

Early field performance of fine flavor cocoa clones for agronomic traits and tolerance to cocoa swollen shoot virus disease

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Abstract: Fine flavor cocoa generates substantial revenue and provides livelihood for millions of people in both Latin America and Africa. Cultivars of fine flavor cocoa were recently introduced into Ghana for breeding purposes. These cultivars were evaluated in two sets of experiments. Experiment 1. involved field evaluation of 30 clones for vigor, precocity, survival potential and heritability of the agronomic traits. Experiment 2. involved evaluation of the 30 clones for tolerance to the cocoa swollen shoot virus disease (CSSD) upon inoculation with the New Juabeng strain of Togo B virus. Results of experiment 1 showed significant ($p<0.05$) variability among the clones for vigor, precocity and percentage survival. Top five stable and precocious clones identified included CRG9006, SCA9, T63/967, GEBP565AF and PA107. Broad-sense heritability estimate ranged from 0.16 for percentage survival to 0.72 for pod number. Pod number had significant and positive correlation ($p<0.01$, $r=0.40$) with stem diameter increment. Similarly, flower intensity correlated positively with pod number ($p<0.01$, $r=0.43$). In the second experiment, the results showed significant differences in the levels of tolerance to CSSD among the 30 clones inoculated. The tolerance score ranged from 1.63 (SCA6) to -1.52 (PA188). Further observations suggested the importance of genetic factors in the management of the disease. Five outstanding clones identified to be tolerant to the cocoa swollen shoot virus disease included PA188, MAN15/60, ICS1, CRG9006 and MAN15-2. It is important that most productive and CSSD tolerant clones identified are considered for future germplasm enhancement.

Keywords: Cocoa, clones, genetic variability, vigor, fine flavor.

Abbreviations: PN _ Pod Number, SDI_ stem diameter increment, SUV _ percentage survival, FI _flowering intensity, CHLC _ chlorophyll content of leaves, CSSD _ Cocoa swollen shoot disease, CSSDS_ Cocoa swollen shoot disease severity score.

Introduction

Cocoa (*Theobroma cacao* L.) is a tropical tree crop with immense economic importance. The beans serve as the primary source of raw material for chocolate production. Cocoa generates substantial revenue and provides employment for millions of people along its value chain. In Ghana cocoa cultivation provides livelihood for more than 800,000 people and also generates over \$2.8 billion revenue annually (GSS 2008; ISSER, 2014). Fine flavor cocoa on the other hand occupies about 5% of the niche market in the world and attracts high premium due to the recognized flavors used in the production of dark chocolate. Cocoa Research Institute of Ghana (CRIG) has identified eight clones certified as fine flavor by the team of Cocoa of Excellence panel standard in Paris, France. The clones include ICS1, ICS16, ICS60, ICS80, PLAYA-ALTA2, VENC4-4, CC11 and SGU50.

Early cocoa introduction in Ghana consisted mainly of Amelonado type from Fernando Po (now Bioko in Equatorial Guinea). This germplasm dominated plantations across Ghana. Subsequently, the Amelonado germplasm was occasioned by the ravages of cocoa swollen shoot disease (CSSD). The situation was further exacerbated by the continuous loss of primary forest cover which has been implicated in the poor establishment abilities of Amelonado germplasm (Posnette, 1940). In the later years, Amelonado was confirmed to lack the potential to contribute significantly to the genetic improvement of the crop. Broadening the genetic base of the crop is key for improvement. Posnette in 1943/44 (Lockwood and Gyamfi 1979) largely made further introductions to widen the genetic base of the crop (mostly bulk flavor cocoa). Studies have shown that between 1946 and 1971, several clones comprising Criollo, Trinitario and Upper Amazon genetic groups were also introduced (Lockwood and Gyamfi 1979). Currently, a little over 1000 different cocoa clones representing the ten genetic groups (Padi et al., 2015) have been successfully introduced into Ghana and are being conserved in the field gene bank at the Cocoa Research Institute of Ghana (CRIG) for further evaluation.

Assessing the breeding potential of all introduced germplasm collections is key in the identification of superior genotypes for genetic enhancement of important traits such as high yield, fine flavor, precocity, field survival and tolerance to the cocoa

swollen shoot disease (CSSD) in hybrid combinations. The CSSD caused by the New Togo B virus is endemic in many plantations, threatening the livelihood of several cocoa farmers in Ghana. Recent reports (Ameyaw et al., 2014, 2023) indicated that the disease is spreading at an alarming rate and devastating cocoa farms across the major production belt in Ghana.

Ameyaw et al. (2019) further indicated that thousands of infected trees are felled yearly in an attempt to curtail the rapid spread of the disease. This approach has not yielded exclusive results despite the high labor cost and laborious nature. Host plant tolerance on the other hand is envisaged as a key approach in mitigating the devastation of farms by the disease. A few germplasm have so far been documented as tolerant to CSSD (Ofori et al., 2015 and Padi et al., 2013) in cocoa breeding studies at CRIG. Further, the continuous change in climatic condition worsens the impact of CSSD on germplasm performance, specifically field establishment potential of most cocoa genotypes. Poor field establishment represses full expression of several important agronomic traits including vigor, precocity and yield. Other studies have also reported that trait expression in hybrid cocoa could be predicted in most cases by parental (clone) performance (Adomako et al., 1999). Therefore, a comprehensive assessment of parental clones under contrasting environments with adequate information on genetic parameters, such as genetic variance and heritability, as well as correlations among agronomic traits is vital in the hybrid enhancement process. Pang et al. (2008) and Padi et al. (2012) have demonstrated the importance of significant correlations between juvenile and mature traits in cocoa and concluded that juvenile traits could aid early identification of promising parents (clones) thereby reducing the huge capital cost required for long term evaluation and selection of parents for hybrid production. It is therefore important to identify and select promising clones (parents) that could be useful in generating superior hybrids for optimum field performance amidst the continuous change in climate.

This study was set up to (1) determine vigor and precocity between bulk and fine flavor clones, (2) assess heritability of traits across two contrasting agro-ecologies, (3) determine the genetic relationship among five agronomic traits and (4), assess and rank clones for tolerance to the new Togo B virus.

Results

Mean squares, heritability and genetic advance of agronomic traits of 30 clones

Replication effect was not significant ($P < 0.05$) for most of the studied traits except for pod number (PN) (Table 3). Variation attributable to clone effect was significant for all studied traits except SUV. The discriminatory influence of location was significant ($P < 0.05$) for all the studied traits (PN, SUV, SDI, FI and CHLC). However, Clone \times Location interaction effect was not significant for most traits except PN.

The environmental co-efficient of variation (ECV %) ranged from 3.29 % (SUV) to 22 % (PN) while the genotypic co-efficient of variation also ranged from 1.22 % (SUV) to 56.4 % (PN). Phenotypic co-efficient of variation and broad-sense heritability ranged from 21 % (SDI) to 96.5 % (CHLC) and 0.16 (SUV) to 0.74 (PN) respectively.

Genetic advance (GA) was highest (3115.9) for PN but lowest (0.54) for SUV, while the genetic advance as a percentage of mean (GAM) ranged from 0.57 (SUV) to 80.99 (FI).

Mean agronomic performance of clones and checks across two contrasting locations

Significant ($p < 0.05$) variation was observed in the mean performance of the clones for all five agronomic traits studied (Table 4). Pod number (PN) was significant ($P < 0.05$) among the clones in both locations. In Tafo, it ranged between 93 pods/ha (EQX78) and 18,825 pods/ha (CRG9006) while in Afosu, it ranged between 0 pods /ha (EQX78) and 20,362 pods/ha (SGU50). Genotypes that significantly outperformed the checks (P30 and T85/799) in Tafo included CRG9006, SGU50, T60/887, SCA9, GU144C, PA150, GEBP565 and T63/967. In Afosu, CRG9006, SGU50, SCA9, ICS43 and CCN51 had significant pod numbers (PN) over the better check (T85/799).

Stem diameter increment (SDI) was significantly different among the clones in Tafo as well as those planted in Afosu. The highest stem diameter increment observed in Tafo was 48.7mm (MAN15/60) while the least increment of 26.8mm was recorded by P30. Consequently, eighty percent (80%) of the clones at Tafo ranked higher than the better check (T85/799). Similarly, in Afosu, the least stem diameter increment (SDI) recorded was among the GU341H (32.5mm) while the highest (48.6 mm) was observed among the SGU50 clones. In general, 27% of the clones ranked higher than the better check (T85/799) while 83.3% ranked better than the worse check (P30).

Percentage survival (SUV) of the clones was relatively high in both locations although Tafo recorded the highest percentage survival for most clones compared to Afosu. In Tafo the percentage survival ranged from 94.4 % to 100% while in Afosu, it ranged from 78% to 100%. Across the locations, only GU225V had 100% survival.

The chlorophyll content significantly varied among the clones. Clone PA150 had the least chlorophyll content (29.1 nmol cm⁻²) while T60/887 recorded the highest (39.6 nmol cm⁻²) in Tafo. In Afosu, the chlorophyll content varied from 39 nmol cm⁻² (GEBP 585AF) to 46.8 nmol cm⁻² (ICS60 and ICS43). Flower intensity score varied from 1 (EQX78) to 6 (T63/967 and T60/887) in Tafo. Similarly, it ranged between 1 (P30) and 6 (CRG9006, SGU50, T60/887, T63/967 and CC11) in Afosu. Top five clones with significant ($p < 0.05$) flower intensity score over the worst check (P30) across locations included T63/967, T60/887, GEBP565AF, CCN51 and ICS60.

Variation in the agronomic performance of fine and bulk flavor clones

Significant ($p < 0.05$) variation was observed between the bulk and fine flavor cocoa clones for pod number (PN) per ha⁻¹ at 36 months after transplanting. The bulk flavor clones produced a significantly higher number of pods per hectare over the fine flavor clones (Fig 1A). No differences were further observed among fine and bulk flavor clones for SDI (Fig 1B).

Table 1. Sources and genetic groupings of clones.

Clone	Source	Genetic group	Clone	Source	Genetic group
CRG9006 (α)	Trinidad	Guiana \times Manus	CC11 (*)	Costa-Rica	-
GU144C (α)	French-Guiana	Guiana	ICS1 (*)	Trinidad	Trinitario
MAN15-2 (α)	Brazil	Purús	PLAYA-ALTA2 (*)	Peru/Brazil	Trinitario
PA150 (α)	Peru	Maranon	SGU50 (*)	Guatemala	Criollo
PA7 (α)	Peru	Maranon	VENC4-4 (*)	Venezuela	-
T60/887 (α)	Trinidad	Maranon \times Nanay	POUND 7 (α)	Peru	Nanay
T85/799 (α) (Check)	Trinidad	Iquitos \times Nanay (Check)	P30 (α)	Peru	Amelonado
CCN51 (α) RUQ 1347	Ecuador	National	ICS16 (*)	Trinidad	Trinitario
EQX78 (α)	Ecuador	Amel \times Iquitos	ICS43 (α)	Trinidad	Trinitario
GEBP565AF (α)	Trinidad	Maranon \times Iquitos	ICS60 (*)	Trinidad	Trinitario
GEBP585AF (α)	Trinidad	Nanay	PA107 (α)	Peru	Maranon
GU125C (α)	French-Guiana	Guiana	PA137 (α)	Peru	Maranon
GU225V (α)	French-Guiana	Guiana	PA188 (α)	Peru	Maranon
GU341H (α)	French-Guiana	Guiana	SCA9 (α)	Peru	Contamana,
MAN15/60 (α)	Brazil	Purús	T63/967 (α)	Peru	Maranon \times Nanay

Clones with asterisk in parenthesis (*) are certified fine flavor clones (FFC) while those with the symbol alpha (α) in parenthesis are considered bulk flavor clones (BFC).

Table 2. Baseline soil nutrient composition determined at 0-20 cm and annual rainfall recordings in Tafo and Afosu from June 2021 to December 2024.

Location/ Sample	Tafo	Afosu	Critical levels*
pH	6.28	4.33	5.60 -7.20
Organic C %	0.85	0.95	2.00
Total N %	0.09	0.10	0.09
Available P μ g/g	14.96	4.03	20.00
Exch. K (Meq/100g)	0.12	0.12	0.25
Exch. Mg (Meq/100g)	1.08	0.47	1.33
Exch. Ca (Meq/100g)	1.23	1.93	7.50
Sand (%)	73.20	66.84	-
Clay (%)	14.80	17.16	-
Silt (%)	12.00	16.00	-
Textural Class (USDA Standard)	sandy loam	Sandy Loam	
	Total Rainfall (mm)		
Period			
Jun 2021- Nov 2021	1116.90	1184.40	
Dec 2021- Feb 2022	44.40	68.40	
Mar 2022- Nov 2022	1318.60	1257.30	
Dec 2022- Feb 2023	241.60	183.40	
Mar 2023- Nov 2023	1546.10	1641.90	
Dec 2023- Feb 2024	205.7	94.5	
Mar 2024- Dec 2024	877	1179.8	

*Critical levels by Ahenkorah *et al.* (1982), (Dadzie *et al.*, 2025).

- Missing value.

Similarly, no differences were observed among the clones for percentage survival (SUV), flower intensity (FI) and chlorophyll content (CHLC) (Figure 1C, 1D and 1E) respectively.

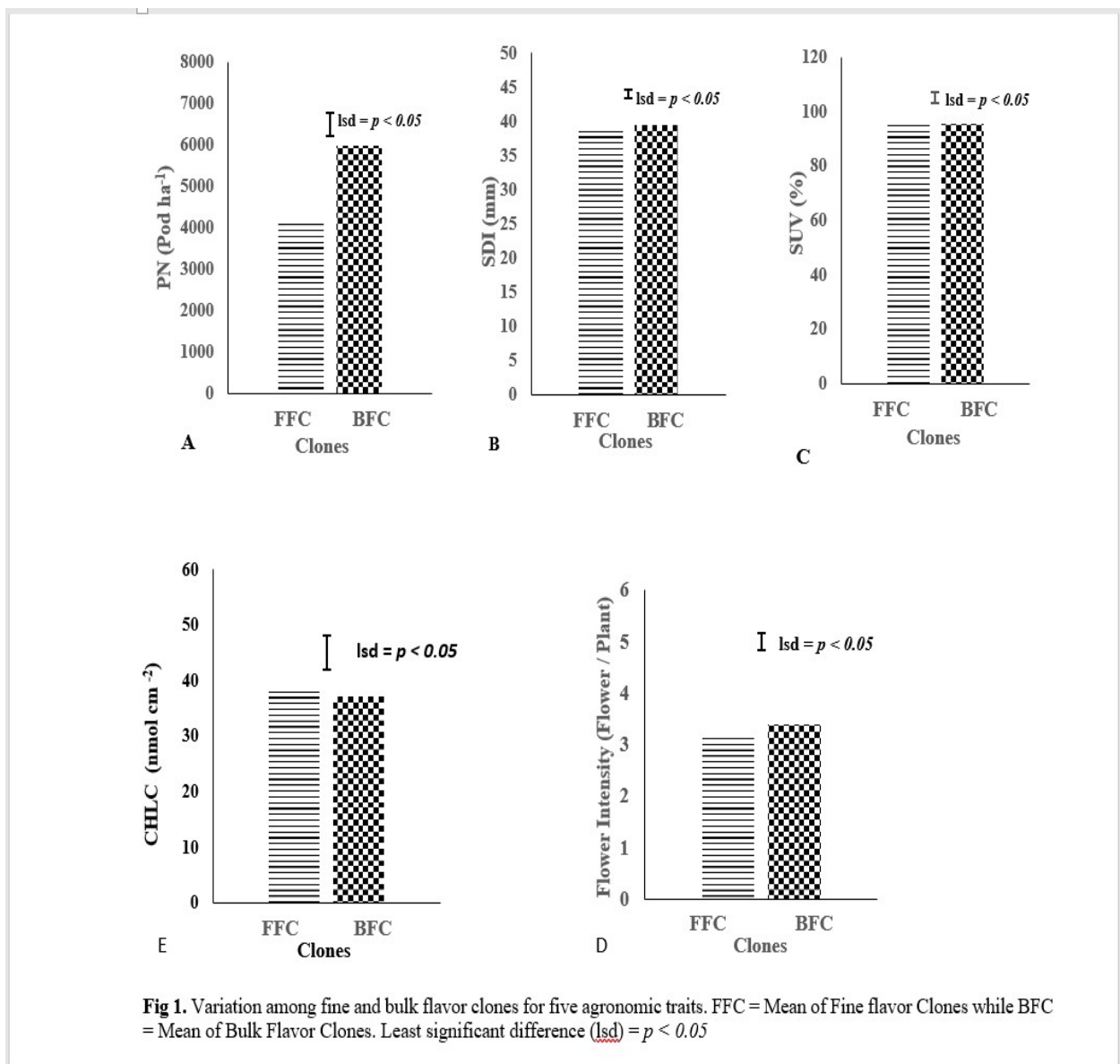
Genotypic correlations among five agronomic traits

Genotypic correlations among pairs of agronomic traits were significant and positive between PN and SDI ($p < 0.05$; $r = 0.40$) (Table 5). Similarly, a significant ($p < 0.05$) and positive relationship was also observed between pod number (PN) and flower intensity (FI) ($p < 0.05$; $r = 0.43$). The stem diameter increment (SDI) also had significant and positive correlations

Table 3. Mean square variances and heritability estimates.

Source	Df	PN (Pods ha ⁻¹)	SDI (mm)	SUV (%)	CHLC (nmol cm ⁻²)	FI (Flower / plant)
Rep	2	131142277**	161.95	18.90	24.35	4.20
Clone	29	3.629E+09***	104.06***	51.83	23.39***	10.77***
Location	1	3.698E+06*	316.17*	816.40**	3167.62***	25.69***
Clone. Location	29	8.575E+08***	33.99	43.69	9.84	2.23
Residual	118	29108572	41.15	44.01	8.14	1.82
ECV (%)		22.00	9.07	3.29	16.37	26.6
GCV (%)		56.40	8.40	1.22	4.00	54.18
PCV (%)		72.52	21.00	29.9	96.50	74.68
<i>h</i> ² <i>bs</i>		0.74	0.63	0.16	0.58	0.53
G A		3115.90	2.48	0.54	3.91	34.12
GAM		50.44	6.30	0.57	10.40	80.99

*, **, ***, Significant at 0.05, 0.01 and ≤ 0.001 probability levels respectively; PN = Pod number; SDI= Stem diameter; SUV= Percentage survival; CHLC = Chlorophyll content; FI= Flower intensity; *h*²*bs* = Broad-sense heritability.



with chlorophyll content as well as flowering intensity. The magnitude and direction of correlations between flower intensity (FI) and chlorophyll content (CHLC) was also significant and positive.

Table 4. Means of six agronomic traits across two contrasting agro-ecologies.

CLONE	PN (Pods ha ⁻¹)		SDI (mm)		SUV (%)		CHLC (nmol cm ⁻²)		FI (Flower / plant)	
	Tafo	Afosu	Tafo	Afosu	Tafo	Afosu	Tafo	Afosu	Tafo	Afosu
CRG9006	18825	18301	41.66	44.90	100	91.70	32.30	41.40	3.33	6.00
SGU50	12128	20362	39.24	48.60	100	87.20	33.10	42.10	3.00	6.00
T60/887	11758	5987	40.13	42.10	100	87.20	39.60	38.40	5.67	6.00
SCA9	11746	13815	29.66	33.30	97.2	88.90	37.80	40.90	3.33	4.70
GU144C	11645	1543	43.74	39.90	94.4	86.10	34.20	41.80	2.67	4.00
PA150	10745	2212	37.81	46.60	97.2	97.20	29.10	39.80	3.67	4.70
GEBP565AF	10715	8394	42.28	41.20	94.4	94.40	33.00	43.20	5.00	5.00
T63/967	9240	9567	42.97	44.70	94.4	100.00	34.40	40.90	5.67	6.00
PA107	8271	6783	44.32	46.80	100	97.20	33.70	44.10	2.33	4.30
GU125C	7870	6697	38.93	40.70	100	97.20	32.70	42.50	2.33	3.00
CCN51	6234	12847	38.70	42.80	94.4	97.20	34.60	44.00	4.67	4.70
MAN15/60	6117	2253	48.66	44.70	94.4	94.40	32.10	41.20	1.67	2.00
GU225V	5802	250	36.24	38.20	100	100.00	33.90	43.10	1.33	2.00
PA137	4531	5246	39.75	39.30	94.4	100.00	32.70	42.20	2.00	5.30
ICS16	4299	1582	39.67	35.40	100	97.20	36.60	42.70	3.67	2.00
POUND7	4228	185	41.79	38.80	100	83.30	29.20	39.00	1.67	1.70
ICS43	3858	15826	35.74	40.50	100	88.90	37.00	46.80	3.00	4.00
CC11	3712	2994	38.03	41.50	97.2	94.40	30.80	42.20	3.67	6.00
MAN15-2	3597	3431	41.86	48.10	100	97.20	35.20	41.20	4.00	5.00
PA7	3426	4598	35.51	36.90	100	94.40	31.80	40.50	2.00	2.00
ICS1	2236	2160	36.26	34.70	94.4	94.40	34.70	43.40	3.00	2.00
GEBP585AF	1930	2097	42.94	38.90	97.2	91.70	31.70	39.00	2.00	2.70
GU341H	1773	3431	27.06	32.50	100	91.70	33.80	40.20	1.33	2.00
PLAYA ALTA2	1666	4249	40.27	44.90	100	97.20	32.70	40.70	3.00	2.30
PA188	1616	3771	28.94	36.50	97.2	88.90	32.90	40.80	3.33	3.00
ICS60	1512	1450	36.64	39.50	100	91.70	35.60	46.80	4.33	4.00
T85/799 (Check)	913	1748	30.21	44.50	94.4	77.80	29.80	40.30	3.00	5.70
P30 (Check)	710	432	26.83	36.10	100	97.20	29.70	39.90	1.67	1.00
VENC4-4	612	595	36.21	40.30	97.2	97.20	35.70	45.00	1.33	3.00
EQX78	93	0	27.65	36.50	100	91.70	30.90	38.60	1.00	1.30
Means	5727	5427	37.66	40.60	98.0	93.10	33.40	41.80	2.96	3.70
Lsd (<i>p</i><0.05)	8444	9305	8.69	11.60	8.38	13.00	5.78	3.50	2.32	2.00

PN = Pod Number; SDI= Stem diameter; SUV= Percentage survival; CHLC = Chlorophyll content; FI= Flower intensity.

Table 5. Genetic correlations among five agronomic traits.

Trait	PN (Pods ha ⁻¹)	SDI (mm)	SUV (%)	CHLC (nmol cm ⁻²)	FI (Flower / plant)
PN (Pods/ha)	-				
SDI (mm)	0.40***	-			
SUV(%)	0.03ns	-0.05ns	-		
CHLC (nmol cm ⁻²)	0.13ns	0.26*	-0.16ns	-	
FI (Flower / plant)	0.43***	0.36**	0.03ns	0.31**	-

*, **, *** Estimates significantly different from zero at 0.05, 0.01 and ≤ 0.001 probability levels respectively. ns = not significant.

Response of 30 clones to CSSD tolerance

The cocoa swollen shoot disease symptom severity expression significantly varied among the 30 clones assessed after inoculation with the New Juabeng strain of the cacao swollen shoot Togo B virus (Table 6). The disease severity symptom expression score was high among the SCA9 ramets which had significant and positive estimate ($p= 5.03E-11$; $estimate=1.63$) for symptom severity score when compared with the check (P30). On the other hand, PA188 ramets also had significant and negative estimates ($p= 6.32E-07$; $estimate = -1.52$) for CSSD severity symptom expression and was the most tolerant clone compared to the rest of the clones including the checks. Four other clones which significantly outperformed the check (P30) based on the magnitude and direction of their estimated values included MAN15/60, ICS1, CRG9006 and MAN15-2 while five most susceptible clones observed in order of increasing susceptibility were PA107, VENC4-4, T63/967, GU144C and SCA9.

Discussion

The search for resilient and productive fine flavor cocoa clones for hybrid development is key in mitigating poor field performance of hybrids for vigor, precocity and survival. Information on the genetic variability among the studied clones and agronomic trait relationship is crucial in selecting potential parents for trait enhancement in cocoa breeding programs. Since the inception of the Cocoa Research Institute of Ghana, a few germplasm clones out of about 1000 collections (Padi et al., 2015) have so far been thoroughly evaluated for various traits such as bean weight, number of beans per pod, pod value

and yield efficiency (Ofori et al., 2016). This present study evaluated 30 clones with special focus on both juvenile and early yield traits across two contrasting agro-ecologies. Initial soil analysis across the locations indicated that nutrient levels across the locations were below the critical levels recommended for cocoa cultivation in Ghana by Ahenkora et al. (1982) and therefore both experimental sites needed soil amelioration with some fertilizer.

The relatively low pH (4.33) in Afosu implied that the soils in Afosu were acidic compared to the near neutral pH (6.28) recorded in Tafo. The acidic pH in Afosu probably affected the available phosphorous level (4.03 $\mu\text{g/g}$) in the soil.

Mean squares of replication was significant for PN. This justified the need for blocking at the experimental sites because the randomized complete block design was appropriate in reducing experimental errors due to the heterogeneity in blocks. Additionally, the significant mean squares of location observed for PN, SDI, SUV, FI and CHLC as well as significant mean squares of clones observed for PN, SDI, FI and CHLC, implied that the test locations were unique and that there was adequate genetic variability among the clones to allow good progress from selection for trait improvement. Similar findings were reported by Ofori et al. (2016) in an assessment of vigor among cocoa clones in Ghana. The significant clone \times location interaction effect observed for PN implied that the ranking order of clones for pod yield was not consistent in the locations. Such observation may partly be due to the significant variation in soil phosphorus in the two locations.

High levels of available phosphorus have been implicated in high flowering intensity in many field crop studies (Dangi et al., 2019). However, it appears our findings were not consistent with the report of Dangi et al. (2019). This is because the clones planted in Afosu where low levels of phosphorus was recorded rather had high flowering intensity than Tafo, suggesting that a complex of factors may be involved in cocoa flowering other than high phosphorous levels.

The environmental co-efficient of variation (ECV) measures the environmental variation in a trait relative to its mean while the genotypic co-efficient of variation (GCV) offers a measure to compare genetic variability in quantitative traits. The phenotypic co-efficient of variation (PCV) on the other hand provides information on the total variation in a trait that can be attributed to both genetic and environmental factors (location). In our present study, there was a close correspondence between genotypic and phenotypic co-efficient of variation for PN and FI.

This implied that these characters are less influenced by the environment (location) and therefore transmission of these characters in hybrid combination would be effective as alluded by Adomako et al. (1999) in their combining ability studies on cocoa.

Environmental co-efficient of variation (ECV) on the other hand was moderately high (22 %) for PN and low (3.29 %) for SUV. However, the heritability estimate (0.16) and magnitude of both ECV (3.29 %) and PCV (29.9 %) relative to GCV (1.22 %) for SUV trait implied that the variation among the clones for SUV was mainly influenced by the environment and therefore not much progress could be realized upon selection within this population for adaptability and survival.

High GCV, coupled with high broad-sense heritability estimate and high genetic advance as percentage of mean (GAM) observed for PN and FI implied that both traits are controlled mainly by genetic factors with minimal environmental effect. Hence, improvement of these precocious traits could be effective through selection.

Among the clones planted in Tafo, CRG9006, SGU50, T60/887, SCA9, GU144C, PA150, GEBP565AF, T63/967, PA107 and GU125C were the top ten most precocious clones in order of decreasing magnitude in PN while in Afosu, SGU50, CRG9006, ICS43, SCA9, CCN51, T63/967, GEBP565AF, PA107, GU125C and T60/887 were the best ten in similar order of decreasing magnitude in PN. Six clones, CRG9006, SCA9, T63/967, GEBP565AF, PA107 and GU125C appeared stable for high PN across the locations. Such stable genotypes could serve as suitable candidates for developing superior hybrids for utilization across multiple environments. High and stable pod yield (PN) have also been reported among hybrids developed from GU clones by Ofori et al. (2014) in their effort to broaden the gene pool for cocoa breeding in Ghana.

It is worth noting that most of the high and stable yielding clones fall under the bulk flavour cocoa (BFC) which produced significantly higher yields than the fine flavour clones (FFC). Such observation was consistent with the report of Fowler, 1999; Ferrão, 2002 who documented fine flavor clones as low yielding and susceptible to diseases. Among the six stable clones identified for high PN production, it was evident that the performance of T63/967 and PA107 which are old introductions corroborated the findings of Padi et al. (2016) who indicated that the core of productive breeding parents in Ghana consisted mainly of Marañón, Nanay and Iquitos genetic groups.

Clone vigor was high among T60/887, MAN15-2, SGU50, PA107 and VENC4-4 compared to EQX78, SCA9 and T85/799 (check). These vigorous clones belong mainly to the Marañón and Iquitos genetic groups and are noted for large stem diameter and bulk flavour except SGU50 and VENCH4-4 which are fine flavour clones that belongs to criollo and an unknown genetic group respectively. Ofori et al. (2017) reported that more vigorous clones survived better than less vigorous ones under drought stress conditions. Our studies did not find differences in vigor between the bulk flavor clones (BFC) and the fine flavor clones (FFC) suggesting that both groups are comparable for vigor and survival with respect to the test locations. Ofori et al. (2017) further indicated that cocoa progenies that showed rapid increase in stem diameter during establishment phase could better adapt to dry weather conditions because their root systems are efficiently developed, enabling them to utilize water efficiently during the dry season.

Several studies have also reported strong positive association between juvenile vigor and high yields (Ofori et al., 2017; Padi et al., 2012), implying that rapid vigor at juvenile stage could effectively serve as a marker in selecting superior parents for further breeding without necessarily conducting a combining ability studies which is expensive and time consuming. Two clones, SGU50 and CRG9006 which demonstrated high vigor relative to the check (T85/799) were also outstanding for high precocity.

Among the studied traits in this population, it was realized that some traits had significantly large correlations enough to ensure correlated response to selection. For example, the significant and positive correlations between pod number (PN) and stem diameter increment (SDI) as well as flower intensity (FI) implied that selection for improvement of vigor (SDI) will indirectly enhance the other two correlated traits (PN and FI). Additionally, significant correlation was also observed

Table 6. Ranking of clones for tolerance to cocoa swollen shoot disease severity (CSSDS).

Progeny	Estimate*	Std. Error	z value	Pr(> z)	Prop
POUND7	-	-	-	-	-
PA188	-1.52	0.30	-4.98	6.32E-07	0.21
MAN15/60	-1.24	0.30	-4.09	4.27E-05	0.28
ICS1	-1.04	0.32	-3.23	0.0012	0.35
CRG9006	-0.61	0.28	-2.14	0.032	0.54
MAN15-2	-0.53	0.28	-1.86	0.063	0.59
P30	-0.41	0.29	-1.43	0.151	0.66
GU341H	-0.36	0.28	-1.26	0.207	0.69
T60/887	-0.31	0.28	-1.09	0.275	0.73
PA137	-0.21	0.28	-0.75	0.453	0.81
PLAYA-ALTA2	-0.19	0.28	-0.72	0.473	0.81
GU125C	-0.13	0.28	-0.46	0.646	0.87
GEPB585AF	-0.12	0.30	-0.39	0.696	0.88
ICS16	-0.10	0.28	-0.37	0.712	0.90
PA150	-0.09	0.28	-0.33	0.730	0.91
PA7	-0.06	0.27	-0.21	0.835	0.94
CCN51	-0.05	0.28	-0.19	0.841	0.94
GU225V	-0.05	0.28	-0.16	0.869	0.95
T85/799	0.21	0.27	0.78	0.436	1.23
ICS60	0.24	0.27	0.89	0.372	1.26
ICS43	0.39	0.27	1.49	0.136	1.48
CC11	0.48	0.26	1.83	0.066	1.60
SGU50	0.72	0.26	2.83	0.004	2.06
GEPB565A-F	0.85	0.26	3.34	0.000	2.34
PA107	1.01	0.26	3.94	8.05E-05	2.75
T63/967	1.06	0.26	4.07	4.63E-05	2.88
VENC4-4	1.09	0.26	4.26	2.04E-05	2.98
EXQ78	1.21	0.25	4.87	1.14E-06	3.34
GU144C	1.59	0.25	6.39	1.69E-10	4.93
SCA9	1.63	0.25	6.57	5.03E-11	5.09

*Higher negative estimates imply better tolerance to CSSD, (-) represents missing value.

between FI and CHLC indicating that high chlorophyll content influenced flowering of plants due to probable efficient photosynthetic systems that might have led to the transportation of assimilates for flower production.

In the second experiment where the clones were assessed for tolerance to the cocoa swollen shoot virus disease using the New Juabeng strain of the cacao swollen shoot Togo B virus, it was realized that all the 30 clones elicited varied responses in terms of symptom severity expression, indicating genetic influence of clones in response to the virulent strain of the virus. Indeed, all the clones showed susceptibility symptoms and were similar to observation made by Ofori et al. (2015) in their assessment of cocoa hybrids for resistance to the cocoa swollen shoot virus disease.

Although many of our test clones were susceptible to the virus, the disease severity expression was highly variable among the clones indicating that genetic factors were underlying the extent of symptom expression. Furthermore, our observation was corroborated by the significant contrast in the magnitude of estimates for severity expression between SCA9 (1.63) and PA188 (-1.52). Clones with significant negative estimates included CRG9006 (-0.61), ICS1(-1.04), MAN15/60 (-1.24) and PA188 (-1.52) in an increasing order of CSSD tolerance over the check. The rest of the clones were not different from the checks except SGU50, GEPB565AF, PA107, T63/967, VENC4-4, EQX78, GU144C and SCA9 which performed worse than the checks.

The SCA9 clone which belongs to the Contamana genetic group was much more susceptible compared to the PA188 clone that belongs to the Marañón genetic group. This observation was not unusual as Marañón and Iquitos genetic materials are noted for contributing tolerant alleles against cocoa swollen shoot virus disease in hybrid cocoa breeding (Padi et al., 2013). Although SCA9 was highly precocious, it may not be very useful in CSSD tolerance breeding. Interestingly, ICS1 was the only certified fine flavor cocoa clone (FFC) observed among the top-five CSSD tolerant genotypes and may be useful for CSSD tolerance breeding. Other clones which performed relatively better than the check (P30) for CSSD tolerance screening included MAN15/60, CRG9006 and MAN15-2 while the five worst clones observed in order of increasing susceptibility included PA107, VENC4-4, T63/967 and GU144C. We therefore conclude here that, most of the bulk flavor cocoa clones tolerate the CSSD better than the fine flavor clones and are potential sources of tolerance alleles for CSSD tolerance breeding.

Materials and Methods

Plant material and nursery conditions

The experimental materials evaluated comprised thirty (30) clones (seven certified fine flavor clones and twenty-three bulk flavor clones) (Table 1.). The clones were generated by budding of scions harvested from fan branches of 30 selected genotypes onto six-months old standard rootstock generated from PA150 × POUND7 cross. The generated clones were kept under shade in the nursery for 6 months during which it was watered every three days. The buddings were then divided into two sub-groups for both field evaluation for vigor, precocity and survival (Experiment 1) and gauze house screening for tolerance to the cocoa swollen shoot disease using the new Togo B virus (Experiment 2).

Field evaluation of clones (Experiment 1)

The 30 clones were transplanted in June 2021, thus six-months after grafting following a randomized complete block design with three replications consisting of 12 plants per clone per block in Tafo and Afosu, respectively. Within each block, all the 30 clones were randomized. The clones were planted at 3.0 m² spacing under both plantains as temporary shade and *Terminalia* spp. as permanent shade at both Tafo (tropical rainforest agro-ecology, with latitude 06° 13' N, 0° 22' W) and Afosu (a transitional belt, latitude 06° 22' N, 01° 10' W), both locations are research stations. The plantain was also planted at 3.0 m² spacing and the permanent shade at 18.00 m² spacing. The shade crops and trees were planted three-months before the cocoa clones were transplanted. Each cocoa plant was subsequently fertilized in July 2021 and 2022, with 70 g nitrogen (N) supplied as ammonium sulphate due to the low levels of soil fertility of the experimental sites. At the beginning of flower production, fertilizers and agro pesticide application was done, following recommended practices for cocoa production in Ghana. Essentially, N: P: K (0:22:18) fertilizer was applied by broadcasting 350 kg/ha in June 2023. Insect pests were controlled using imidacloprid (200 SL) as Confidor (BayerCrop Science, Monheim, Germany) at the rate of 150 ml/ha from April to December each year.

Assessment of cocoa clones for tolerance to cocoa swollen shoot disease (CSSD) severity (Experiment 2)

Six-months old budded cocoa clones comprising 30 genotypes were transferred to a screen house for inoculation and screening following the protocol described previously by Ofori et al. (2015). The clones were laid out in a single clone randomization procedure with five plants per replication, using three different cubicles in the screen house as replications. Each clone was represented by 15 plants and inoculated with the New Juabeng strain of the cacao swollen shoot Togo B virus (CSSTBV) by allowing 10 viruliferous mealybug nymphs, [*Planococcoides njalensis* (Laing), Homoptera: pseudococcidae] to feed on the leaves of the clones.

The inoculation procedure was repeated 2 weeks after the first for all the plants to ensure high levels of infection. Symptom expression after inoculation in the first, second, third and ninth flush leaves were evaluated using a 1–9-point scale of increasing severity with 1 = no symptom; 2 = red vein banding; 3 = chlorotic vein flecking; 4 = chlorotic vein clearing; 5 = green vein banding; 6 = diffused flecking; 7 = fern pattern; 8 = swollen stem; 9 = dead plant. Where multiple symptoms were expressed in the same flush of leaves, the score of the leaf or leaves with the highest severity rating was used as described in Padi et al. (2013).

Data collection

Data collected for analysis included Pod Number (PN), stem diameter increment (SDI) (mm), percentage survival (SUV), flowering intensity (FI) (Average number of flowers per plant), chlorophyll content (CHLC) of leaves (nmol cm⁻²), Cocoa swollen shoot disease (CSSD) severity score (CSSDS).

Data on pod number (PN), average number of matured pods produced per plant recorded at two monthly intervals from 18 to 30 months after transplanting was determined and used to estimate total pod yield per hectare. Pod yield per hectare was estimated by computing average pods produced per clone, multiplied by 1,111 trees per hectare.

Stem diameter measurement (SD) was carried out with an electronic caliper at 15 cm above-ground surface at 3 months after transplanting and subsequently measured at six monthly intervals for 30 months.

Stem diameter increment (SDI)(vigor) was determined 30 months after transplanting by computing the difference between stem diameter at 30 months and the initial stem diameter recorded at 3 months after transplanting.

Percentage survival (SUV) was obtained by counting the number of surviving plants in each plot 24 months after transplanting and expressed as a percentage.

Flowering intensity (FI) from 24 to 30 months after transplanting was scored on a 0 to 6-point scale: 0 = absence, 1 = 1 to 10 (very low), 2 = 11 to 20 (low), 3 = 21 to 30 (moderately low), 4 = 31 to 40 (medium), 5 = 41 to 50 (high) and 6 = >50 (very high) (modified from Eskes et al., 2000). Three cycles of scoring at monthly intervals were completed.

Leaf chlorophyll content (CHLC) was determined with SPAD Chlorophyll Meter (SCM-Minolta, Japan). Two CHLC readings were recorded in the early mornings (between 6 and 9 am) on fully expanded third, fourth or fifth leaves from the apex of all plants (18 and 24 months after transplanting).

Soil samples were randomly taken in early June 2021 from 12 different spots at 0 – 20 cm depth at each location. The soil samples were mixed thoroughly and then analyzed for their physical (textural class) and chemical properties (pH, % N, % C and available P) (Table 2). With the exception of total % N components had values lower than the recommended critical.

Data analysis

The Statistical Analysis System (SAS) software version 9.2 (SAS Institute, 2012) was used to perform analysis of variance on the data for each location. Normality test of data was conducted using residual error graphs obtained from the analysis of variance.

A combined analysis of variance (ANOVA) was performed on the data based on best linear unbiased estimates (BLUE) of plot means, with blocks and location \times genotype combinations considered random effects, whereas genotypes were treated as fixed effects. Mean separation was performed using the least significant difference (LSD). Mean squares for each location and across locations were estimated using the 30 clones in RCBD. The measured traits were computed from the mean values adjusted for block effects for each location and across locations. The statistical model used for the combined analysis across environments is as follows:

$$Y_{ijk} = \mu + l_i + r_j + g_k + lg_{ik} + e_{ijk}$$

where Y_{ijk} is the BLUE of genotype k in location i and replication j , μ is mean, l_i is the effect of the i^{th} location, r_j is the effect of the j^{th} replication, g_k is the genetic effect of the k^{th} genotype, lg_{ik} is the interaction effect, and e_{ijk} is the experimental error term. The REML (restricted maximum likelihood) method (Corbeil and Searle, 1976) was used to determine the variance components of the various sources including genotypic variance and the error variance. All effects were regarded as fixed except replication. The variance components were used to estimate the broad-sense heritability (h^2bs) on the clone mean basis.

The phenotypic, genotypic (clone) and environmental variances were calculated according to the formula suggested by Singh and Chaudhary (1999) as follows:

$$\text{Environmental variance } (\sigma^2 E) = MSE$$

$$\text{Genotypic variance } (\sigma^2 G) = \frac{MSG - MSE}{b}$$

$$\text{Phenotypic variance } (\sigma^2 P) = \sigma^2 G + \sigma^2 E$$

$h^2 b = \frac{\sigma^2 G}{\sigma^2 P}$ where $h^2 b$, $\sigma^2 G$, and $\sigma^2 P$ are broad-sense heritability, genetic variance and phenotypic variance, respectively.

Confidence intervals for heritabilities were estimated following Singh et al. (1993) as follows:

$$\frac{\left(\frac{MSG}{MSE - \left(1 - \frac{\alpha}{2}\right)} \right)}{\left(\frac{MSG}{MSE + (b - 1) - \left(1 - \frac{\alpha}{2}\right)} \right)} \leq h^2 \leq \frac{\left(\frac{MSG}{MSE - \alpha/2} \right)}{\left(\frac{MSG}{MSE + (b - 1)\alpha/2} \right)}$$

where MSE is error mean square (environmental variance), MSG is mean square attributable to clone, b is the number of blocks and $\alpha/2$ is the probability point of the F distribution, respectively. Treatment means were separated via the Duncan's multiple range test at $p = 0.05$ level of significance.

Genotypic, Phenotypic and Environmental coefficient of Variation were calculated as:

$$GCV\% = \frac{\sqrt{\sigma^2 G}}{\bar{x}} \times 100$$

$$PCV\% = \frac{\sqrt{\sigma^2 P}}{\bar{x}} \times 100$$

$$ECV\% = \frac{\sqrt{\sigma^2 E}}{\bar{x}} \times 100$$

Where, GCV % = Genotypic Coefficient of variation; PCV % = Phenotypic Coefficient of variation; ECV % = Environmental Coefficient of variation; $\sigma^2 G$ = Genotypic Variance; $\sigma^2 P$ = Phenotypic Variance; $\sigma^2 E$ = Environmental Variance; \bar{x} = Mean of trait

The expected Genetic Advance for each trait was calculated as: $GA = K\sqrt{\sigma^2 P h^2 b}$

Where, $K = 1.40$ at 20% selection intensity for trait; $\sigma^2 P$ = Phenotypic variance for trait; $H^2 b$ = Broad-sense Heritability of trait; Genetic Advance as percentage of mean (GAM) was estimated as: $GAM = \frac{GA}{\bar{x}} \times 100$

Pearson's correlation coefficients among the evaluated traits were estimated and categorized as weak. (-0.30 to 0.30), moderate (-0.5 to -0.3 and 0.30 to 0.50), strong (-0.9 to -0.5 and 0.50 to 0.90) and very strong (-1.0 to -0.9 and 0.90 to 1.0) (Cohen 1992).

Cocoa swollen shoot disease symptom severity scores were analyzed as multinomial (ordered) distribution which utilized cumulative logit link function in the model to compute the cumulative odds for each category of response (O'Connell et al., 2008).

Conclusion

Significant genetic variation was observed among the 30 clones for all the studied traits including vigor and precocity. The most vigorous clones included T60/887, MAN15-2, SGU50, PA107 and VENC4-4 while the most stable and precocious clones were mainly the bulk flavour types which included, CRG9006, SCA9, T63/967, GEBP565AF, PA107 and GU125C. Broad-sense heritability estimates were low (0.16) for percentage survival (SUV), moderate (0.53), (0.58) for flowering intensity (FI) and chlorophyll content (CHLC) respectively. Pod number (PN) had a high heritability estimate (0.74) coupled with high % GCV relative to % ECV suggesting that considerable selection responses could be expected for pod number. Moderate and positive correlation was observed among SDI, PN, and FI suggesting that direct selection for one of those significantly correlated traits would give a favorable response to the other traits. All 30 clones inoculated with the New Juabeng strain of the Togo B virus showed varied severity responses indicating the importance of genetic factors in the management of the disease. Five most outstanding clones for CSSD tolerance included PA188, MAN15/60, ICS1, CRG9006 and MAN15-2.

Declaration of competing interest: The authors hereby declare that the study was carried out without any financial and/or commercial commitments that could result in a potential conflict of interest.

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