J. Appl. Genet. 44(3), 2003, pp. 339-353

Assessment of the value of doubled haploids as progenitors in cocoa (*Theobroma cacao* L.) breeding

Olivier SOUNIGO[1], Philippe LACHENAUD[1], Philippe BASTIDE[1], Christian CILAS[1],
Jeanne N'GORAN[2], Claire LANAUD[1]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement,
Montpellier, France
[2] Centre National de Recherche Agronomique, Abidjan 01 Côte d'Ivoire, France

Abstract. In order to evaluate twelve doubled haploids (DHs) of *Theobroma cacao* L. used as parents, a trial was set up in Côte d'Ivoire. Several traits were observed, such as yield, vigour, yield/vigour ratios, resistance to the black pod disease caused by *Phytophthora*, percentage of flat beans and mean weight of 100 cocoa beans. Out of the three progenies derived from crosses between two DHs, two showed severe drawbacks. A reduction of the heterogeneity within these progenies was occasionally observed for some of the traits, but failed to be consistent. When tested as female parents in combination with diploid testers, some of the DHs showed a significantly higher combining value than their parents for traits such as the mean weight of 100 beans and the yield/canopy surface ratio. The results showed the potential of DHs to improve selected parents in only one cycle of selection but more crosses between two DHs need to be tested in order to evaluate potential of the resulting F_1 progenies.

Key words: combining value, doubled haploid, *Theobroma cacao* L.

Introduction

Cocoa (*Theobroma cacao* L.) is a diploid (COPE 1984) preferentially allogamous tropical tree species, belonging to the family Sterculiacae (CUATRECASAS 1964). Its geographical place of origin is South America (CHEESMAN 1944, MOTAMAYOR et al. 2002), where many diverse wild populations range from Amazonia to the Guianese region. Most of the traditionally cultivated varieties belong to three major groups: the Criollo, the Forastero and the Trinitario, according to their morphology and geographic and genetic origins. The Criollo have been

---

Received: January 23, 2003. Accepted: April 28, 2003.

Correspondence: O. SOUNIGO, CIRAD BP 5035 34032 Montpellier, France, email: olivier.sounigo@cirad.fr

cultivated for a long time in Central America and in the north of South America. The first cultivated Forastero originated from the Lower Amazonia and were mainly cultivated in Brazil and Venezuela. One particular morphological type, called Amelonado, was introduced to Africa and has provided the material traditionally cultivated in this continent, before being partially replaced by other types of selected material during the last forty years.

During the last sixty years, cocoa trees from the Upper Amazonia were widely used in breeding programmes (POUND 1938) in most of the cocoa producing countries, because they are sources of resistance to different diseases, such as the black pod disease, caused by several *Phytophthora* species (TOXOPEUS 1985, IWARO et al. 2001), the witches' broom disease, caused by *Crinipellis perniciosa* (TOXOPEUS 1985), and the cocoa swollen shoot virus (TOXOPEUS 1969). The Trinitario is a hybrid group originating from crosses between some Criollo and Lower Amazon Forastero genotypes (MOTAMAYOR 2001).

Since the late 1960's, Côte d'Ivoire has conducted a breeding program based on crosses between Upper Amazon Forastero genotypes and Forastero genotypes from the Lower Amazonia (Amelonado), introduced in West Africa or Trinitario varieties, followed by distribution of the seeds resulting from the best crosses to cocoa farmers (BESSE 1977).

Even if some varieties, such as Amelonado, are poorly heterozygous, most of the others, among the Upper Amazon Forastero, and especially among the Trinitario genotypes, are highly heterozygous (LANAUD 1987c, LAURENT et al. 1995). In order to increase the level of homogeneity of the progenies distributed to cocoa farmers, the use of several cycles of selfing progenitors, to increase their homozygosity, was not regarded as a promising method. Indeed, this is an excessively time-consuming method, since between four and five years are required per cycle. Thus, the use of doubled haploids appears as a faster method to obtain homozygous parental genotypes, among which some might have fixed favourable alleles.

As early as 1972, DUBLIN (1972) started a program on spontaneous haploids of *Theobroma cacao* L., and their diploidization (DUBLIN 1978) in Côte d'Ivoire. This program resulted in the production of several dozens of haploids, which were then diploidized, before being planted in collection fields, where they were evaluated (LANAUD 1987a, b) for fertility, fruit and bean characteristics and self-incompatibility. Then, they were crossed among themselves and with several diploid genotypes, the progenies obtained being set up in a first trial, planted in Côte d'Ivoire (LANAUD et al. 1988). Several other such trials were later set up in this country, and are still under investigation.

This first trial, reported in the present work, includes three progenies derived from crosses between two DH parents, and allows us to compare the behaviour of these progenies, in terms of performance and of homogeneity for several useful traits. It also includes progenies issued from crosses between DHs and their parents with a common diploid tester. This allows to compare the DHs with their parents for combining value.

Material and methods

Plant material

DHs tested as progenitors

Haploids were produced from two cocoa genotypes, after large scale sowing, followed by morphological screening (the haploid plants are stunted) and counting of the chromosomes. These genotypes are T85/799 and UPA603, two Upper Amazonian Forastero, selected as good progenitors and used as parents of two progenies distributed to cocoa farmers in Côte d'Ivoire.

Five DHs were from T 85/799: H 943, H 1167, H 985, H 815 and H 967, and five DHs were issued from UPA 603: H 1258, H 492, H 809, H 381 and H 478.

Progenies

– Three of the tested progenies were issued from crosses between two DHs.
– Seventeen of the tested progenies were issued from crosses between IMC 67, used as a tester, and the DHs and their parents. IMC 67 is an Upper Amazonian Forastero, widely used in cocoa breeding.
– Four progenies were issued from crosses between SNK 12 used as a tester, and four DHs and their parent, T 85/799. SNK 12 is a Trinitario selected in Cameroon.
– A progeny derived from the cross UPA 603 * UF 667 was used as a standard in the trial, because this progeny has been widely distributed to cocoa farmers in Côte d'Ivoire. UF 667 is a Trinitario selected in Costa Rica.

More details about the tested progenies are given in Table 1. In the case of progenies marked with "(R)", the reciprocal was also analysed.

Methods

Trial

The progeny trial was conducted on a plot set up in the CNRA (Centre National de Recherche Agronomique) research station at Divo, in the south-east of Côte d'Ivoire.

Traits observed

Yield. The measurements started five years after planting. On each tree, the ripe pods were harvested during five consecutive years, and all the ripe healthy pods of each tree were weighed at each harvest. These pod weights were transformed into fresh bean weight according to the method proposed by LACHENAUD (1984). Cumulative yield was obtained by adding the bean weights obtained at each harvest.

Resistance to black pod disease caused by *Phytophthora palmivora* Butl. – estimated by the contribution of rotten fruits to all the pods harvested during a four consecutive years period, measured in percent. Only the trees on which at least 30 pods were harvested during this period were used to analyse this trait.

Bean characteristics. (1) Percentage of flat beans: percentage of seeds which are empty or have an abnormal embryo with poorly developed cotyledons (DUBLIN 1973). Flat beans are thus unusable for cocoa preparation. This defect is often observed on DHs and is a major drawback, since it results in a reduced cocoa amount and in the necessity of sorting the beans before fermentation. The percentage was evaluated for 200 to 1,000 beans, collected on a number of trees ranging from 3 to 13 per progeny. (2) Mean weight of 100 fermented and dried cocoa beans: this trait is important since small cocoa beans (lighter than one gram) are generally rejected by cocoa buyers.

Vigour, measured by trunk circumference, at a height of 50 cm, and canopy surface, estimated by the product of the diameters of two perpendicular axes, represented by the main branches.

Yield/vigour ratios measured by the yield/trunk circumference ratio, and the yield/canopy surface ratio. These traits indicate how the tree dispatches its resources between vegetative and reproductive development. In addition, the high-yielding trees which show a low vigour are particularly interesting since they result in a lower level of competition with the neighbouring trees and can be adapted to high-density planting (LOTODE, LACHENAUD 1988, LACHENAUD, MONTAGNON 2002) .

Statistical design

Each progeny was represented by 40 trees and planted according to a single tree randomisation design (LOTODE 1971). In this case, each tree is a replication for the "progeny" effect.

Statistical analyses

Statistical analyses were performed using SAS software. Coefficient of variation was evaluated and analyses of variance was performed for observed traits. The Newman-Keuls test was used for comparison of studied progenies.

Coefficient of variation. In the case of the progenies issued from crosses between a DH and IMC 67 or SNK 12, comparisons were made with the progenies issued from crosses between the parents of the DHs and IMC 67 or SNK 12.

In the case of the progenies derived from crosses between two DHs, comparisons were made with all the other tested progenies. Indeed, it was not possible to obtain the most adequate control (T 85/799 * UPA 603 and reciprocal) for these progenies because of the cross-incompatibility between these two clones (LANAUD 1987c).

Variance analyses. Three effects were studied for each trait:
– sense of the cross: comparing the same DHs as female and male progenitors in combination with IMC 67;

– parent of the DH: comparing the value of the DHs from T 85/799 and UPA 603 in combination with IMC 67;
– progeny: comparing all the progenies, to evaluate the progenies issued from crosses between two DHs and to compare the different DHs as progenitors in combination with IMC 67 and SNK 12.

Results

Homogeneity of the different types of progenies

The coefficients of variation (CV) observed for the different types of progenies are indicated in Table 1.

Only one of the three DH * DH progenies (H 1167 * H 492) presents a lower CV value than the other types of progenies for yield and yield/vigour ratios.

Among the progenies issued from crosses between a DH from UPA 603 and IMC 67, the one issued from H 1258 * IMC 67 presents CV values similar to or higher than UPA 603 * IMC 67 for all the traits observed, while the three other progenies present lower CV values for several traits than UPA 603 * IMC 67. Among the progenies issued from crosses between a DH from T 85/799 and IMC 67, the one issued from H 985 * IMC 67 shows a clearly lower CV value than T 85/799 * IMC 67 for the percentage of rotten pods. Among the progenies issued from a cross between a DH and SNK 12, none presents a lower CV value than the one issued from T 85/799 * SNK 12.

Value of the progenies issued from crosses between two doubled haploids

The three DH * DH progenies present different results (Table 2):
(1) H 967 * H 492 shows several disadvantages, such as a low yield, a high % of rotten pods, a high % of flat beans and a low yield/trunk circumference ratio;
(2) H 1258 * H 1167 shows performance similar to that of UPA 603 * UF 667 (progeny distributed to cocoa farmers), except a low survival rate of the trees in the field;
(3) H 1167 * H 492 presents performance similar to that of UPA 603 * UF 667, and even better in the case of the percentage of rotten pods.

The only progeny with a high percentage of flat beans is issued from a cross between two DHs with a high percentage of flat beans, while the other two DH * DH progenies present a low percentage of flat beans.

Analyses of the progenies issued from crosses between DH and IMC 67

Evaluation of reciprocal effects

When comparing values of the progenies issued from DH * IMC 67 and IMC 67 * DH, no significant effect appears for the sense of the cross. This shows that DHs can be used in combinations as female or as male progenitors.

Table 1. Coefficients of variation (%) observed for several traits in cocoa

Progeny	Fresh bean yield	Rotten pods	Mean weight of 100 beans	Trunk circumference	Canopy surface	Yield / trunk circumference	Yield / canopy surface
UPA 603 * UF 667	50	49	20	17	47	44	46
H 1258 * **H 1167**	58	55	11	14	54	46	89
H 1167 * H 492	35	53	6	15	49	31	36
H 967 * H 492	62	55	8	20	51	49	60
UPA 603 * IMC 67	54	51	11	20	45	50	51
H 1258 * IMC 67 (R)	59	46	9	22	54	51	80
H 809 * IMC 67 (R)	43	34	9	14	38	38	42
H381 * IMC 67 (R)	45	44	8	16	28	35	43
H 478 * IMC 67 (R)	43	44	12	17	49	34	72
T 85/799 * IMC 67	65	43	11	24	57	52	55
H 943 * IMC 67 (R)	54	50	10	20	51	41	46
H 1167 * IMC 67 (R)	52	60	8	17	68	44	55
H 985 * IMC 67 (R)	59	33	10	20	53	53	61
H 815 *IMC 67	63	54	11	20	46	45	57
T 85/799 * SNK 12	40	55	8	12	38	37	50
H 815 * SNK 12	47	48	6	20	45	44	49
H 943 * SNK 12	38	58	15	17	35	37	92
H 985 * SNK 12	44	54	10	13	47	39	58

(R) indicates that the reciprocal cross was also analysed, but the data are not shown.
The DHs from UPA 603 are underlined and the ones from T 85/799 are indicated in bold.

Value of the DHs and of their parents in combination with IMC 67

A highly significant "progenitor" effect is observed for all the traits while a highly significant effect of the parents (UPA 603 or T 85/799) of the progenitors is observed for all the traits except the mean weight of 100 fermented and dried beans.

The values of the progenies issued from crosses between eight DHs and their two parents as female progenitors and IMC 67 as male progenitor, are shown in Table 3. The traits observed are: yield, percentage of rotten pods and mean weight of 100 fermented and dried beans.

UPA 603 and its DHs confer a significantly higher yield to their progenies than T 85/799 and all its DHs except H 1258. On the other hand, no significant difference is observed between DHs from the same parent or between any DHs and its parent. In the case of the % of rotten pods, the only significant difference is observed between one DH from UPA 603 (H 381) and two DHs from T 85/799 (H 1167 and H 815).

Table 2. Mean values of the three progenies issued from crosses between two cocoa DHs (DH * DH progenies) and of the control (UPA 603 * UF 667)

Progeny	Survival rate (%)	Fresh bean yield (kg)	Rotten pods (%)	Flat beans (%)			Mean weight of 100 beans (g)	Trunk circum ference (cm)	Canopy Surface (m^2)	Yield / trunk circum ference (kg/cm)	Yield / canopy surface (kg/m^2)
				progeny	female parent	male parent					
H 967 * H 492	90	5.8	21.7	9.9	30.9	17.7	116	32.9	5.1	0.16	1.29
H 1258 * H 1167	57.5	8.6	16.9	3.3	2.7	n.d	105	38.6	6.2	0.22	1.59
H 1167 * H 492	87.5	8.7	13.7	4.3	n.d	17.7	108	33.9	7.7	0.25	1.58
UPA 603 * UF 667	100	10.9	22.1	4.7	0.9	0.75	116	40	9.0	0.28	1.36

Mean values of the DH progenies are underlined when significantly different from the mean value of the control (UPA 603 * UF 667).
The DH from UPA 603 are underlined and the ones from T 85/799 are indicated in bold.
n.d. = not determined

Table 3. Combining value of cocoa DHs. Mean values and grouping according to the Newman and Keuls test at 5% of the progenies with DHs as female parents in combination with IMC 67

Cumulative fresh bean yield (kg)			Rotten pods (%)			Mean weight of 100 cocoa beans (g)		
H 478	13.5	a	H 381	20.8	a	**H 985**	127	a
H 809	13.5	a	H 1258	19.0	ab	H 1258	123	ab
H 381	12.9	a	UPA 603	18.7	ab	**H 943**	121	ab
UPA 603	12.3	a	H 809	18.2	ab	UPA 603	120	ab
H 1258	10.5	ab	**T 85/799**	17.8	ab	H 381	118	ab
H 1167	8.4	bc	**H 985**	16.1	ab	H 809	113	abc
T 85/799	7.7	bc	H 478	16.0	ab	H 1167	112	abc
H815	7.6	bc	**H 943**	14.7	ab	H 478	111	abc
H943	7.3	bc	**H 1167**	14.1	b	**T 85/799**	110	bc
H985	5.5	c	**H 815**	13.6	b	**H 815**	100	c

The DHs from UPA 603 are underlined and the ones from T 85/799 are indicated in bold.
The letters indicate the groups of non significantly different means to which the mean values belong, according to the Newman-Keuls tests at 5%.

Table 4. Percentage of flat beans observed in some of the cocoa DHs and in the progenies with DHs as female parents

Progeny	Flat beans (%)	
	progeny	female parent
H 815 * IMC 67	0.5	n.d
H 809 * IMC 67	0.9	3.4
H 985 * IMC 67	1.2	n.d
H381 * IMC 67	1.3	39.9
H 1258 * IMC 67	1.3	2.7
UPA 603 * IMC 67	1.7	0.9
H 1167 * IMC 67	2.2	n.d
H 478 * IMC 67	2.4	5.8
H 943 * IMC 67	3.4	n.d
T 85/799 * IMC 67	7.4	3.8

The DHs from UPA 603 are underlined and the ones from T 85/799 are indicated in bold.
n.d = not determined

In the case of the mean weight of 100 fermented and dried beans, significant differences are observed between H 815, originating from T 85/799, and DHs from UPA 603, such as H 1258 and H 381. Also, significant differences are observed among DHs from T 85/799, and, more importantly, between H 985 and its parent, T 85/799. Indeed, H 985 confers a significantly higher mean weight of 100 fermented and dried beans to its progeny than its parent does.

The progenies with one DH as female parent do not show high percentages of flat beans (Table 4). The progeny T 85/799 * IMC 67 shows a higher percentage of flat beans than the four progenies with a DH originating from this clone as female parent. Also, UPA 603 * IMC 67 shows a higher percentage of flat beans than three of the progenies with a DH originating from this clone as a female parent. Even the progeny derived from the cross with H 381, the clone which produces a high % of flat beans (39.9 %), produces a low % of flat beans (1.3%).

When tested as progenitor, UPA 603 confers a significantly higher trunk circumference and canopy surface than T 85/799. Similarly, the DHs from UPA 603 generally confer a higher value to their progenies for these traits than the DHs from T 85/799 do, and several significant differences are found between the DHs from different parents. No significant difference is observed between DHs from the same parent for canopy surface. On the other hand, for trunk circumference, H 985, issued from T 85/799, shows a significantly lower combining value than those of the three other DHs from the same parent. For the two traits, no significant difference is observed between any DH and its parent (Table 5).

UPA 603 also confers a significantly higher yield/trunk circumference ratio to its progeny than T 85/799 does. Similarly, three of the DHs from UPA 603 confer a significantly higher value to their progenies for this trait than T 85/799 and its four DHs. Significant differences are observed between DHs from UPA 603 for this trait. No DH shows a significantly different combining value from its parent for this trait.

In the case of the yield/canopy surface ratio, the only significant difference observed is between H 478 and all the other progenitors, including its parent (UPA 603). Indeed, this DH confers the highest value to its progeny for this trait.

Value of the DHs and of their parents in combination with SNK 12

Significant differences between the tested female progenitors were observed only for three traits: mean weight of 100 fermented and dried beans, trunk circumference and canopy surface (Table 6).

H 815 confers a significantly lower mean weight of 100 fermented and dried beans than the other two tested DHs and its parent (T85/799).

H 985 confers a lower vigour to its progenies than the other DHs (significant difference for both trunk circumference and canopy surface) and than its parent (T 85/799) (significant difference only for trunk circumference).

Table 5. Combining value of cocoa DHs. Mean values and grouping according to the Newman and Keuls test at 5% of the DHs as female parents in combination with IMC 67

Trunk circumference (cm)			Canopy surface (m^2)			Yield / trunk circumference (kg / cm)			Yield / canopy surface (kg / m^2)		
H 381	44.9	a	H 381	9.5	a	H 478	0.34	a	H 478	2.07	a
H 809	43.1	ab	H 809	9.0	a	H 809	0.30	a	**H 1167**	1.59	b
UPA 603	43.1	ab	UPA 603	8.7	a	UPA 603	0.28	ab	H 809	1.54	b
H 1258	42.9	ab	H1258	8.4	ab	H 381	0.28	ab	H 1258	1.50	b
H 815	40.4	abc	H 478	8.2	ab	H 1258	0.23	bc	UPA 603	1.44	b
H 478	39.8	abc	**H 943**	6.3	bc	**H 1167**	0.21	cd	**T 85/799**	1.40	b
H 943	39.2	bc	**H 1167**	6.2	bc	**T 85/799**	0.19	cd	H 381	1.40	b
H 1167	37.6	c	**H 815**	6.2	bc	**H 815**	0.17	cd	**H 985**	1.26	b
T 85/799	35.8	cd	**T 85/799**	5.7	c	**H 943**	0.17	cd	**H 815**	1.26	b
H 985	32.3	d	**H 985**	4.7	c	**H 985**	0.15	d	H 943	1.19	b

The DHs from UPA 603 are underlined and the ones from T 85/799 are indicated in bold.
The letters indicate the groups of non significantly different means to which the mean values belong, according to the Newman-Keuls tests at 5%.

Table 6. Combining value of cocoa DHs. Mean values and grouping according to the Newman and Keuls test at 5% of progenies derived from T 85/799 and some of its DHs as female parents in combination with SNK 12

Mean weight of 100 fermented and dried beans			Trunk circumference (cm)			Canopy surface (m^2)		
H 943	1.57	a	H 943	45.8	a	H 815	12.1	a
H 985	1.49	a	H 815	44.4	a	H 943	11.6	a
T 85/799	1.49	a	T 85/799	43.1	a	T 85/799	9.9	ab
H 815	1.22	b	H 985	39.6	b	H 985	8.5	b

The letters indicate the groups of non significantly different means to which the mean values belong, according to the Newman-Keuls tests at 5%.

Discussion

The purpose of this trial was to evaluate the DHs as parents, when crossed with other DHs and with naturally diploid genotypes.

Out of the three DH * DH tested progenies, two presented some disadvantages, while the third one was acceptable for all the traits observed and even significantly better than the control (progeny distributed to cocoa farmers) for the resistance to the black pod disease. In addition, this progeny showed a lower coefficient of variation than the other progenies for some of the traits observed, such as yield, mean weight of 100 beans and yield/vigour ratios. On the other hand, such a reduction in the coefficient of variation failed to be consistently shown by the three DH * DH progenies, for all the traits, as could have been expected. SUGIMOTO (2002) also found that radish progenies derived from crosses between two DHs could perform as well as some commercial varieties but failed to show a consistently higher homogeneity.

One hypothesis for explaining this lack of consistent homogeneity improvement in our case is that the two parents of the DHs are not highly heterozygous at the level of the loci involved in the observed traits. It would be interesting to observe progenies derived from crosses between two DHs derived from Trinitario genotypes. Indeed, the Trinitario have been shown to be highly heterozygous when analysed by isozyme electrophoresis (LANAUD 1987c) or RFLP (LAURENT et al. 1995).

When compared to their parents for their combining value with IMC 67, the DHs generally did not transmit high percentage of flat beans to their progenies. Similarly, LASHERMES et al. (1994a, b) observed fertility problems in DHs from *Coffea canephora* P., but these problems were not transmitted to their progenies.

The combining values of the DHs were in agreement with those of their parents, for all the traits analysed, except for the mean weight of 100 fermented and dried beans. LASHERMES et al. (1994b) found the same trend on *Coffea* *canephora* P. This result strongly suggests that the search for DHs should be restricted to the clones with a high combining value for useful traits.

Despite this trend, significant differences could be observed between the combining values of DHs originating from the same parent for some of the traits observed, showing that DHs can be a tool to create diversity from selected genotypes. Similarly, a high level of variation was observed for both performance and combining ability of DHs from the same parent, at the level of several useful traits on *Coffea canephora* P. (LASHERMES et al. 1994a, b). Similarly, MENTEWAB and SARRAFI (1998) found a high level of variability for the performance at the level of several traits among DHs issued from F_1 obtained from a cross between two slightly different spring wheat parents.

In addition, for two traits (mean weight of 100 fermented and dried beans and yield/canopy surface ratio), it was possible to observe DHs with a significantly higher combining value than their origin. Due to the very low number of DHs tested per parent (only four), this result is very encouraging, since it indicates the possibility of significantly improving selected genotypes for their combining value in only one cycle of selection, by fixing favourable alleles. LASHERMES et al. (1994b) also found it possible to select DHs from *Coffea canephora* P. with a better combining value than their parents. On the other hand, when evaluating the combining values of several wheat DH lines, CSEUZ et al. (1990) found that they were similar or significantly lower than those of their parents. When evaluating DH lines for their performance, several authors could identify some DH lines showing better performance than their parents for traits like yield (KASHA et al. 1977, SUENAGA 1994), mean weight of 1000 seeds (BA BONG, SWAMINATHAN 1995), resistance to diseases (FICCADENTI et al. 2002) and oil quality (BURBULIS et al. 2001).

Another information from this trial was the absence of reciprocal effect, in the case of the crosses between DHs and IMC 67. Indeed, for the large-scale distribution to cocoa farmers of a progeny issued from a cross between a DH parent and a diploid parent, it is more advantageous to use the DH as the male parent. Indeed, because of their generally low vigour, DHs would be more easily used as pollen donors than as fruit bearers.

Anyhow, the results obtained here must be confirmed with larger numbers of DHs. Such a confirmation should be possible in a few years, when enough data from other trials planted in Côte d'Ivoire are available.

Two major limitations of our study are the limited sources of haploids and the low numbers of haploids available for each source. These limitations are due to the scarcity of occurrence of haploids in natural conditions. Attempts were made in order to increase the efficiency of haploid production, but neither the anther and ovary tissue culture nor the use of irradiated pollen (FALQUE 1994), were successful. If the potentials of DHs as progenitors are confirmed by the results of the trials that will be analysed in the near future, important efforts will need to be made in order to improve the production of haploids.

Conclusion

The results obtained in our study indicate that it is possible to create promising progenies by crossing two DHs, assuming that the two DHs used as parents do not produce fruits with a high percentage of flat beans. But obtaining such progenies does not necessarily result in a consistent increase in homogeneity, as compared to progenies issued from crosses between two diploid parents. On the other hand, our results indicate that obtaining DHs from promising progenitors seems to be an efficient way to improve them in a single cycle of selfing, by fixing some favourable alleles. Anyhow, these results remain to be confirmed by the trials established more recently.

REFERENCES

BA BONG B., SWAMINATHAN M.S. (1995). Magnitude of hybrid vigor retained in doubled haploid lines of some heterotic rice hybrids. Theor. Appl. Genet. 90: 253-257.

BESSE J. (1977). La sélection générative du cacaoyer en Côte d'Ivoire, bilan et orientation des recherches en 1975. 5ème Conférence internationale sur les recherches cacaoyčres. IBADAN. 1-9. Sept. 1975: 95-103.

BURBULIS N., MALINAUSKAITE R., KOTT L. (2001). Oil quality improvement through *in vitro* mutagenesis and haploid selection in Lithuanian winter *Brassica napus* germplasm. Proc. Latvian Academy of Sciences. Section B, Natural, Exact and Applied Sciences. 55, 5/6: 197-200.

CHEESMAN E.E. (1944). Notes on the nomenclature: classification possible and relations of cocoa populations. Tropical Agriculture. 21: 144-159.

COPE F.W. (1984). Cacao *Theobroma cacao* (Sterculiaceae). In: Evolution of crop plants. Longman, London: 285-289.

CSEUZ L., KERTESZ Z., PAUK J. (1990). Combining ability of doubled haploid wheat lines. Cereal Res. Commun. 18(1-2): 45-50.

CUATRECASAS J. (1964). Cacao and its allies: a taxonomic revision of the genus *Theobroma*. Bulletin of the United States National Museum. Smithsonian Institution (Washington), 35: 379-614.

DUBLIN P. (1972). Polyembryonie et haploďdie chez *Theobroma cacao*. Café. Cacao. Thé. 16(4): 295-311.

DUBLIN P. (1973). Les "fèves plates": une nouvelle source d'haploďdie chez le cacaoyer (*Theobroma cacao*) . Café. Cacao. Thé. 17(1): 25-36.

DUBLIN P. (1978). Haploïdes diploïdisés et obtention de genotypes homozygotes fertiles chez les cacaoyers cultivés (*Theobroma cacao*). Café. Cacao. Thé. 22(4): 275-284.

FALQUE M. (1994). Fécondation et développement des fruits et des graines chez le cacaoyer *Theobroma cacao* L.PhD thesis. INP. Toulouse. France.

FICCADENTI N., SESTILI S., ANNIBALI S., CAMPANELLI G., BELISARIO A., MACCARONI M., CORAZZA L. (2002). Resistance to *Fusarium oxysporum* f. sp. *melonis* race 1, 2 in muskmelon lines Nad-1 and Nad-2. Plant Diseases 86(8) : 897-900.

KASHA K.J., SONG L.S.P., PARK S.J., REINBERGS E. (1977). Fixation of heterosis: comparison of F_1 hybrids with their respective homozygous lines developing using doubled haploid procedures. Cereal Res. Commun. 5(3): 205-214.

IWARO A.D., SINGH V., BARATH S., JUGMOHAN N. (2001). Germplasm evaluation at the International Cocoa Genebank. Trinidad for resistance to *Phytophthora* pod rot. Annual Report 2000 of the Cocoa Research Unit. The University of the West Indies, Trinidad: 34-40.

LACHENAUD P. (1984). Une méthode d'évaluation de la production de fèves fraîches applicable aux essais entièrement « randomisés ». Café. Cacao. Thé. 28(2): 83-88.

LACHENAUD P., MONTAGNON C. (2002). Competition effects in cocoa (*Theobroma cacao* L.) hybrid trials. Euphytica 128: 97-104.

LANAUD C. (1987a). Doubled haploids of cocoa (*Theobroma cacao* L.). I. Observations of fertility. Plant Breeding 99(3): 187-195.

LANAUD C. (1987b). Doubled haploids of cocoa (*Theobroma cacao* L.). II. Observation of monogenic and polygenic characters. Plant Breeding 99(3): 196-202.

LANAUD C. (1987c). Nouvelles données sur la biologie du cacaoyer (*Theobroma cacao* L.): diversités de populations. systèmes d'incompatibilité. haploïdes spontanés; leurs conséquences pour l'amélioration génétique de cette espèce. PhD Thesis. Université Paris XI. Orsay, France.

LANAUD C., LACHENAUD P., SOUNIGO O. (1988). Behavior in the growth of of doubled haploids of cacao plants (*Theobroma cacao*). Can. J. Bot. 66(10): 1986-1992.

LASHERMES P., COUTURON E., CHARRIER A. (1994a). Doubled haploids of *Coffea canephora*: development, fertility and agronomic characteristics. Euphytica 74: 149-157.

LASHERMES P., COUTURON E., CHARRIER A. (1994b). Combining ability of doubled haploids in *Coffea canephora* P. Plant Breeding 112: 330-337.

LAURENT V., N'GORAN J.A.K., ESKES A.B., LANAUD C. (1995). Estimation of the level of heterozygosity and genetic diversity with RFLP markers. Ingenic Newsletter 1: 5-7.

LOTODÉ R. (1971). Possibilités d'amélioration de l'expérimentation sur cacaoyers. Café. Cacao. Thé. 15(2): 91-104.

LOTODÉ R., LACHENAUD P. (1988). Méthodologie destinée aux essais de selection du cacaoyer. Café. Cacao. Thé. 32(4): 275-292.

MENTEWAB A., SARRAFI A. (1998). Performance of androgenic doubled haploid spring wheat lines for excised-leaf water status and agronomic traits in comparison with their parents. Cereal Res Commun. 26(2): 137-143.

MOTAMAYOR J.C. (2001). Etude de la diversité génétique et de la domestication des cacaoyers du groupe Criollo (*Theobroma cacao* L.) à l'aide de marqueurs moléculaires. PhD thesis. Université Paris XI.

MOTAMAYOR J.C., RISTERUCCI A.M., LOPEZ P.A., ORTIZ C.F., MORENO A., LANAUD C. (2002). Cacao domestication I: The origin of the cacao cultivated by the Mayas. Heredity 89: 380-386.

POUND F.J. (1938). Cacao and witchbroom disease (*Marasmius perniciosus*) of South America with notes on other species of Theobroma. Yuille's printery. Trinidad. (reprint in « Archives of Cocoa Research ». 1. 1982. 9-49).

SUENAGA K. (1994). Doubled haploid system using the intergeneric crosses between wheat (*Triticum aestivum*) and maize (*Zea mays*). Bull. Natl. Inst. Agrobiol. Resour. 9: 83-139.

SUGIMOTO K. (2002). F1 hybrid production using doubled haploid of microspore-derived ‘Moriguchi’ radish (*Raphanus sativus* L.). J. Japan. Soc. Hort. Sci. 71(2): 187-191.

TOXOPEUS H. (1969). Cacao. *Theobroma cacao* L. In: Outlines of perennial crop breeding in the tropics (F.P. Ferwerda, F. Wit, eds). Veenman and Zonen, Wageningen, Netherlands: 79-109.

TOXOPEUS H. (1985). Planting material. In: Cocoa (4th edn) (G.A.R. Wood, R.A. Lass, eds). Longman London U.K.: 80-92.