but without being properly tested; b) the intensive participation of large farms in the evaluation scheme, stimulating the test of a large number of selections of high potential or clones with good flavor quality.

At the fourth step, clones selected in the LSC trials are transferred to a unit of mass vegetative propagation – the Biofactory – where the clones are distributed as rooted cuttings or as grafts. Up to now, 39 clones have been released to farmers by CEPEC and more than 150 thousand hectares of susceptible varieties have been renewed with resistant clones in Southern Bahia. Some characteristics of these clones are described in Table 1 and the pedigrees presented in Figure 3.

FINAL CONSIDERATIONS

In spite of the shortage of resources and of available staff to CEPEC's cacao breeding program on these last years, and despite the introduction of the witches´ broom in Bahia, which demanded considerable changes in the program's focus, the success achieved was great, having as results the generation of more than 30 thousand trees, the testing of more than 500 clones and the releasing of 39 clones and three hybrids to farmers.

The completion of the sequence of two cacao genomes in 2010 open new doors to improve the efficiency of identifying new segregants carrying genes of interest in breeding populations. The involvement of breeders in the exploitation and interpretation of these sequences and in the development of markers for selection is essential for the success of using the results in breeding the varieties of the future. It is believed this will be key when pyramiding genes of resistance to diseases.

In summary, the quality of the future cacao varieties will depend on a successful plan done in the present, the use of new (and old) tools available, without ignoring that, besides these tools, breeding varieties requires recombination, recurrent accumulation of good gene combinations, selection and testing; so requires breeders.

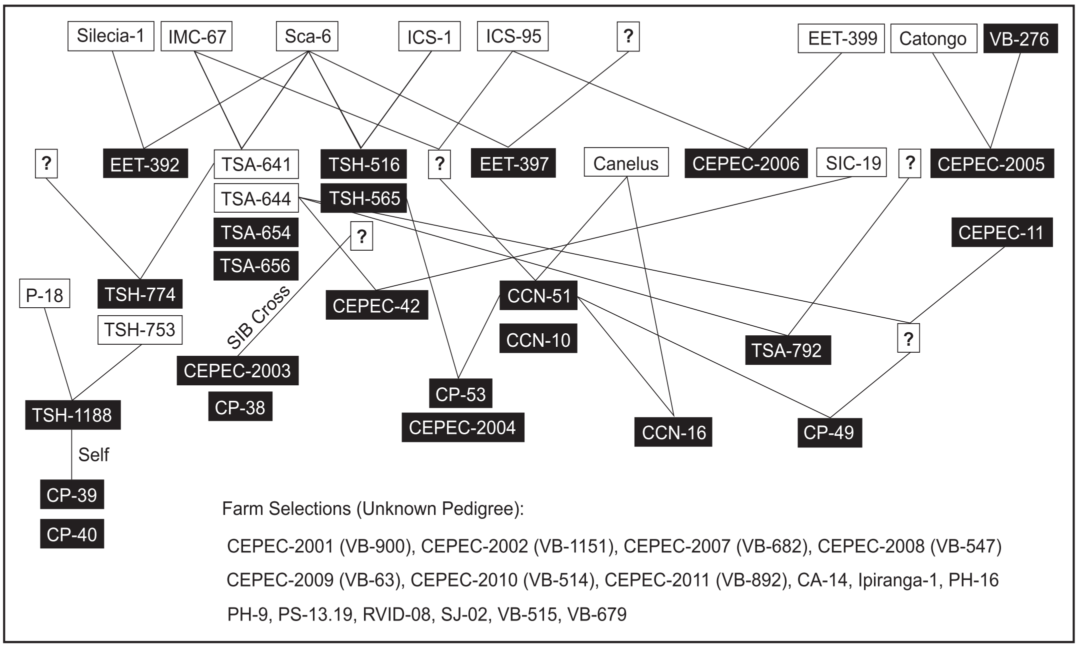

Figure 3. Pedigree of clonal varieties released (black boxes) to farmers by the Cacao Research Center from 1995 to 2010.

Table 1. Description of the cacao clones recommended by the Cacao Research Center

Release*	Clone	Synonym	IR	NBPP	SBW	BWPP (g)	Husk (%)	Yield (kg ha^{-1})	YSS (%)	NVB	NCB	WBPI (%)	BPIP (%)
1995	CEPEC-42	-	SI	45.4	1.1	52.0	70.1	1287	32	1.4	3.5	7.5	1.1
	EET-397	-	SI	31.0	1.3	40.2	77.2	1110	35	1.0	5.5	4.5	2.3
	TSH-1188	-	SI	45.0	1.4	63.2	68.9	1186	35	3.3	0.6	8.2	4.6
	TSH-516	-	SI	47.6	1.1	52.7	72.4	1178	33	3.2	2.7	7.7	2.9
	TSH-565	-	SI	46.7	1.3	58.5	73.0	1083	32	3.1	1.2	10.8	3.7
1998	TSA-654	-	SI	30.8	1.1	34.5	76.2	837	24	3.5	4.2	6.9	2.9
	TSA-656	-	SI	40.8	1.1	44.6	72.5	805	24	4.2	5.8	6.9	2.9
	TSA-792	-	SI	49.8	1.2	58.0	61.5	1199	23	4.8	5.5	7.9	1.2
	TSH-774	-	SI	38.8	1.0	38.2	74.5	730	22	3.0	7.9	8.1	2.5
2001	CEPEC-2001	VB-900	SI	39.7	0.8	30.1	65.4	632	3	2.0	0.1	5.2	3.3
2002	CEPEC-2002	VB-1151	SC	41.2	0.8	32.3	74.4	1681	51	4.3	5.2	10.3	3.3
	CEPEC-2003	CP-37	SC	38.6	0.5	20.2	85.9	1157	50	2.1	0.6	9.5	2.2
	CEPEC-2004	CP-46	SC	33.0	0.9	29.2	76.5	1094	34	0.0	7.6	0.0	2.6
	CEPEC-2005	CP-55	SC	38.0	0.9	35.3	53.4	862	34	3.8	4.6	4.6	0.9
	CEPEC-2006	CP-50	SC	36.6	1.1	41.5	72.8	-	-	-	-	-	-
	CEPEC-2007	VB-681	SC	34.5	1.1	38.9	74.2	1187	54	3.2	3.9	5.6	2.8
	CEPEC-2008	VB-547	SI	23.2	1.3	29.7	85.9	1091	29	6.2	7.3	11.7	3.3
	CEPEC-2009	VB-663	SI	29.7	1.1	32.5	77.6	1036	28	0.0	9.4	0.0	3.7
	CEPEC-2010	VB-514	SI	36.8	0.7	26.4	69.5	934	19	4.6	5.5	11.9	8.4
	CEPEC-2011	VB-892	SI	30.1	1.1	33.9	80.9	-	-	-	-	-	-
2003	CCN-10	CP-02	SC	34.4	1.9	64.0	67.1	1387	39	3.3	3.7	9.9	7.0
	CCN-51	CP-01	SC	38.6	1.6	61.3	60.1	1614	47	1.5	0.0	8.6	12.1
	CP-38	-	SC	34.3	0.9	31.4	80.1	-	-	-	-	-	-
	CP-39	-	SC	32.0	1.1	35.0	-	-	-	-	-	-	-
	CP-40	-	SC	31.8	1.1	35.0	-	-	-	-	-	-	-
	CP-49	-	SC	37.0	1.2	45.1	79.2	1250	45	3.7	10.5	21.4	2.1
	CP-53	-	SC	38.6	1.1	41.7	72.1	-	-	-	-	-	-
	EET-392	-	AI	35.8	1.3	46.5	-	-	-	-	-	-	-
	PH-16	-	SC	38.0	1.1	41.8	68.3	1359	42	3.3	8.8	3.4	0.0
	VB-276	-	SI	41.4	1.3	54.7	71.3	1174	1	2.4	1.1	8.7	1.5
	VB-515	-	SI	21.3	0.9	19.1	81.1	1462	14	2.2	12.5	0.0	1.0
	VB-679	-	SI	31.0	1.0	31.7	83.7	885	41	4.1	12.7	11.2	2.9
2004	PS-13.19	-	SC	42.3	1.1	46.2	70.1	1427	51	6.6	4.1	19.7	5.5
	RVID-08	-	SC	58.9	1.0	57.3	64.9	1198	41	7.4	5.6	9.9	3.3
	SJ-02	-	SC	43.4	1.0	42.5	69.7	1774	47	27.8	16.9	16.3	1.1
2006	CA-1.4	-	SC	-	-	-	-	1430	42	1.0	0.0	7.0	7.3
	CCN-16	-	SI	41.0	2.1	86.0	-	1208	31	4.1	6.3	11.8	3.1
	Ipiranga-1	-	SC	41.0	1.6	64.0	-	1106	40	4.4	4.9	9.2	4.4
2007	PH-9	-	SC	43.0	1.2	51.6	-	1535	61	2.6	1.9	3.2	2.4

*Year of release, synonym, incompatibility reaction (IR), number of beans per pod (NBPP), single bean weight (SBW), bean weight per pod (BWPP), the percent of husk in relation to the total pod weight (husk), productivity at 4 years of age (yield), percent of yield in the second semester of the year (YSS), number of vegetative (NVB) and of cushion brooms per plant per year (NCB), witches´ broom (WBPI) and black pod incidence in pods (BPIP).

Melhoramento do cacau na Bahia, Brasil - estratégias e resultados

RESUMO - *Cacau foi introduzido na Bahia em 1756, tornando-se posteriormente o estado maior produtor no país. Visando dar suporte ao plantio de cacau na região, um programa de melhoramento foi estabelecido pelo CEPEC no início dos anos 1970. Por longo tempo, o programa consistiu em testar novos híbridos (irmãos completos) e distribuir uma mistura dos melhores para os produtores. Posteriormente, particularmente após a chegada da vassoura-de-bruxa à região, em 1989, estratégias de melhoramento recorrente foram implementadas visando principalmente o desenvolvimento de clones. De 1993 a 2010, mais de 500 progênies, acumulando mais de 30 mil árvores, foram desenvolvidas por cruzamento de muitos progenitores com resistência à vassoura-de-bruxa, alta produção e outros caracteres. Neste período, mais de 500 clones foram colocados em ensaios e 39 deles e três híbridos foram recomendados para os produtores. Neste artigo as estratégias e os resultados obtidos pelo programa são revistos. De modo geral o programa tem boa interface com os programas de fitopatologia e genômica.*

Palavras-chave: *Theobroma cacao*; estratégias de melhoramento; métodos de seleção.

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