Genetic analysis and hybrid performance of recommended cocoa (*Theobroma cacao* L.) clones for bean yield in Ghana

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Abstract

Attempts to develop high yielding varieties in Ghana have mostly relied on the introduction of new clones to broaden the range of planting materials for yield improvement. The objective of this study was to estimate the genetic variation and heritability for bean yield of six recommended cocoa clones using these as males in crosses with five seed garden parents. Twenty-four families obtained from a 5×6 North Carolina II (NC II) incomplete factorial mating design together with 19 high yielding single crosses, of which four were standard mixed hybrids, were planted in a randomized complete block design with four replications and evaluated for bean yield over 6 years. To account for the serial correlation among yield data collected from the same plants over years, six models with different covariance structures were tested. The general covariance model emerged appropriate based on Akaike information criterion values with significant ($P < 0.01$) family × year interaction. Average bean yield was highest in the NC II families followed by the specific crosses then the standard mixed hybrids. Combining ability analysis among the NC II was significant for female, male and female × male interaction along with a narrow-sense heritability of 0.26. Clone CRG 6035 among the males which had good general combining ability could be added to the seed garden parents, while the promising hybrids (NA 33 × SCA 9, SCA 6 × Pound 10, T60/887 × PA 121 and T79/501 × CRG 6035/103) undergo stability tests before release.

Introduction

Cocoa (*Theobroma cacao* L.) is a humid tropical tree crop grown in areas of high annual rainfall. Even though, it originated from Amazon valley in South America (Motamayor *et al.*, 2008), cocoa is cultivated in large areas of most West African countries. In Ghana, it is estimated to occupy an area of 1.9 million ha of land and there are approximately 800 000 cocoa farm families that depend on the crop (CMS, 2023). Most of the cocoa farmers in Ghana are smallholders who harvest the produce on 2–3 ha with annual average yield range of 400–450 kg/ha (Baah *et al.*, 2011). Relatively, higher yield of 1000 kg/ha and above have been reported in Ecuador and other South American countries (Amores *et al.*, 2011). Cocoa is a major contributor to the tax income of the major countries involved in the production of the crop, accounting for 8.2% of Ghana GDP and 30% of total export earnings (Asante-Poku and Angelucci, 2013). Cocoa is mainly cultivated for its seeds known as beans which are commercially used in chocolate and beverage worldwide, and one of the richest sources of polyphenols (Wollgast and Anklam, 2010). Locally, cocoa by-product can be used to produce soap, gin, fertilizer, animal feed, jam, vinegar and biscuits.

Cocoa cultivation in Ghana and other West African countries started when the Amelonado and Trinitario pods were introduced into the West African coastal area by the Portuguese and Spaniards in 1722 from Bahia in Brazil (Anon, 1963). Other introductions were made from Fernando Po in 1879 by Tetteh Quarshie (Posnette and Todd, 1951), and São Tomé in the 1890s by Governor Griffiths (Legg, 1972). The bulk of the cocoa in Ghana before formal research descended from these early introductions of which about 95% of the cocoa trees in Ghana comprised the Lower Amazon Forastero variety, now known as West African Amelonado. The rest were descendants of Trinitario which is a hybrid between Criollo and Forestaro and called 'local Trinitarios' (Glendinning, 1964). These formed the base of the popular 'West African Amelonado and Trinitario' population in most parts of West Africa. They are late low yielding and susceptible to black pod and cocoa swollen shoot virus (CSSV) diseases (Brunt, 1975; Adu-Ampomah *et al.*, 1999).

To overcome these problems, a research centre known as West African Cocoa Research Institute (WACRI) was established to formally research into the crop. Several cocoa collections from the cocoa-growing areas were made for evaluation and utilization. This revealed that the cocoa cultivars being grown were mainly of Amelonado and Trinitario origin and uniform in most of the economic traits (Posnette, 1943), suggesting that the locally available materials

cannot make significant contribution to genetic improvement of the crop. Subsequently, different introductions to broaden the genetic base of the cocoa varieties being grown in West Africa were made. In 1943/1944, Posnnette introduced F1 seeds derived from 102 pods of cocoa from Trinidad and Tobago (Glendinning, 1957). After that, several clones belonging to the Criollo, Trinitario and Upper Amazon genetic groups were also introduced between 1946 and 1971 (Lockwood and Gyamfi, 1979). Later introductions occurred between 1972 and 2004 and largely comprised the LCT-EEN, AGU and GU populations. Materials introduced after 2004, generally referred to as recent introductions, were dominated by the RIM, DOM and PNG clones (Abdul-Karimu *et al.*, 2006).

Along with the introductions, different breeding programmes resulted in the development of several varieties such as Approved T, Series II hybrids and the Inter Amazon hybrids (Edwin and Masters, 2005). The release hybrid varieties over the period are the main source of planting materials used in Ghana by the Seed Production Division (SPD) to produce hybrid pods. Presently, about 27 clonal seed gardens of these planting materials have been established across the country by the SPD. Each seed garden is planted between four and six parental clones which are used to generate hybrid pods and supplied to farmers as pods or seedlings. The clones are specified as either female or male parents at the time of establishment of the seed gardens, and seed pods are generated through manual pollination following the tree identity.

Cocoa is generally a self-incompatible and cross-pollinated crop with high genetic diversity within varieties. Different methods such as full-sib selection or the development of population cultivars have been exploited in cocoa improvement. Among these methods, full sib selection makes direct use of combining ability and heterosis (Lambeth *et al.*, 2001). Several studies on combing ability and heterosis for yield and yield components among different cocoa population have been studied. Dias and Kagayama (1995) using 5×5 diallel mating design observed significant differences in general combining ability (GCA) and specific combining ability (SCA) for yield component traits. In a 5×12 incomplete factorial mating design among Upper Amazon cocoa parents, Padi *et al.* (2016) observed a larger non-additive effect than their corresponding additive variance for vigour and yield. Cervantes-Martinez *et al.* (2006) with 6×6 diallel mating design of Costa Rica parental clones observed the importance of both GCA and SCA in controlling disease resistance and yield. The importance of both additive and non-additive effects in controlling black pod incidence, yield and its components was reported among 3×8 factorial mating design observed (Ofori *et al.*, 2020). Combining abilities and heritability for growth and yield traits in 12 underrepresented cocoa parents of diverse genetic groups in crosses with three tester parents also observed that both additive and dominant effects are important in the inheritance of traits (Ofori *et al.*, 2019).

The development and release of cocoa varieties takes about 15 years of bi-parental testing in locations representative of the cocoa production belt (Eskes, 2011). The cocoa tree starts to produce pod after two to three years of establishment and harvesting of pods on the same tree will continue throughout the life span of the crop. For yield collected in different years on the same plant, the assumption of independence between observations used to analyse data in analysis of variance is no longer valid. A linear mixed model of repeated measures analysis that considers multiple correlations and combines all measurements into a single complex model that specifies the correlated structure of the experimental data is required. This is because yield increased over years and the correlation between yield from the different years is high when years are close compared to those that are farther apart (Stroup *et al.*, 2018). Repeated measures usually result in reduced standard error of the means, which then produce narrower confidence intervals and increased statistical power.

Improving high-yielding materials are crucial to meeting the ever-increasing global demand for cocoa. This is especially important given the significant resources challenges, such as limited cocoa farmland and competing land-use options available to farmers. More recent efforts to develop cocoa varieties that yield better than what is currently in the seed garden have focused on selecting suitable male parental clones from genetic groups other than the Upper Amazon which forms the bulk of parental clones currently in the seed gardens. The potential benefit of any hybrid breeding programme depends on combining genetically distant genotypes to exploit heterosis (Falconer and Mackay, 1996). This will offer the opportunity to broaden the genetic base of available planting materials in the seed garden. The overall goal of the study is to determine the GCA, SCA and heritability of some recommended cocoa clones from major cocoa-producing countries for yield. The specific objectives of this study were (1) to determine combining abilities of recommended clones as males in combination with seed garden parents used in Ghana and (2) compare the per se performance of the hybrid families with four standard mixed hybrids for yield in Ghana.

Materials and methods

Plant materials

Crosses were made between five female and six male clones design (Table 1) using a NC II mating. The female clones were selected because they are among the dominant clones in the Seed Gardens in Ghana and much of West Africa. They have previously been used as females in other breeding projects at Tafo (Padi *et al.*, 2016; Ofori and Padi, 2020; Ofori *et al.*, 2020) to select suitable male parents that are compatible with the seed gardens female parents. The males in this study are recommended clones across major cocoa-producing countries (Eskes, 2011) except for Pound 10. The 11 cocoa clones were genotyped using single-nucleotide polymorphism (SNP) markers to obtain estimates of genetic diversity and pedigree relationships in the progeny. Based on their distribution across the 10 chromosomes of cocoa and level of polymorphism, 140 SNP markers were selected from 1560 candidate SNP markers that had been developed using cDNA sequences from a wide range of cocoa tissues (Argout *et al.*, 2008). The SNP fingerprinting was performed at LGC Genomics using the competitive allele-specific PCR KASPar chemistry (LGC Genomics, Hoddesdon, Hertfordshire, UK). Following manual pollination conducted between May and June 2012, seed pods were harvested at maturity, and seedlings from these were nursed for six months before transplanting to the field. While generating seed pods for the experiment, pods from six crosses (PA 7 × RUQ 1347, T60/887 × RUQ 1347, SCA 6 × CRG 6035, PA 7 × PA 121, SCA 9 × T60/887 and SCA 9 × SCA 6) were lost due to damage caused by infection from *Phytophthora* spp., leading to 24 of the expected 30 F1 crosses (Supplementary Table S1). Fifteen other specific crosses (Table 2) of best-performing hybrids selected from experiments conducted over different periods were equally added to obtain a total of 39 families. Four standard mixed hybrids typically

Table 1. Description of clones used in the experiment by source and parentage

Clone	Designation	Source and parentage
PA 150	Female	A clone of Parinari origin, belonging to the Marañón genetic group
PA 7/808	Female	A clone of Parinari origin, belonging to the Marañón genetic group
T79/501	Female	Derived from NA 32 × PA 7 made in Trinidad and collected by Posnette in 1944
T60/808	Female	Derived from PA 7 × NA 32 made in Trinidad and collected by Posnette in 1944
SCA 6	Female	A clone of Scavina origin, belonging to the Cantanama genetic group
RUQ 1347	Male	A clone obtained through the Reading University Quarantine centre, of Trinitario parentage, previously mislabelled as CCN 51
MAN 15/2	Male	A Brazilian clone selection from an international clone trial[a]
CRG 6035	Male	A clone selection made in Ghana from SCA 9 × T63/971
PA 121	Male	A clone of Parinari origin, belonging to the Marañón genetic group
Pound 10	Male	Material selected in the headwaters of the Amazon by Pound
SCA 9	Male	A clone of Scavina origin, belonging to the Cantanama genetic group

[a]Eskes (2011).

Table 2. List of specific crosses evaluated together with families generated from 5 × 6 incomplete North Carolina II design

Specific crosses
AMAZ 15-15 × MAN 15-2
AMAZ 15-15 × PA 150
GU 144/C × MAN 15-2
GU 144/C × POUND 10
MAN 15-2 × CCN 51
NA 33 × POUND 10
NA 33 × SCA 9
NA 33 × T79/467
PA 107 × POUND 10
PA 150 × POUND 7
SCA 9 × MAN 15-2
T63/967 × CCN 51
T63/971 × SCA 9
T85/799 × PA 150
T85/799 × T79/501

composed of mixed hybrid progenies, derived from the Apedwa, Bunso, Pankese and Oyoko seed gardens, were also included as controls and their pedigree are indicated in Supplementary Table S2.

Field establishment and plant management

The trial is located within the experimental area at CRIG, Tafo (6° 13′ 89″ N; 0° 21′ 04″ W). A plot that has previously supported cocoa for 30 years was grubbed in January 2013 and used for establishment of the experiment. The plot was planted with plantain in April 2013 at a spacing of 2.5 × 2.5 cm to provide temporary shade to the cocoa seedlings. The shade was augmented by planting stakes of *Gliricidia sepium* in alternate rows of the plantain. *Terminalia* sp. was planted at the rate of 1 tree per 500 m^2 (20 trees per ha) to provide permanent shade to the cocoa. To know the characterization of experimental soil, samples were taken in May for analyses of soil physical and chemical properties. Samples were taken from soil depths (0–30 cm) within each of four blocks per field. The size of soil particles was analysed, and the soils were classified using the USDA soil textural triangle for assigning soil texture either as sandy loam, loamy sand or sandy clay loam (Supplementary Table S3). Consistent with the long cropping histories of the trial sites, contents of soil organic carbon were low at all soil samples, typically less than 2% in the top 20 cm of soil. Similarly, soil total nitrogen and available phosphorus contents were low, with acidic soil reaction.

Cocoa seedlings were transplanted to the field in June 2013 within rows of the plantain at a spacing of 2.5 × 2.5 m to obtain a population of 1600 plants per hectare. Seedlings of progenies were established randomly in a single row of 20 trees per progeny per block in each of the four blocks. One month after transplanting, the cocoa plants were fertilized with 70 g N per plant as sulphate of ammonia. Weeds were controlled by manual slashing. Following the start of flower production, N:P: K (0 : 22 : 18) fertilizer was applied by broadcasting 350 kg/ha in May each year. Insect pests were controlled using imidacloprid (200 SL) as Confidor (Bayer CropScience, Monheim, Germany) at the rate of 150 ml/ha from August to December each year. Black pod disease was controlled by applying Ridomil Gold (Syngenta; 60% copper oxide and 6% mefenoxam) at the rate of 3.4 g/l of water and applied to drench developing pods on trees. The rate of fungicide used was therefore dependent on the number of pods per tree at the time of fungicide application. The mean annual rainfall recorded from 2014 to 2022 at the location was 1461.6 mm with a bimodal pattern of April–July and September–November (Supplementary Table S4).

Field data collection

Three years after transplanting to the field, yield of the various families was estimated. Annual dry bean yield was determined from the total number of healthy pods produced per plot (typically in four separate annual harvests) divided by the pod index. Harvested beans were fermented and dried to about 7.0% moisture content to estimate average dry bean yield. The pod index was obtained from the number of pods required to obtain 1 kg of dry cocoa beans, estimated from the dry weight of a random sample of 30 pods per plot. As black pod disease was controlled by following recommended regime of fungicide sprays, incidence of the black pod disease was minimal and diseased pods were excluded from yield estimations.

Statistical analyses

Principal coordinate analysis (PCoA) on the 11 NC II design parental clones to observe the pattern of genetic relatedness between

them was performed using the Past 3 software (Hammer *et al.*, 2001). Pairwise Euclidean distances were calculated on 110 single-nucleotide polymorphism (SNP) data, which was used to conduct the PCoA using a covariance matrix with data standardization option. For the yield data, analysis of variance (ANOVA) was performed on 43 cocoa hybrid families using GenStat statistical software version 11(VSN International Ltd., Hemel Hempstead, UK). First, a separate ANOVA for each year was conducted for the 6-year data collection period using a mixed model with family considered fixed and replication as random. Variance components were evaluated using the restricted maximum likelihood (REML) procedure. Normality of residuals was evaluated and confirmed graphically as recommended by Kozak and Piepho (2018). The model used was:

$$Y_{ij} = \mu + rj + gi + e_{ij} \tag{1}$$

where Y_{ij} is the phenotypic measurement of family i in replication j; μ is the population mean, rj is the effect of block j; gi is the effect of family i; and e_{ij} is the experimental error. The procedure of comparing averages was performed using the Tukey test ($P \leq 0.05$) with the specific residual variance value for each year after verifying that the ratio between the largest and smallest mean square of the residue of the years did not exceed the ratio 7:1 (Pimentel-Gomes, 2009).

In determining the relationship among yield across years, a repeated measure analysis was conducted. In repeated measures, the errors can be correlated and/or have uneven variances, so the function allows incorporating different correlation structures adapted for yield over years. Thus, generalized linear models were evaluated with the following correlation structures: composite symmetry (CS), auto regressive (AR1), heterogeneous composite symmetry (CSH), heterogeneous auto regressive (ARH1), auto regression moving average (ARMA 1,1), heterogeneous auto regression moving average (ARMAH 1,1) and unstructured (UN). The best model was selected according to the lowest value of the Akaike Information Criterion (AIC) (Akaike, 1974), which was used to verify the effects of the interaction between the factors. The package nlme (Pinheiro and Bates, 2020) of the R software which has the function LMN, and adjusts a linear model using generalized least squares was used for the analysis.

To estimate combining abilities, mean squares of GCA of female (GCAf), GCA of male (GCAm), and SCA of female × male (SCAf × m) was performed for the 5 × 6 NC II incomplete crossing. The analyses used the average trait values across years. The model was as follows:

$$Yjnp = \mu + yj + fn + mp + fmnp + ejnp \tag{2}$$

where $Yjnp$ is defined as the effect of crosses between parents n and pin year j, μ is the overall mean, yj is the effect of the jth year, fn is the random effect of the nth female, mp is the random effect of the pth male, $fmnp$ is the interaction of female and male and $ejnp$ is the error term. Variance components of the various sources were obtained using restricted maximum likelihood (REML) methods. The additive variance ($\sigma^2 A$), dominance variance ($\sigma^2 D$) and environmental variance ($\sigma^2 E$) were computed using the variance components of female GCA ($\sigma^2 f$), male GCA ($\sigma^2 m$), female × male SCA ($\sigma^2 f \times m$), and error ($\sigma^2 e$) and determined, respectively, as follows: $\sigma 2A = 4(\sigma^2 f + \sigma^2 m)/2$, $\sigma^2 D$

$= 4(\sigma^2 f \times m)$, and $\sigma^2 E = \sigma^2 e$. Narrow-sense heritability ($h^2 ns$) estimate was calculated as

$$h^2 ns = (s^2 A)/(\sigma^2 A + \sigma^2 D + \sigma^2 E) \tag{3}$$

Narrow-sense heritability estimates were categorized as follows: 0–30% = low; 31–60% = moderate; >60% = high, as described by Robinson *et al.* (1949). The significance of the female GCA, male GCA and SCA male × female interaction effects were assessed using the associated standard error of the effect values. ∗significant at the 0.05 probability level was determined when the values were two times greater than SE; and ∗∗significant at the 0.01 probability level when the values were three times greater than SE.

Results

Parental diversity of the 11 NC II parental clones

In the PCoA of six parents as males and five seed garden clones as females, the first two principal coordinates explained 55.9% of the total variation (Fig. 1). The two-dimensional diagram allows the discrimination of the parental clones based on the genetic distances among them. The diversity among the males was higher than that of the females. All the five female seed garden clones clustered in the same area, except one (SCA 6) which was identified in a different quadrant. The male parents on the other hand were widely separated among the four different quadrants.

Repeated measure analysis for bean yield

The repeated analysis results showed that the unstructured model had the lowest AIC estimate, indicating that this structure is the one that best fits yield data collected over years for repeated measures analysis for the data (Table 3). The correlation between years was necessary not higher for those that were close compared to those farther apart. The unstructured error analysis of the 43 cocoa families evaluated in 6 years observed significant ($P < 0.01$) interaction between family and year (Table 4), demonstrating the existence of different behaviour of families over the years.

Mean performance of families for bean yield

Among the 24 NC II hybrid families, 15 specific crosses and the four standard mixed hybrids from the seed gardens, significant differences were observed within the years for bean yield (Table 5). Across years, yield increased from 2017 to 2020 after which it started to decline. Variation among families was highest in 2020 and lowest in 2017, and family NA 33 × SCA 9 was consistently the best in 2019 and 2021. On average, the 24 NC II families outperformed the 15 specific crosses by 2.5% and standard mixed hybrids by 8.7%. Progenies with the lowest yield were dominated by those with MAN 15/2 and T79/501 as either parent, whereas the progenies with the highest yield were typically those with T60/887 and SCA 9 as parents. Families SCA 9 × MAN 15-2 and NA 33 × SCA 9 among the specific crosses had the lowest (652 kg/ha) and the highest of (992 kg/ha) bean yield, respectively. Of the four standard mixed hybrids obtained from the seed gardens at Apedwa and Pankese had the lowest bean yield, with that of Bunso having the highest yield of 881 kg/ha. Families SCA 6 × Pound 10, T60/887 × MAN 15-2, T60/887 × PA 121, SCA 6 × PA 121, PA 150 × CRG 6035/103 and T79/501 × RUQ 1347 and PA 150 × Pound 10 among the NC II
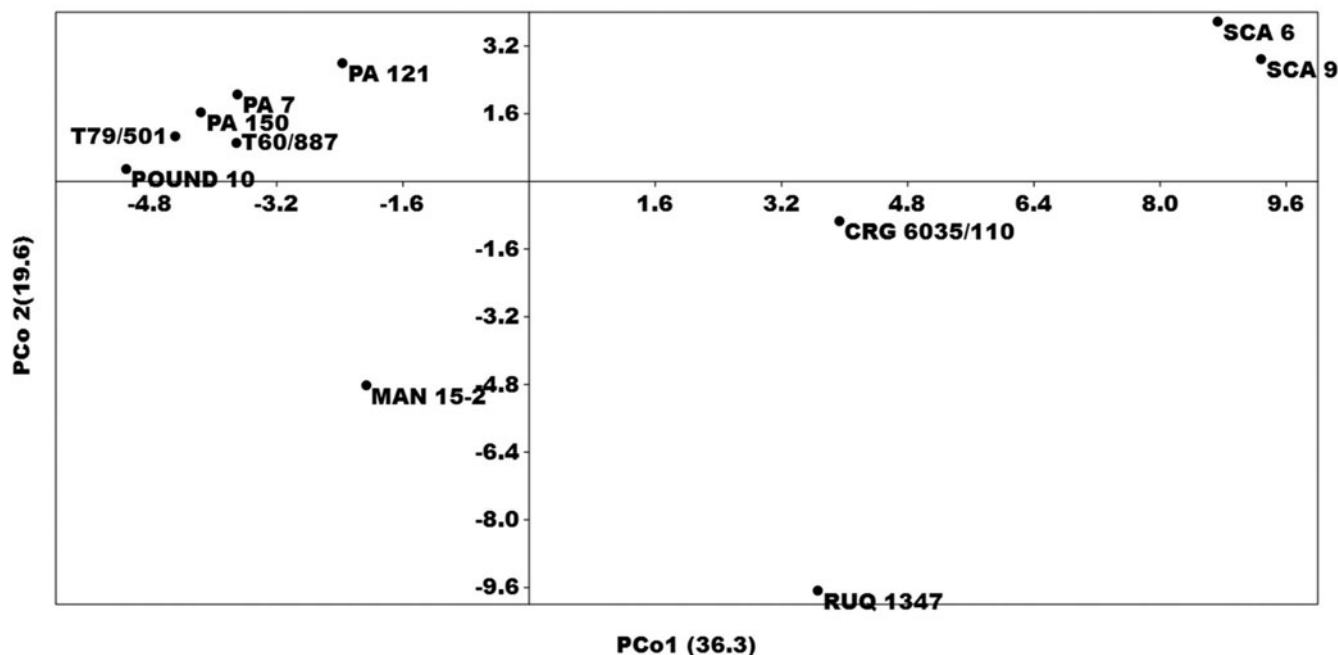
Figure 1. Principal coordinate (PCo) plot of 11 cocoa parental clones (five male and six female) genotyped at 140 SNP loci.

crosses, and NA 33 × SCA 9, T63/971 × SCA 9, MAN 15-2 × RUQ 1347 and T85/799 × PA 150 among the specific crosses significantly yielded higher than the mean standard mixed hybrid.

Genetic parameter estimates and combining ability effects of the 24 incomplete NC II families

Combined ANOVA showed highly significant ($P < 0.05$) variation for female GCA, male GCA and female × male SCA for yield (Table 6). The female and male parent effects were much lower than the female × male interaction effect as indicated by the mean square values for the trait. Genetic variance estimates observed higher dominance variance than their corresponding additive variance, indicating that non-additive gene action seems to control more the expression of bean yield. The narrow sense heritability estimate for bean yield was low.

The GCA estimates of the five females (Table 7) had T60/887 significantly contributing positively and T79/501 significantly contributing negatively to bean yield. Of the remaining three, PA 150 and SCA 6 had positive, and PA 7 had negative effects. For the male clones, CRG 6035 and Pound 10 had significantly ($P < 0.05$) positive GCA effects and MAN 15/2 had significantly ($P < 0.05$) negative GCA effect for bean yield. Clones RUQ 1347 and PA 121 had negative effects and SCA 9 had positive effects. The SCA of the 24 hybrid families had seven being significantly

positive and five negatives for yield (Table 8). In general, values of SCA effects could be predicted based on the parental GCA values. Parental clones T60/887, Pound 10, CRG 6935 and SCA 6 with positive GCA effects were involved in crosses PA 150 × MAN 15/2, PA 7 × CRG 6035, PA 7 × Pound 10, SCA 6 × PA121, SCA 6 × Pound 10 and T60/887 × MAN 15-2 that had significant positive SCA effects. Also, progenies such as PA 7 × MAN 15/2, T79/501 × Pound 10, T79/501 PA 121 and SCA × MAN 15/2 which showed significant negative SCA estimates correspondingly had negative GCA estimates for at least one of the parents.

Discussion

Cocoa productivity is directly related to management of the plants including diseases and pest infestations. As capsid infestation and black pod disease were controlled by following recommended regime of insecticide and fungicide sprays, incidence of the black pod disease and pest damage were minimal and were not included in the data collection. The gene pool of cocoa varieties currently cultivated in most of the cacao-growing regions in West Africa is narrow, and predominantly of Upper Amazon origin (Opoku *et al.*, 2007; Aikpokpodion *et al.*, 2009). This agrees with this study in that in the diversity analysis, four out of the

Table 3. Comparative values of Akaike information criterion obtained from models with different error structures

CS	AR (1)	CSH	ARH (1)	ARM (1,1)	ARMAH (1,1)	UN
11 684	11 682	11 533	11 527	11 684	11 529	11 476

CS, composite symmetry; AR (1), auto regressive; CSH, heterogeneous composite symmetry; ARH (1), heterogeneous auto regressive; ARMA (1,1), auto regression moving average; ARMAH (1,1), heterogeneous auto regression moving average; UN, unstructured.

Table 4. Fixed effects test considering a general error structure for dry bean yield of 43 cocoa hybrids evaluated over 6 years

Source	DF	*F*-value	*P* value
Replications	3	2083.6	<0.001
Hybrids	42	34.6	<0.001
Years	5	1008.6	<0.001
Hybrids × Years	210	1.9	<0.001

DF, degrees of freedom.

Table 5. Mean performance of cocoa hybrids for bean yield (kg/ha) over 6 years in Tafo, Ghana

Hybrid	Periods						Across years
	2017	2018	2019	2020	2021	2022	
PA 150 × RUQ 1347	175	677	690	1147	1132	1255	846
PA 150 × CRG 6035/103	357	658	932	1370	1198	986	917
PA 150 × MAN 15-2	281	713	780	1477	1077	1043	895
PA 150 × PA 121	277	737	792	1288	1210	990	882
PA 150 × POUND 10	129	836	889	1355	1156	1107	912
PA 150 × SCA 9	318	767	880	1453	1030	961	901
PA 7 × CRG 6035/103	195	849	802	1301	1342	957	907
PA 7 × MAN 15-2	156	846	930	1047	1117	926	837
PA 7 × POUND 10	168	750	787	1265	972	990	822
PA 7 × SCA 9	248	783	876	1440	1136	890	896
SCA 6 × RUQ 1347	230	825	1059	1276	1003	964	893
SCA 6 × MAN 15-2	132	716	594	894	988	1276	767
SCA 6 × PA 121	303	865	888	1462	1133	919	928
SCA 6 × POUND 10	350	829	938	1176	1235	1199	954
T60/887 × CRG 6035/103	158	738	749	1243	1342	1057	881
T60/887 × MAN 15-2	359	860	661	1327	1233	1136	929
T60/887 × PA 121	247	736	919	1542	1262	997	950
T60/887 × POUND 10	215	776	864	990	1346	1261	909
T79/501 × RUQ 1347	151	653	673	1251	1117	1168	836
T79/501 × CRG 6035/103	148	785	942	1369	1258	1182	947
T79/501 × MAN 15-2	120	676	768	841	1016	1162	764
T79/501 × PA 121	176	616	759	1308	933	998	798
T79/501 × POUND 10	249	662	696	1522	1128	985	874
T79/501 × SCA 9	244	717	795	1224	1255	1129	894
Mean of NC II progenies	224	753	819	1274	1151	1064	881
T85/799 × PA 150	180	715	831	1320	1259	1195	917
T85/799 × T79/501	228	877	687	1302	1123	1137	892
T63/967 × RUQ 1347	147	623	664	1313	1077	1174	833
T63/971 × SCA 9	282	819	859	1339	1279	1162	957
AMAZ 15-15 × MAN 15-2	290	698	941	913	1269	1193	884
AMAZ 15-15 × PA 150	116	810	745	1315	1160	804	825
GU 144/C × MAN 15-2	248	731	762	1454	863	975	839
GU 144/C × POUND 10	269	858	969	1203	900	1106	884
MAN 15-2 × RUQ 1347	144	758	912	1324	1195	1171	917
NA 33 × POUND 10	211	653	793	1033	1096	979	794
NA 33 × SCA 9	328	826	988	1352	1348	1109	992
NA 33 × T79/467	171	689	644	1030	1219	851	767
PA 107 × POUND 10	126	688	727	1340	1163	1081	854
PA 150 × POUND 7	242	738	612	1377	1205	1116	882
SCA 9 × MAN 15-2	200	548	587	745	946	881	652
Mean of specific crosses	212	735	781	1224	1134	1062	859

(*Continued*)

Table 5. (*Continued.*)

Hybrid	Periods						Across years
	2017	2018	2019	2020	2021	2022	
BUNSO	176	747	918	1208	1192	1040	880
APEDWA	180	805	772	1200	1015	1028	833
OYOKO	171	626	650	1320	1089	1052	818
PANKESE	145	616	623	798	1076	1003	710
Mean of standard varieties	168	698	741	1132	1093	1031	810
LSD (5%)	120	234	326	72	334	222	100

five female parents selected from among the seed garden clones in Ghana clustered in the same area. On the other hand, the six male parents which are recommended clones selected across major cocoa-producing countries including South America were highly diverse and separated in the different quadrants. This explains the need for broadening the range of planting materials in West Africa because the potential benefit of heterosis in any hybrid breeding programme depends on combining genetically distant genotypes (Falconer and Mackay, 1996).

The tree of cocoa starts to produce pods in the second to third year after establishment (Padi *et al.*, 2016) and due to the exponential growth of the plants, yield and other components increase substantially over years. Petithuguenin (1995) showed that the main yield production phase of cocoa in the life cycle of the crop is after the fifth year of establishment. For most tree crop such as cocoa for which data are collected on the same plant from year to year, it is appropriate to evaluate the response of plants using models that capture the autocorrelation and take dependence over time into account (Keselman *et al.*, 1998; Hoffmann, 2016). In this study, the unstructured model had the lowest AIC estimate, indicating that it is the model that best fits yield data analysis over years for the study. The significant year and family × year interaction effects showed that the phenotypic value of bean yield is not consistent in different years. The mean yield differences were 529 kg/ha between 2017 and 2018, 42 kg/ha between 2018 and 2019, 391 kg/ha between 2019 and 2020 then a little decrease of 39 kg/ha between 2020 and 2021, and another decrease of 60 kg/ha between 2021 and 2022. The growth in tree architecture after it starts to produce pods may explain the increase in bean yield between 2017 and 2020. As expected, due to the exponential growth of trees, yield and other components increase in subsequent years.

In Ghana, seed gardens have been established with three to six different parental clones in all the cocoa districts to generate hybrid pods for farmers to plant. For comparison, we included four seed garden mixed hybrids from the Apedwa, Bunso, Pankese and Oyoko districts. Performance of the 24 NC II hybrid families and 15 specific crosses were higher than that of the standards (seed garden mixed hybrids) for yield. Seven of 24 NC II hybrid families and five of the specific crosses yielded higher than the best standard mixed hybrid, indicating that the recently developed hybrids holds adequate potential to perform better than current seed garden parents even though there is the need to evaluate them across different locations. This agrees with Padi and Ofori (2016) who observed that a high proportion of the new varieties, selected for positive SCA for establishment were superior to varieties currently supplied to farmers regarding plant survival, growth and precocity. Variation in yield among the seed gardens was quite high, 710 kg/ha in Pankese, 818 kg/ha in Oyoko, 833 kg/ha in Apedwa and 881 kg/ha in Bunso. The

Table 6. Analysis of variance of GCA and SCA, genetic variance, and heritability of the 24 cocoa progenies developed from a five-by-six incomplete factorial mating-design for bean yield evaluated over 6 years in Ghana

Source	DF	Yield
GCA (female)	4	27 095**
GCA (male)	5	50 681**
SCA (female × male)	14	17 066**
Error	69	1726
Genetic variance		
σ^2_f		797
σ^2_m		1979
$\sigma^2_{f \times m}$		3547
σ^2_A		5552
σ^2_D		14 188
σ^2_A/σ^2_D		0.39
$\sigma^2 E/(\sigma^2 A + \sigma^2 D)$		0.07
h^2ns		0.26

DF, degrees of freedom; f, female; m, male; $\sigma^2 A$, additive variance; $\sigma^2 D$, dominance variance; $\sigma^2 E$, environmental variance; h^2ns, narrow-sense heritability; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level.

Table 7. GCA effects for bean yield of five female and six male cocoa clones evaluated over 6 years in Ghana

Female	Effect	Male	Effect
PA 150	12.4	CRG 6035	29.27*
PA 7	−11.33	RUQ 1347	−3.64
SCA 6	9.58	MAN 15/2	−55.48**
T60/887	17.89*	PA 121	−18.52
T79/501	−28.54**	PD 10	45.88**
		SCA 9	2.49
SE$_{0.05}$	8.60		14.6

SE, standard error for comparison of GCA effect values; *, ** significance when effects were twice and thrice greater than the SE, respectively.

Table 8. Specific combining ability (SCA) effects for bean yield of 24 cocoa crosses derived from an incomplete factorial mating design of five females and six males over 6 years in Tafo, Ghana

PA 150 × RUQ 1347	8.36
PA 150 × CRG 6035/103	−2.4
PA 150 × MAN 15-2	37.98**
PA 150 × PA 121	1.62
PA 150 × POUND 10	17.49*
PA 150 × SCA 9	−7.83
PA 7 × CRG 6035/103	28.94**
PA 7 × MAN 15-2	−122.23**
PA 7 × POUND 10	47.07**
PA 7 × SCA 9	−4.24
SCA 6 × RUQ 1347	6.23
SCA 6 × MAN 15-2	−89.14**
SCA 6 × PA 121	26.23**
SCA 6 × POUND 10	99.35**
T60/887 × CRG 6035/103	12.44
T60/887 × MAN 15-2	76.41**
T60/887 × PA 121	−18.87*
T60/887 × POUND 10	9.67
T79/501 × RUQ 1347	2.71
T79/501 × CRG 6035/103	−10.35
T79/501 × MAN 15-2	−2.45
T79/501 × PA 121	−42.17**
T79/501 × POUND 10	−91.36**
T79/501 × SCA 9	16.53
$SE_{0.05}$	8.5

SE, standard error for comparison of SCA effect values; *, ** significance when effects were twice and thrice greater than SE, respectively.

different parent combinations in use at the different cocoa stations of the Seed Garden may account for the large differences among seed garden mixed hybrids. Oyoko had C42-T60/887, Apedwa had C67-T79/501 Pankese had C74-T63/971 and Bunso had C 85 - PA 7.

For the 24 NC II families and 15 specific families, variation for yield ranged from 651 kg/ha in SCA 9 × MAN 15-2 to 992 kg/ha in NA 33 × SCA 9 among the specific crosses and 768 kg/ha in T79/501 × MAN 15-2 to 955 kg/ha in SCA 6 × Pound 10 among the NC II families. A similar range of 672 kg/ha in T60/887 × IMC 78 to 1075 kg/ha in T60/887 × PA 150 among 20 cocoa families was reported in a previous study (Adomako and Adu-Ampomah, 2003). The ten best-performing families with highest bean yield had six from the incomplete NC II families and four from specific single crosses. Surprisingly, SCA 9 as male performed very well in the single crosses group but not the case in the NCII crosses. Families T63/971 × SCA 9 and NA 33 × SCA 9 among the single crosses were the first and second highest yielding and the highest yielding SCA 9 cross (PA 150 × SCA 9) among the NCII families was not even among the best 10 families.

Results of the 5 × 6 incomplete NCII mating design analyses, showed that genetic variance was influenced by both GCA and SCA, suggesting the importance of both additive and non-additive genetic effects in controlling bean yield. So, for the same trait, some of the parents pass on the average level of their trait to its progeny while in some cases the progeny generated between pair of parents are either higher or lower than what the two parents are passing on to their progeny. This observation has been reported for yield in earlier studies (Dias and Kagayama, 1995; Cervantes-Martinez et al., 2006; Pereira et al., 2017; Ofori et al., 2019, 2020; Ofori and Padi, 2020). This suggests a predominance of additive variance over dominance variance for the yield in dry bean yield and PBPI traits.

Variations among the female parents were lower than that of the males for yield, and this agrees with the diversity analysis. The female parents were selected from the seed garden parents available in Ghana, as in much of West Africa. All these parents descended from a small number of genetic groups of the Upper Amazon origin that made significant contributions to cocoa production during the early years of formal research (Posnette, 1951). The male parents on the other hand are clones of different genetic background and the large additive variation observed in this study is expected. CRG 6035 is a very high yielding clone selection made in Ghana from SCA 9 × T63/971, RUQ 1347 is a clone obtained through the Reading University Quarantine centre, of Trinitario parentage, MAN 15/2, is a very high yielding Brazilian clone selection from an international clone trial, PA 121 is a clone of Parinari origin belonging to the Marañón genetic group, Pound 10 is a clone selected in the headwaters of the Amazon by Pound and SCA 9 is a clone of Scavina origin, belonging to the Cantanama genetic group.

Non-additive was more important than additive variances as indicated by the additive to dominance variance ratio, which was below 0.50, and the importance of specific cross combinations. This is in accordance with the narrow-sense heritability of 0.26 for bean yield, which indicate the difficulty in direct selection for bean yield. This implies that further yield improvement by selecting within the same genetic group is likely to be poor. Broadening the genetic base through crossing with clones from other genetic groups will be the best options to promote further yield improvement as employed by this study. In previous studies (Cervantes-Martinez et al., 2006; Padi et al., 2016; Ofori et al., 2019) a high non-additive genetic control was observed for bean yield.

Among the females, parent T60/887 derived from PA 7 × NA 32 contributed positive GCA effect, indicating that it combines well with the male parents which includes the recent (RUQ 1347, MAN 15/2, PA 121) and old (Pound 10 and SCA 9) introductions, and CRG 6035 developed at CRIG. Similar in previous combining ability studies among GU clones (Ofori et al., 2014), T60/877 had positive effects for growth and precocity traits. Such a clone is very important in cocoa production because the crop is out-crossing and productivity depends on the acceptance or compatibility of pollens among neighbouring plants. Families derived from T60/887 would be characterized by high productivity and they should be used as parents in breeding programmes that seeks to improve on yield.

Parent T79/501 derived from NA 32 × PA 7, a reciprocal of T60/887 on the other hand contributed negative GCA female effect, indicating that it does not combine well with all the male clones. This is a clear indication of reciprocal differences, which is probably influenced by maternal effect or role of maternal

parents in controlling traits of economic importance in cocoa (Ofori *et al.*, 2020). Families T60/887 × MAN 15-2, T60/887 × PA 121 and T60/887 × Pound 10 generated from T60/887 as females showed predominantly higher bean yield than T79/501 × MAN 15-2, T79/501 × PA 121, T79/501 × Pound 10 and T79/501 × RUQ 1347 that were generated from T79/501. This indicates that T79/501 parent is not compatible with both the new and old introductions, even though it has been in the seed garden for many years. It was among the 11 open pollinated F2 seed trees that were selected and distributed to farmers in Ghana and other West African countries in the early 1950s to replace the Amelonado cocoa that was being destroyed by the swollen shoot disease (Glendinning, 1957; Brunt, 1975; Adu-Ampomah *et al.*, 1999). They were released without testing for their combining ability potential and this may account for their poor GCA effects in this study.

Among the male parents, Pound 10 and CRG 6035 contributed positive GCA effects for bean yield. This agrees with the mean performance, where hybrid families derived from Pound 10 and CRG 6035 were associated with higher bean yield. Parent CRG 6035 is a new variety developed at CRIG and Pound 10 is an old seed garden parent, all from the upper Amazon genetic group. Using them as parents in breeding programmes that seeks to improve on yield on one hand, and their addition as male parental clones to those currently used in producing cocoa varieties in the seed gardens on the other hand is recommended based on the present observations.

With respect to the international clones (RUQ 1347, MAN 15/2 and PA 121) used as males, they all had negative GCA influence for yield. This indicates that they generally combine poorly with the seed garden parents used as females in Ghana and other West African countries. This implies that high yielding clones do not necessarily result in good GCA in their progenies. To better inform on the relevance of clones MAN 15-2 and PA 121 in cocoa breeding in Ghana and West Africa at large, further genetic analysis of these clones in combination with clones other than those used in this study may be required. This is evidenced as some specific-cross progenies such as MAN 15-2 × RUQ 1347, T60/887 × MAN 15-2 and T60/887 × PA 121 generated from the international clone were among the high yielding families.

The significant SCA effects observed for yield with seven being positive and five negatives among the families showed strong variation in SCA. This is consistent with the observation that in crosses T60/887 × MAN 15-2, SCA 6 × PA 121 and PA 150 × MAN 15-2 that recorded significant positive SCA estimates correspondingly recorded high bean yield. They are potential candidates that could be exploited through heterosis and multi-locational trials to determine their adaptation to different environments. Similarly, crosses PA 7 × MAN 15-2, SCA 6 × MAN 15-2 and T79/501 × PA 121 with significantly negative SCA effects were among the low yielding families. This suggests that these families will contribute low productivity to yield and are not useful for breeding programmes that aim at developing progenies for high yield.

Conclusion

The general covariance model that emerged appropriate with significant family × year interaction effects indicates that the phenotypic value of bean yield is not very consistent in different years. The yield reported in the present study are much higher than the average yield obtained from the seed garden varieties used as standards, suggesting the potential for productivity gains from the use of hybrid families in the present study. Among the recommended cocoa clones used as males, only CRG 6035 had good GCA, indicating it potential in developing progenies with high bean yield. It is recommended to the seed garden breeding system for production of high yielding cocoa varieties for farmers.

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