

Full Length Research Paper

Assessment of cocoa trees (*Theobroma cacao* L.) for their productivity and tolerance to cocoa swollen shoot virus disease in Côte d'Ivoire

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In Côte d'Ivoire, cocoa trees were submitted to several production constraints, including cocoa swollen shoot disease (CSSD). The aim of this work is to select cocoa trees with good agronomic traits and CSSD tolerance. Ten families of cocoa trees have been evaluated with 7 descriptive parameters such as the number and weight of total fresh beans and also the weight of dry beans. These families were also evaluated against CSSD. Results showed that families F8, F6 and F5 have the best for average number of normal fresh beans per pod, with 43.97, 42.64, and 42.28 beans, respectively. F6 (42.64 beans) and F5 (42.28 beans) also had the best average fresh bean weights, with 138.39 and 122.89 g, respectively. F9, F6 and F10 have been characterized by the highest value of merchantable beans, with respectively 1.30, 1.20 and 1.18 g per dry bean. F6, F4 and F8 showed the best tolerance to CSSD in the greenhouse, with 0% infection rate and good vegetative growth. The agronomical performances of these hybrids must be confirmed in multilocal trials in infected areas before their introduction into seed gardens. This result constituted an indicator for selecting cocoa trees for CSSD-infected areas.

Key words: Assessment, *Theobroma cacao*, cocoa swollen shoot disease (CSSD)-tolerance, Côte d'Ivoire.

INTRODUCTION

Cacao tree is a perennial plant from the humid tropics of Central and South America, belonging to the Malvaceae family and the *Theobroma* genus (Alverson et al., 1999). *Theobroma cacao* L., is cultivated for its beans, which are also used in the manufacture of chocolate, cosmetics and pharmaceutical products (Braudeau, 1969).

Côte d'Ivoire is the world leading producer, with an annual output of around 2,000,000 tonnes of

merchantable cocoa (Statistica, 2014; ICCO, 2014), around 35% of global supply. Cocoa farming plays an important role in the Ivorian economy. Indeed, Ivorian production contributes 15% of Gross Domestic Product (GDP). Despite this remarkable performance, cocoa farming has many production constraints. These include failure to follow adapted technical aging orchards and low levels of use of improved planting material (Koua et al.,

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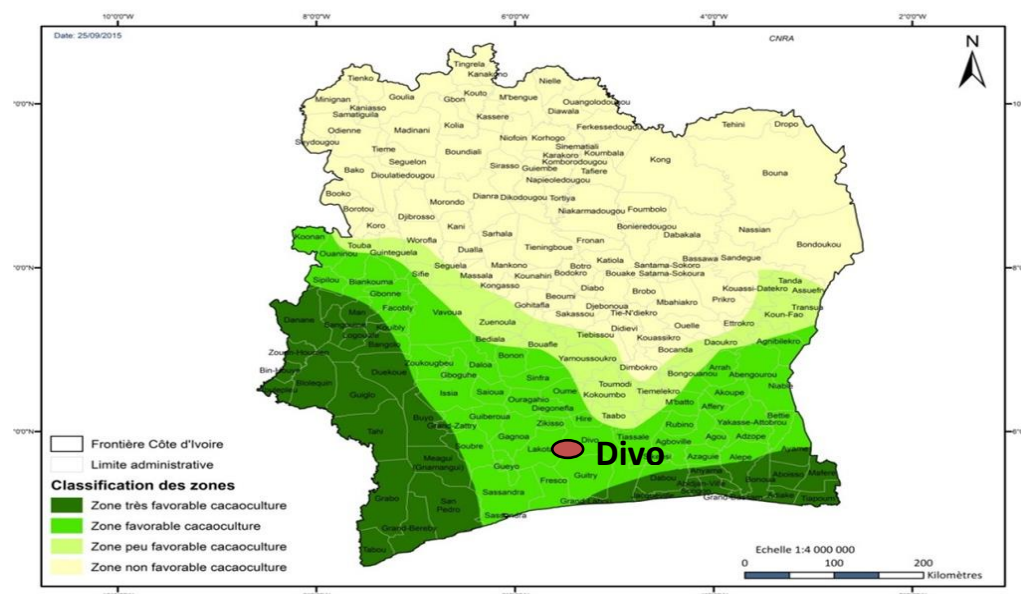


Figure 1. Map of Côte d'Ivoire presenting study site.

2018; Tahi et al., 2017). There are also high pressure from insect pests (N'Guessan and Coulibaly, 2000), black pod rot and swollen shoot diseases.

Indeed, since its resurgence in 2003, the swollen shoot disease has caused considerable production losses and the destruction of orchards in several of the country's production zones (Kébé, 2006; Kouakou, 2014). Chemical, agronomic and biological control methods have proved insufficient to control this disease.

Ivorian agronomic research through the National Agronomic Research Center (CNRA) decided to find solutions for stop this disease. These solutions inevitably involve indexing, identification (Muller and Sackey, 2005) and selection of CSSD-tolerant cocoa varieties (Adu-Ampomah et al., 1999; Guiraud, 2019). The aim is to supply farmers with varieties that combine productivity, resistance and tolerance to CSSVD, and are adapted to new production environments.

CNRA has a genetic improvement program for selecting cocoa trees with good agronomics traits. The effectiveness of this program is based on the existence, on the Divo research station, of a collection of clones used as parents to create high-performance hybrids. However, the agronomic and CSSD susceptibility characteristics of some hybrid families are still poorly known. It is therefore necessary to evaluate hybrid families currently under selection, in order to guide breeders' choices for replanting and rehabilitating destroyed orchards. The objective of this work is to identify families of cocoa hybrids with good agronomic productivity and resistance to CSSD. Specifically, the aim is to determine the agro-morphological performances of 10 hybrid families and their susceptibility to CSSD in greenhouse.

MATERIALS AND METHODS

Study site

The study was carried out at the Divo research station (Figure 1). It is forest area favorable to cocoa production (Kassin et al., 2012). This zone has two rainy and two dry seasons (Roose and Jardin, 1969). The long rainy season extends from April to July, and the short rainy season from October to November. The long dry season is December to March and the short dry season from August to September (Montagnon, 2000). The soils are deep, dark brown, clayey-sandy or humus-bearing, depending on the type of relief. They have low potassium content and an acid pH of between 5.2 and 6 (Montagnon, 2000).

Plant

Plant material is composed of 10 families of cocoa hybrids resulting from manual crosses between parents installed at the Divo station, within the CNRA's main cocoa collection. The list of families and their genealogies are shown in Table 1. The ten 'hybrids have been also evaluated in greenhouse by inoculation with CSSD-Badnavirus isolates.

Entomological and viral material

Entomological material used for this work consists of scale insect (mealybug) larvae of the *Planococcus njalensis* species, which is main vector of swollen shoot. The viral strain used for greenhouse inoculations is strain CSSTBV, identified as the most aggressive of all known strains in Côte d'Ivoire (Kouakou, 2014).

Agromorphologic assessment of cocoa trees in field

Ten hybrids family were characterized in field for agromorphological traits in field. This cacao trees were been evaluated for their tolerance to CSSD in a greenhouse.

Table 1. List of plant material used for cocoa evaluation.

Num	Crossing	Genealogy	Evaluation
F1	PA 150 × GU 284 A	HA × TRINITARIO	Field and greenhouse
F2	PA 150 × GU 175 A	HA × AMELONADO	Field and greenhouse
F3	PA 150 × T79/501	HA × TRINITARIO	Field and greenhouse
F4	PA 150 × T60/887	HA × CRIOLLO	Field and greenhouse
F5	PA 150 × IFC 5	HA × AMELONADO	Field and greenhouse
F6	P 7 × PA 150	HA × CRIOLLO	Field and greenhouse
F7	P 7 × GU 284 A	HA × AMELONADO	Field and greenhouse
F8	P 7 × T60/887	HA × AMELONADO	Field and greenhouse
F9	P 7 × IFC 1	HA × AMELONADO	Field and greenhouse
F10	T 79/501 × IFC 1M	HA × CRIOLLO	Field and greenhouse

*HA: Upper Amazonian.

A16	B	B	B	B	B	B	B
A15	B	F2	F4	F6	F7	F9	B
A14	B	F2	F4	F6	F7	F9	B
A13	B	F2	F4	F6	F7	F9	B
A12	B	F2	F4	F6	F7	F9	B
A11	B	F2	F4	F6	F7	F9	B
A10	B	F2	F4	F6	F7	F9	B
A9	B	F2	F4	F6	F7	F9	B
A8	B	F1	F3	F5	F8	F10	B
A7	B	F1	F3	F5	F8	F10	B
A6	B	F1	F3	F5	F8	F10	B
A5	B	F1	F3	F5	F8	F10	B
A4	B	F1	F3	F5	F8	F10	B
A3	B	F1	F3	F5	F8	F10	B
A2	B	F1	F3	F5	F8	F10	B
A1	B	B	B	B	B	B	B

A16	B	B	B	B	B	B	B
A15	B	F2	F4	F6	F7	F9	B
A14	B	F2	F4	F6	F7	F9	B
A13	B	F2	F4	F6	F7	F9	B
A12	B	F2	F4	F6	F7	F9	B
A11	B	F2	F4	F6	F7	F9	B
A10	B	F2	F4	F6	F7	F9	B
A9	B	F2	F4	F6	F7	F9	B
A8	B	F1	F3	F5	F8	F10	B
A7	B	F1	F3	F5	F8	F10	B
A6	B	F1	F3	F5	F8	F10	B
A5	B	F1	F3	F5	F8	F10	B
A4	B	F1	F3	F5	F8	F10	B
A3	B	F1	F3	F5	F8	F10	B
A2	B	F1	F3	F5	F8	F10	B
A1	B	B	B	B	B	B	B

Figure 2. Experimental design for agromorphological assessment of 10 hybrids families of cocoa trees.

Experimental design in field

The experimental design for agromorphological assessment of 10 hybrids families was a complete randomized block with two sub-blocks. Each sub-block corresponds to a replication and is made up of seven lines, with 2 families per line and 7 trees per family. The trial was set up on an area of 0.17 ha for 2 blocks. A total of 112 plants per block and 224 cocoa trees, were planted on two blocks with 3 m between lines and 2.5 m between trees. This plot is installed at June 2018 on CNRA research station at Divo (Figure 2).

Data collected in field

20 healthy pods were collected from each cocoa tree. These pods were labelled and transported to laboratory. Six descriptors were used to assess agromorphological performance of cacao trees. These were:

i) Volume of harvested pods per tree (VOLCab): Using the Walters

and Hunter (1928) formula, the average volume of pods for each family is calculated as follows:

$V = L (\pi \cdot D)^2 / 22$, where L = length of pod; D = diameter of pod; v= volume of pod.

ii) Cortex thickness (CE): This was measured using a caliper, after opening a pod.

iii) Number of normal fresh beans (NFFN) per pod: This parameter was determined on a sample of 20 pods per tree. Each pod was opened and the normal beans counted.

iv) Number of fresh flat beans (NFFP) per pod: This is the number of empty or embryo-free beans per pod. It was determined on a total of 20 healthy pods.

v) Normal fresh bean weight (PFFN) per pod: The weight of fresh beans per pod was determined on the same sample of 20 pods per tree by weighing the fresh beans from each pod using a precision 0.01g electric balance.

vi) Weight of a merchantable cocoa bean (P1FEVM): Preparation of merchantable cocoa beans was necessary before determining this parameter. A sample of fresh beans from healthy, ripe pods from



Figure 3. Mealybug collection and mealybug trapping device for virus acquisition on a cocoa tree infected with CSSTBV strain.

each family was used and placed in carefully labelled openwork cloths before being fermented in specially designed tanks. Fermentation lasted six days. The beans were turned every two days. The fermentation process was stopped on day 7, and the fermented beans from each sample representing each family were placed in the sun on drying racks. This last stage took around seven to eight days, depending on the degree of sunlight. After drying, the dried beans were transported to the laboratory, where a sample of 100 merchantable cocoa beans per family was individually weighed using a precision electric balance.

Cocoa swollen shoot assessment in greenhouse

Collecting mealybugs

The mealybugs used as vectors for bean inoculation were collected at the Divo Research Station, in cocoa groves free of CSSD (Figure 3). Mealybug colonies were collected with brushes directly from the pods the day before inoculation, and placed in petri dishes.

Virus acquisition

After transfer to the Bouaflé greenhouse, the mealybugs were removed from the pods and fasted for 24 h to increased hunger. The mealybugs were then transferred to a trapping device (Figure 3) on the flushes of trees identified as carrying the CSSTBV strain. Seventy-two hours were required for virus acquisition and the production of viral nymphs.

Preparation of beans for inoculation

Ten beans per family were selected for evaluation. To prepare them, the beans were cleaned of their mucilaginous pulp by rubbing with sawdust. The shell and translucent film covering the cotyledon were then removed, exposing the interstices of the beans.

Beans inoculation

Ten virus-inoculated nymphs were deposited in the interstices of each bean per family. The inoculated beans were then stored in tea glasses for 24 h to transfer the virus to the beans.

Experimental design in greenhouse

The experimental set-up used to evaluate 10 hybrid families for their susceptibility to CSSD was also a complete randomized block

made up of five bins, each representing a replication. Each tray comprises three lines of 10 plants. Each line is made up of two families, each represented by five contiguous plants, giving a total of 6 families. Each family is represented in the trial by 3×5 randomly distributed plants (Figure 4).

Data collect in greenhouse

This work involved collecting data in the greenhouse on each of the inoculated plants. The following parameters were collected.

Number of germinated plants: This parameter was determined by the number of plants germinated per family. It was used to determine the germination rate and mortality rate per family.

Number of infected plants: The number of infected plants in each family was counted to determine the infection rate per family assessed.

Latency time: This is the number of days it takes for the first symptom to appear in each family.

Plant length: Plant length was measured with a tape measure three months after sowing;

Plant diameter: Diameter was measured with a caliper three months after sowing;

Number of leaves per family: This is the number of leaves that appeared on the plants three months after sowing.

Statistical analysis

Data were analyzed using SAS 9.4 software. Hybrid families were compared by analysis of variance (ANOVA) with a classification criterion, with a threshold of $P = 0.05$, and by post ANOVA analysis. ANOVA significant at the 0.05 threshold was followed by the Student-Newman-Keuls (SNK) test to classify hybrid families into homogeneous groups for the trait studied.

RESULTS

Comparison of cocoa trees for pods agromorphological traits

Table 2 compares 10 cocoa families according for agromorphological traits. Variance analysis at the 5%

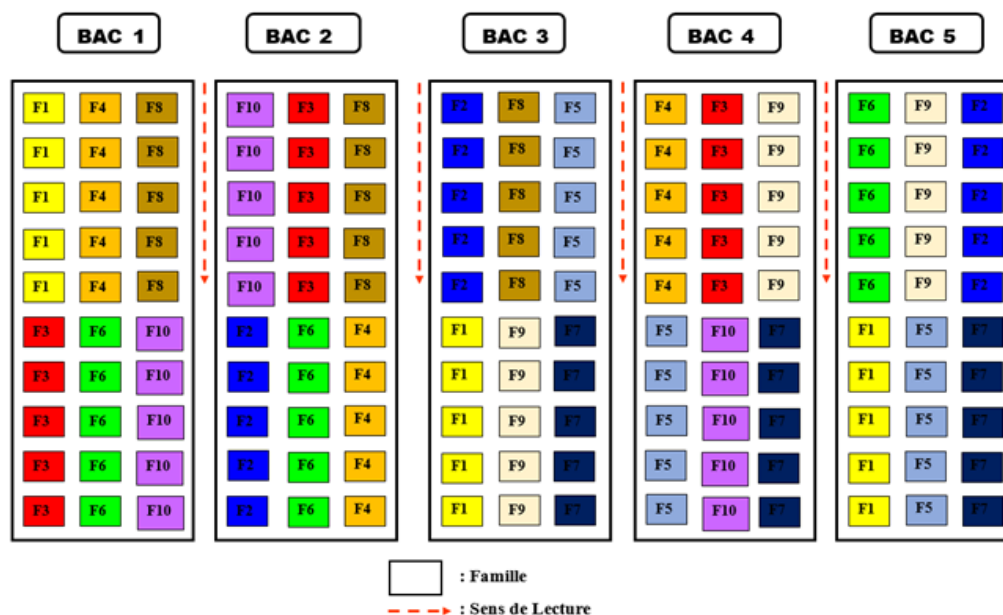


Figure 4. Experimental design used for CSSD-Badnavirus inoculation in greenhouse.

Table 2. Comparison of hybrid families for agromorphological parameters related to pods.

Family	VOLCAB (cm ³)	CE (cm)
F6	738.09±232.26 ^a	1.76±0.40 ^a
F1	617.88±202.04 ^{ab}	1.51±0.29 ^{abc}
F4	581.06±133.26 ^{abc}	1.50±0.26 ^{abc}
F2	580.63±151.44 ^{abc}	1.48±0.24 ^{bc}
F7	557.50±224.34 ^{bc}	1.62±0.47 ^{ab}
F5	546.51±168.56 ^{bc}	1.42±0.18 ^{bc}
F8	512.82±135.86 ^{cd}	1.46±0.26 ^{bc}
F9	508.81±176.15 ^{cd}	1.44±0.34 ^{bc}
F10	472.95±133.58 ^{de}	1.41±0.23 ^{bc}
F3	438.00±123.15 ^e	1.22±0.19 ^c
<i>P</i>	<0.0001	<0.0001

P: Probability; CE: cortex thickness.

threshold, revealed a highly significant family effect ($P < 0.0001$) for all traits studied. The ranking of hybrid families for average pod volume showed that averages ranged from 438.00 (F3) to 738.09 cm³ (F6). Families F6 (P7 × PA150), F1 (PA 150 × GU 284 A) F4 (PA 150 × T60/887) had the largest pods with values of 738.09 cm³, 617.88 cm³ and 581.06 cm³, respectively. Concerning average thickness of the cortex of pod (CE), the results showed that the averages for the families studied ranged from 1.76 (F6) to 1.22 cm (F3), with an overall average of 1.50 cm. The thickest families for this parameter were F6, F1 and F4 with respective averages of 1.76, 1.51 and 1.50 cm.

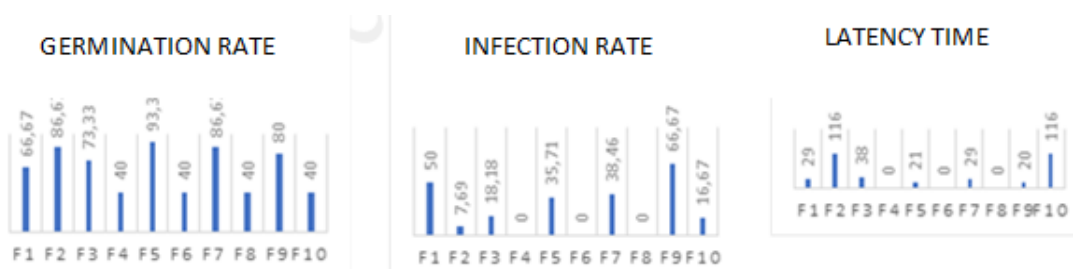
Comparison of cocoa trees for beans agromorphological traits

Table 3 compares 10 families of cocoa hybrids following 4 agromorphological traits related to beans: average number of fresh normal beans (NFFN), average number of fresh flat beans (NFFP), average weight of fresh normal beans in a pod (PFFN) and average weight of a usable bean (P1FevM). The results of the analysis of variance performed on each trait at the 5% level revealed a highly significant family effect ($P < 0.0001$) on all four traits studied. The average number of normal fresh beans per pod (NFFN) ranged from 43.97 to 37.87 beans.

Table 3. Comparison of families for agro-morphological parameters related to beans.

Family	NFFN	PFFN (g)	P1FevM (g)
F6	42.64±9.48 ^{ab}	138.39±45.37 ^a	1.20±0.21 ^{ab}
F1	40.59±8.71 ^{bcd}	116.04±39.96 ^{de}	1.14±0.24 ^{bc}
F7	38.82±8.55 ^{cd}	116.28±31.92 ^{de}	1.12±0.26 ^{bc}
F4	37.88±7.83 ^d	106.12±28.32 ^{de}	1.15±0.22 ^{bc}
F9	40.76±8.03 ^{bcd}	120.63±40.61 ^{cd}	1.30±0.32 ^a
F5	42.28±5.61 ^{ab}	122.89±39.88 ^{bc}	1.14±0.28 ^{bc}
F8	43.97±7.95 ^a	116.81±31.75 ^{de}	1.14±0.16 ^{bc}
F2	38.17±7.95 ^{cd}	104.47±29.02 ^{ef}	1.03±0.18 ^c
F10	41.14±6.45 ^{bc}	107.31±26.57 ^{ef}	1.18±0.20 ^{ab}
F3	37.87±7.56 ^d	82.00±24.56 ^f	0.96±0.21 ^c
P	<0.0001	<0.0001	<0.0001

P: Probability.

**Figure 5.** Germination rate, infection rate and latency time of hybrid families after CSSTBV inoculation in greenhouse.

Family F8 (P 7 × T60/887), F6 (P 7 × PA 150) and F5 (PA 150 × IFC 5) showed the best values respectively with 43.97, 42.64 and 42.28. With regard to the average weight of normal fresh beans per pod, the results show that the average values of the families varied from 138.39 to 82.00 g. Families F6 and F5 had the heaviest fresh beans per pod with 138.39 and 122.89 g respectively. Finally, the average weight of a dry bean varied from 1.30 to 0.96 g. F9, F6 and F10 were characterized by the highest value of merchantable beans, with respectively 1.30, 1.20 and 1.18 g per bean.

Comparison of 10 cocoa trees for susceptibility to CSSD

Results concerning the behavior of hybrid families after inoculation in the greenhouse are shown in Figure 5. Analysis of the histograms shows that the germination rate of the 10 families varied from 93.33 to 40%, with an overall average of 64.67%. Families F5, F2, F7 and F9 had an average germination rate of over 80%. Two families (F3 and F1) had germination rates between 80 and 50%, and four families (F4, F6, F8 and F10) had

germination rates below 50%. This percentage has been determined three weeks after sowing. Infection rates for the ten families ranged from 66.67 to 0%, with an overall average of 23.34%. Three families, F4, F6 and F8, with 0% infection rate, are free of swollen shoot symptoms. Families F2, F10 and F3 have less than 20% infected plants. F5, F7 and F1 have infection rates of between 20 and 50%. Family F9 has an infection rate of over 50%.

The latency period ranged from 20 to 116 days, with an overall average of 53 days. Families F9 and F5 had the shortest latency, with 20 and 21 days of virus incubation. Families F7, F1 and F3 have a latency period of between 29 and 38 days. Families F2 and F10 have a latency time of over 90 days (3 months). The results relating to the growth and development of plants inoculated in the greenhouse are shown in Table 4. Analysis of the table shows a significant difference between the number of leaves, plant diameter and plant length for all families evaluated six months after inoculation ($P < 0.05$).

The F1 family had the most leaves per plant, with an average of 14.22 leaves per plant. Families F5, F9, F4, F7, F6, F2, F8 and F3 had an intermediate number of leaves, ranging from 13 to 10.3 leaves per plant. The collar diameter of family ranged from 5.89 to 3.17 cm,

Table 4. Average values for the number of leaves, plant diameter and plant length of the families evaluated

Family	Number of leaves	Plant Diameter (cm)	Plant length (cm)
F1	14.22 ^a	5.89 ^a	27.56 ^a
F5	13 ^{ab}	4.93 ^{ab}	23.29 ^{abcd}
F9	12.75 ^{abc}	5.83 ^a	26.9 ^{ab}
F4	12.33 ^{abcd}	3 ^d	15.33 ^e
F7	12.15 ^{abcd}	5.54 ^{ab}	26.85 ^{ab}
F6	11.8 ^{abcd}	4.8 ^{abc}	23.8 ^{abc}
F2	10.92 ^{bcd}	3.31 ^{cd}	20.92 ^{bcd}
F8	10.83 ^{bcd}	4 ^{bcd}	25 ^{abc}
F3	10.3 ^{cd}	3 ^d	20 ^{cde}
F10	10 ^d	3.17 ^d	16.83 ^{de}
P	0.0265	0.0001	0.0014

P: Probability.

with an overall average of 4.48 cm and a coefficient of variation of 35.47%. Families F1 and F9 had the largest diameters, averaging 5.89 and 5.83 cm. Families F7, F5, F6, F8 and F2 have intermediate diameters ranging from 5.54 to 3.31 cm. F10, F4 and F3 have the smallest plants at 3.31 and 3 cm. Family F1 has the longest plants, averaging 27.56 cm. It is followed by families F9 and F7 with an average of 26.9 cm. Families F6 and F8, F5, F2, F3 and F10 have average plant lengths ranging from 23.8 to 16.83 cm. Family F4 has the shortest plants, averaging 15.33 cm per plant.

DISCUSSION

The aim of evaluating the agro-morphological characteristics of 10 hybrid families is to identify cocoa trees that combine several characteristics of agronomic interest. The results showed significant differences ($P < 0.0001$) between the 10 families for all the parameters studied. This suggests that, for each trait, families can be ranked from best to worst performers. Results showed that families F6 (P7 × PA150), F1 (PA 150 × GU 284 A), F4 (PA 150 × T60/887) have the largest pods, with 738.09, 617.88 and 581.06 cm³ respectively. This performance of these hybrid families is partly linked to their high Amazonian genetic origin (Motamayor et al., 2003).

Families F8 (P 7 × T60/887), F6 (P 7 × PA 150) and F5 (PA 150 × IFC 5) were ranked among the best for average number of normal fresh beans per pod, with 43.97, 42.64 and 42.28 beans respectively. Families F6 and F5 also had the best average fresh bean weights, with 138.39 and 122.89 g respectively. These results demonstrate good pod filling in these families, as shown by the work of Lachenaud et al. (2006), whose results showed good pod filling in the IFC5 clone, with an average of 40 beans per pod. In addition, the good filling of cocoa pods is attributable to a sufficient quantity of compatible pollen at good pollination of the flowers

(Braudeau, 1969; Lachenaud, 1984; Lanaud, 1987, Lachenaud, 1994).

In terms of average pod cortex thickness, families F6 (P7 × PA150), F1 (PA 150 × GU 284 A), F4 (PA 150 × T60/887) topped the rankings with 1.76, 1.51 and 1.50 cm respectively. The great thickness of the cortex of pods from these three families would indicate good resistance to brown rot (Tahi et al., 2000). In fact, this great thickness delays the progression of *Phytophthora palmivora* zoospores into the pericarp tissues of the pod, which is an advantage for preserving cocoa beans from field infections caused by brown rot (Tahi et al., 2000).

Regarding seediness, evaluated in this study by the average weight of a dry cocoa bean (P1FevM), all families except F3 presented average dry bean values between 1.03 (F2) and 1.30 g (F9). These values exceed the industry standard of 1 g per commercial cocoa bean. Families F9, F6, and F10 exhibited the highest values of merchantable beans, with 1.30, 1.20 and 1.18 g per bean, respectively, indicating very good graining. This is consistent with Tunbull and Eskes (2010), who reported that cocoa trees with marketable cocoa bean weights ranging from 1.48 to 1.20 g can be considered to have very good seediness. In the greenhouse evaluation of CSSD-badnavirus tolerance, families F6 (P7 × PA150), F4 (PA150 × T60/887), and F8 (P7 × T60/887) showed the best tolerance, with good vegetative growth and 0% infection rate over three months after inoculation. These results align with Partiot (1978), who found that the P7 × T60/887 hybrid exhibited good tolerance to CSSD under artificial inoculation conditions in Togo. Families F2 (PA150 × GU175A) and F10 (T79/501 × IFC5) showed infection rates of 7.69 and 16.67% and a latency time of 116 days, indicating moderate tolerance to CSSD (Kouakou, 2014; Guiraud et al., 2022).

Conclusion

This study aimed to identify cocoa trees with desirable

agronomic traits to address emerging production constraints. The results showed that families F6 (P7 × PA150), F9 (T85/799 × T79/501), and F10 (SCA 6 × MOQ 413) exhibited very good graining. Additionally, families F6 (P7 × PA150), F4 (PA150 × T60/887), and F8 (P7 × T60/887) demonstrated excellent tolerance to CSSD, characterized by healthy vegetative growth and a 0% infection rate. Although preliminary, these findings provide valuable insights for selecting families to introduce into seed fields for dissemination in CSSD-infected areas.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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