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Breeding Value of Cocoa (*Theobroma cacao* L.) for Pod and Bean Traits: A Consequential Advance in Nigerian Cocoa Breeding Program

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Abstract

The understanding of the generated hybrids' breeding value gives a measure of probable advances in a breeding program. Four cocoa genotypes (testers) were crossed with two distinct lines (T65/7 and T86/2). The parents and the hybrids were separately established in randomized complete block design with six replications. The fourteen genotypes were evaluated for pod length (PL), weight (PW), beans/pod (NoB), bean length (BL), width (Bwi) and thickness (BT). The treatment, parent, crosses and Line x Tester sources of variation were significant (P ≤ 0.05). Hybrids from the same maternal parent differed significantly (P < 0.05) for the six traits. Contribution to the total variance of PL, PW and Bwi were in the following order: Tester > Line x Tester > Lines. The highest general combining ability (0.42) occurred in T65/7 for PW; the least (-0.081) occurred in T86/2 for PL. Cross combination T65/7xT57/22 produced the highest specific ability of combination (4.33) for NoB. Variance of GCA and SCA were significant (P < 0.05) for the six traits. The GCA/SCA ratios revealed the inheritance of PL, PW, Bwi and BT to be additive. Non-additive gene effect controlled NoB and BL. Heterosis for the six traits ranged between -17.82% for BT (T65/7xT57/22) to 52.40% for PW (T65/7xT53/8). Increased productivity in cocoa is possible through hybrid breeding programs.

Keywords: combining abilities, heterosis, line and tester analysis, parents, progenies

Introduction

It is known that breeding success at tree crops is slow; scaling the process of the right parents' choice for generating high quality hybrids is another challenge. A concerted breeding effort on tree crops, such as cocoa, often leads to significant improvement in quality, yield and other economic traits. Effective breeding will take advantage of available genetic resources into an improvement program to achieve high level of heterosis for desired traits. Hladni *et al.* (2008) remarked that the act of obtaining plant genetic resources with desirable traits for pairing as male and female parents, to produce superior F1 progeny over the existing genotypes, is a main precondition for designing a model in hybrid breeding program. Therefore, the success in crop breeding through hybridization is dependent on the identification, selection and utilization of the right parents.

Line and Tester analysis (Kempthorne, 1957) is significantly relevant in the testing procedure of highly performing parents' selection. The knowledge of the general and specific combining abilities (GCA and SCA) is useful to assess heterosis among genotypes. The critical information guides the choice of the right parent(s) and elucidates the nature and magnitude of gene actions determining the phenotypic expression of traits in the resultant crosses (Rashid *et al.*, 2007; Salgotra *et al.,* 2009; Sanghera and Hussain, 2012; Sarker *et al.,* 2002). The concept of combining ability is fundamental in enhancing productivity among different crops, especially superior F1 hybrids production (Ali *et al.*, 2012). The identification of proven parents for further breeding programs and expansion of existing seed garden hectarage for continuous hybrid pod generation remains a contextual annual objective in the cocoa breeding scheme.

The production of hybrid varieties is a feasible strategy to achieve increased yield in crops. The cultivation of hybrid cocoa seedling in Brazil led to a heave in the production of the country over the years (Dias *et al.*, 2003). Hybrid breeding programs have remarkably contributed to yield, disease/pest resistance, nutritional qualities improvement, adaptability etc. The breeding value of an offspring or hybrid is measured by heterosis and heritability, most reliably the narrow sense heritability. Heterosis is an unexpected but beneficial and significant positive deviation

from the average of the two parental lines (Kendall *et al.*, 2013). Heterosis conceals inferior genes in a hybrid; its utilization is effective for the genetic improvement of different traits.

The generation of high quality hybrid pods, to raise seedlings for new cocoa plantations, is ensured by the establishment and upgrading of cocoa seed gardens with high quality parental stock. Information on combining abilities through Line x Tester mating design guide breeders to select parental stocks with good GCA and SCA for polyclonal and biclonal seed gardens, respectively. Therefore, the objectives of the present study include: to investigate combining ability of six cocoa clones for some pod and bean traits, to identify parents with desirable GCA, cross combinations with desirable SCA and to understand the nature of gene action involved in the studied pod and bean characters.

Materials and methods

In the bid to investigate the breeding value of some cocoa hybrids for pod and bean traits, a Line and Tester mating design was carried out on six parental cocoa clones. Hybridization through hand pollination was carried out between two Lines and four Testers to generate hybrid pods, in 1999. Budwood from the six parents was collected and clonal seedlings were generated through patch budding. Seedlings from the hybrid pods and the parental clonal seedlings were established in the planting season of 2000. The parent and progeny plots were settled separately in the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria in a randomized complete block design with six replications. Tab. 1 shows the genetic identity of the parents and the offspring. Owing to the fitness of the experiment, to the assumption of uniformity in development (Kempthorne, 1957; Singh and Chaudhary, 1985) of the Line and Tester analysis; fifteen uniformly matured and ripe cocoa pods were selected and harvested from each genotype/plot in the parent and hybrid plots in 2011. Pod length, pod weight, number of beans per pod, length, width and thickness of dried beans were recorded. The data was subjected to Line and Tester analysis following the procedure of Singh and Chaudhary (1985), leading to the estimates of the general and specific combining abilities (GCA and SCA), their standard errors and proportion of contribution to total variance etc. The significance of GCA and SCA were tested following the procedure of Abrha *et al.* (2013) such that the corresponding GCA and SCA values were divided by their respective standard error to obtain the t-calculated value. T-calculated was compared with the t-tabulated value at error degree of freedom. Mid-parent heterosis was calculated as follows:

Heterosis = [F1-(P1+P2)/2]/[(P1+P2)/2]*100;

where: F1 is the value of the hybrid for a trait, and P1 and P2 are the values of each parents for the same trait.

Results and discussion

The means of the two lines differed significantly (P≤ 0.05) for five of the six pod and bean traits (Tab. 2). Line 1 (T65/7) had a higher mean for PL, PW, BL, Bwi and BT. Significant (P≤ 0.05) differences existed among the means of the four testers for each of the six traits except for the

number of beans per pod as shown in Tab. 2. Variation in treatment and the component of the treatment (parent, crosses and parent vs. crosses) differed significantly (P ≤ 0.01) for all the characters except the bean length (Tab. 3). However, significant (P ≤ 0.05) variation due to parent and parent vs. crosses was limited to four and three of the six quantitative traits respectively (Tab. 3). Moreover, in Tab. 3, significant (P ≤ 0.05) and highly significant (P ≤0.01) differences occurred among line x tester interaction for bean width, number of beans per pod and bean thickness. The proportion of the variance of GCA to SCA was higher than one (1.00) in PL, PW, Bwi and BT, however, GCA/SCA proportion was lower than 1.00 in NoB and BL (Tab. 3).

Tab. 4 presents the proportional contribution of the Lines, Testers and the interaction of both with respect to the six studied traits. The Tester evidently played an important role toward the phenotypic expression of pod length (85.45%) and pod weight (80.77%). Contribution to the phenotypic expression of the line and the tester was fairly the same for bean width and bean thickness; although, the Testers had higher proportional contribution by magnitude (Tab. 4). The contribution of Line x Tester interaction was highest for NoB (66.15%), BL (49.13%) and BT (36.06%). The results of the general combining ability for the Lines and Testers are presented in Tab. 5. Significant GCA mean squares for Lines occurred only for PW, Bwi and BT (P≤ 0.05). Among the Testers GCA, significant variance was observed in T3 for PL, PW, NoB, and BT, T4 for BT, T5 for PL, PW and NoB and T6 for BL and Bwi (P≤ 0.05). From Tab. 5, while the GCA effects of T4 (BT), T5 (PL, PW and NoB) and T6 (Bwi) were negative, positive and significant GCA were observed for T3 (PL, PW and NoB) and T6 (BL).

The cross combinations between L1 and L2 with T3 and T4 gave opposite (positive and negative) significant SCA estimates for PL, PW, Bwi and BT (P≤ 0.05). The SCA estimates for L1xT6 and L2xT5 were positive for NoB, BL, Bwi and BT. However, negative but significant SCA estimates was observed for the same traits in the crosses combination between L1xT5 and L2xT6 (Tab. 6). The best specific combiners for optimum pod and bean length were: L2xT3 and L1xT6 respectively. While L2xT4 then L1xT3 were the best combiners for PW, L1xT4 then L2xT4 were identified from Tab. 6 as the best specific combiners for NoB. Three and four respective good specific cross combiners were identified for BT and Bwi in Tab. 6.

Percent mid-parent heterosis (MPH) for the six traits is presented in Tab. 7. The percent heterosis varied from cross to cross and between traits. Generally, the percentage range of MPH in this study was -17.82 (L1xT4 for BT) to 52.40 (L1xT6 for PW). For the six traits, the eight crosses exhibited both positive and negative MPH. The percentage MPH ranges for the eight crosses for each of the six traits were: 10.96 (PL), 65.00 (PW), 28.81 (NoB), 33.67 (BL), 24.54 (Bwi) and 34.27 (BT). The least and highest variation occurred in PL and PW respectively (Tab. 7). Among the eight crosses, only L2xT4 (T86/2xT57/22) displayed positive MPH of approximate 1.00% – 12.5% for the six traits. The trait with the highest cumulative MPH (16.94%) in this study was PW; the least (-3.32%) occurred in BT (Tab. 7). Heterosis for PW (36.49%) was higher in all the crosses relating to Line 1. Crosses, which had Line 2 as the

maternal parent showed quite high heterosis for NoB, BT and BL (Tab. 7). Based on the heterosis value in Tab. 7, the respective best crosses for each trait were: L1xT4 (PL), L1xT6 (PW), L2xT5 (NoB), L1xT6 (BL), L2xT3 (Bwi) and L2xT3 (BT). The worst crosses were: L1xT3 (PL), L2xT3 (PW), L1xT4 (NoB), L1xT5 (BL), L1xT5 (Bwi) and L1xT4 (BT).

The identified significant variation among the Lines and the Testers presents a justified platform for the selection of the six parents. The outcome of the 2 x 4 crosses was the generation of new cocoa variants with improved performances. Use of parents with wider genetic distance for hybridization program assures the creation of new variants and the increase of genetic resources in a germplasm (Adewale *et al.*, 2013). The significant differences observed among the parents and their F1 hybrids for most of the traits studied indicates the presence of genetic differences among the genotypes. The higher pod weight of T65/7 and the higher bean width and thickness of T86/2 may have informed their choice as female parents. Moreover, according to Abrha *et al.* (2013), the non-significant mean square observed in the Lines and Testers for some of the traits in this study may suggests that the Lines and the

Testers used had comparable potential for the studied traits.

While the GCA represents the fixable component of genetic variance and is important to develop superior genotypes, the SCA represents the non-fixable component of genetic variation, which is important to provide information on hybrid performance (Mohammed, 2009). Hladni *et al.* (2008) and Mohammed (2009) specifically observed significant variation between the GCA for Lines and the Testers and SCA for the crosses. The greater than 1.00 value from the GCA:SCA for pod length, weight, bean width and thickness obtained in this study agrees with the results of some authors e.g. Ganesen and Rangaswamy (1997) and Singh *et al.* (1996). Therefore, with reference to the remarks of Hladni *et al.* (2003), Sarker *et al.* (2002) and Rashid *et al.* (2007), the responsible gene action for pod length, weight, bean width and thickness in the studied cocoa genotypes is additive. Identification of the preponderance of additive gene action makes progeny selection very effective because heritability of such traits will be very reliable (Hladni *et al.*, 2003; Abrha *et al.*, 2013).

Testers were more important in the determination of the phenotypic expression of pod length and weight; indicating predominant paternal influence for the two traits. Maternal genetic influence from the Lines was poor in influencing the expression of most of the studied traits. Proportionally high Line x Tester contribution to variance indicates higher estimates of specific combining abilities variance effects (Sanghera and Hussain, 2012). This proved to be true for number of beans per pod and bean length in this study. However, with reference to the less than a unit (< 1.0) GCA/SCA ratio for number of beans per pod and bean length, the implicated gene action is non-additive. Hladni *et al.* (2008) remarked that non-additive genetic component was responsible for the inheritance of plant length and the total leaf area in sunflower. The presence of non-additive genetic variance is the primary justification for initiating hybrid program (Pradhan *et al.*, 2006; Sanghera and Hussain, 2012). Fortunately, T65/7xT57/22 and T65/7xT9/15 were two of the eight cocoa hybrids released to Nigeria farmers in 2011 as CRIN Tc 4 and CRIN Tc 8 respectively (CRIN, 2011).

Negative GCA is undesirable for all the studied characters. An initial study (Adewale *et al.*, 2013) identified strong and significant positive correlation among some of the traits in the present study. The positive and significant GCA effect of T9/15 (for pod length, weight, number of beans per pod and bean thickness) and T53/8 for bean

Tab. 1. Genetic identity of the cocoa genotypes

Codes	Pedigree	Line and Tester Identity
G36	T65/7	L1
G39	T86/2	L2
G35	T9/15	T3
G26	T57/22	T4
G28	T22/28	T5
G32	T53/8	T6
G3	T65/7 x T9/15	L1xT3
G7	T65/7 x T57/22	L1xT4
G1	T65/7 x T22/28	L1xT5
G18	T65/7 x T53/8	L1xT6
G12	T86/2 x T9/15	L2xT3
G24	T86/2 x T57/22	L2xT4
G15	T86/2 x T22/28	L2xT5
G20	T86/2 x T53/8	L2xT6

NB: L = Line(s), T = Tester(s)

Tab. 2. Descriptive statistics of the two lines and the four testers with respect to the six traits

Traits	Means of Lines			Means of Testers				
	L1	L2	LSD(0.05)	T3	T4	T5	T6	LSD(0.05)
PL	20.28	19.29	0.9111	25.64	19.36	18.85	21.15	2.3271
PW	26.23	24.11	0.5394	30.10	24.36	27.69	28.53	2.0386
NoB	36.84	35.50	4.92	36.98	34.59	30.61	35.58	7.5913
BL	2.41	2.18	0.1308	2.44	2.16	2.54	2.45	0.1615
Bwi	1.30	1.24	0.0544	1.30	1.29	1.44	1.33	0.0771
BT	1.06	0.911	0.0820	1.00	0.90	0.91	0.94	0.0776

NB: PL = Pod length, PW = Pod weight, NoB = Number of beans, BL = Bean length, Bwi = Bean width and BT = Bean thickness

Tab. 3. Analysis of variance summary of the two lines by four testers

Sources of variation	DF	Traits/Mean Squares					
		PL	PW	NoB	BL	Bwi	BT
Treatment	13	19.498**	23.196**	68.214**	0.284	0.029**	0.029**
Parent	5	38.292**	28.856**	32.801	0.141	0.027**	0.027**
Crosses	7	8.732**	15.044**	90.853**	0.360	0.027**	0.035**
Par Vs Cro	1	0.893	51.958*	86.806**	0.467	0.045**	0.0004
Line	1	0.311	8.577	8.227	0.134	0.058	0.075
Tester	3	17.410	28.352	69.006	0.382	0.022	0.027
Line x Tester	3	2.860	3.891	140.242**	0.412	0.022*	0.030**
Error	65	2.364	1.704	18.168	0.235	0.006	0.006
σ^2gca		0.709	1.929	7.106	0.0067	0.0045	0.0059
σ^2sca		0.036	0.202	14.502	0.013	0.0017	0.0027
σ^2gca/σ^2sca		19.430	9.531	0.489	0.547	2.531	2.183

NB: Par Vs Cro = Parent Vs Crosses, σ^2 = Mean squares (variance), PL = Pod length, PW = Pod weight, NoB = Number of beans, BL = Bean length, Bwi = Bean width and BT = Bean thickness

Tab. 4. Proportion of contribution to the variance

Compo nents	Traits					
	PL (%)	PW (%)	NoB (%)	BL (%)	Bwi (%)	BT (%)
Line	0.51	8.14	1.29	5.34	30.65	30.59
Tester	85.45	80.77	32.55	45.53	35.43	33.35
Line x Tester	14.04	11.09	66.15	49.13	33.92	36.06

NB: PL = Pod length, PW = Pod weight, NoB = Number of beans, BL = Bean length, Bwi = Bean width and BT = Bean thickness

length makes both potential paternal parents for the identified traits. Selection of the genotypes for the corresponding traits will be effective in subsequent hybrids development for productive pod and bean qualities. Owing to the prolific status of the studied genotypes as reflected by their high GCA, a polyclonal seeds garden plot may be initiated to ensure different hybrid pod generation and distribution to farmers for planting.

The existence of both positive and negative SCA effects had been also reported in many crops (El-Gazzar and Khalil, 2012; Kanagarasu et al., 2010; Mohammed, 2009; Sanghera and Hussain, 2012). A significant SCA effects among crosses indicates significant deviation from predictable parental performances (Abrha et al., 2013). These crosses with highly positive and significant estimates for SCA could be selected for use in cocoa breeding programs. The understanding of the SCA status of cocoa clones is a requisite for initiating biclonal seed garden plots; as this leads to the generation of high quality hybrid pods. While a specific hybrid remains the derivative from the plot, hand pollination may become unnecessary for hybrid pod generation in such a plot.

Heterosis differs among genotypes (hybrids) of plants and animals for various quantitative traits. The observed differences in the value of heterosis for each of the eight crosses with respect to the six traits is consistent with the result of many authors (Alam et al., 2004; Ali et al., 2012; Rashid et al., 2007). The ultimate aim of hybrid breeding

Tab. 5. Estimates of the general combining abilities of the lines and the testers for each of the six traits

	PL	PW	NoB	BL	Bwi	BT
	GCA for Lines					
L1	0.0805	0.4227*	-0.4140	0.0529	-0.0349*	-0.0396*
L2	-0.0805	-0.4227*	0.4140	-0.0529	0.0349*	0.0396*
SE(g$_i$)	0.632625	0.537	1.753675	0.199475	0.030725	0.03125
SE(g$_i$-g$_i$)	0.4439	0.3768	1.2305	0.1400	0.0216	0.0219
	GCA for Testers					
T3	1.6387**	2.0638**	2.5126*	-0.0310	0.0571	0.0654**
T4	-0.1327	-0.5896	1.4967	-0.0915	0.0099	-0.0468*
T5	-1.2607**	-1.5721**	-2.5795*	-0.1371	-0.0301	0.0004
T6	-0.2454	0.0979	-1.4299	0.2596*	-0.0368*	-0.0190
SE(g$_i$)	0.8947	0.759425	2.480075	0.2821	0.04345	0.0442
SE(g$_i$-g$_i$)	0.3139	0.2664	0.8701	0.0990	0.0152	0.0155

NB: L = Line, T = Tester, PL = Pod length, PW = Pod weight, NoB = Number of beans, BL = Bean length, Bwi = Bean width and BT = Bean thickness

Tab. 6. Specific combining abilities of each line with each tester for the six traits

Crosses	PL	PW	NoB	BL	Bwi	BT
L1xT3	-0.5843*	0.4969*	-0.8380	-0.1457	-0.0351*	-0.0354*
L1xT4	0.5050	-0.7854*	4.3326**	-0.0163	0.0410*	0.0001
L1xT5	-0.2007	-0.0394	-3.9245**	-0.1040	-0.0379*	-0.0349*
L1xT6	0.2800	0.3278	0.4299	0.2660*	0.0321*	0.0701*
L2xT3	0.5843*	-0.4969*	0.8380	0.1457	0.0351*	0.0354*
L2xT4	-0.5050	0.7854*	-4.3326**	0.0162	-0.0410*	-0.0001
L2xT5	0.2007	0.0394	3.9245**	0.1040	0.0379*	0.0349*
L2xT6	-0.2800	-0.3278	-0.4299	-0.2660*	-0.0321*	-0.0701*
SE(g_i)	0.759165	0.644415	2.10441	0.23937	0.03687	0.037515
SE($S_{ij}-S_{kl}$)	0.6278	0.5329	1.7401	0.1979	0.0305	0.0310

NB: L = Line, T = Tester, PL = Pod length, PW = Pod weight, NoB = Number of beans, BL = Bean length, Bwi = Bean width and BT = Bean thickness

Tab. 7. Mid-parent heterotic performance of the eight hybrids for the six traits

Hybrids	PL	PW	NoB	BL	Bwi	BT
L1xT3	-4.572	25.242	7.986	-1.07	-3.017	-16.272
L1xT4	6.385	37.216	-14.858	8.104	-0.445	-17.819
L1xT5	-0.854	31.088	6.59	-5.268	-14.222	-16.743
L1xT6	3.496	52.401	-8.684	28.403	-6.233	-13.075
Mean	1.114	36.487	-2.242	7.542	-5.979	-15.977
L2xT3	1.671	-12.601	13.057	11.597	10.323	16.447
L2xT4	3.443	12.494	2.472	10.066	0.972	7.141
L2xT5	2.501	-6.483	13.95	3.595	-1.323	15.344
L2xT6	-0.145	-3.833	9.121	6.279	-3.127	-1.607
Mean	1.867	-2.605	9.650	7.884	1.711	9.331
Grand Mean	1.491	16.941	3.704	7.713	-2.134	-3.323

NB: PL = Pod length, PW = Pod weight, NoB = Number of beans, BL = Bean length, Bwi = Bean width and BT = Bean thickness

program is to gain heterosis for the trait of interest. Heavier pods in most cases contain many beans and or few bigger beans. In this study, T65/7xT53/8 and T86/2xT22/28 were outstanding among the eight crosses for pod weight and number of beans per pod; they can therefore be considered for further studies. Moreover, their release as hybrids would be ideal (Jain and Sastry, 2012). Hybrids with T65/7 as a maternal parent produced positive heterosis for pod length, weight and bean length. All hybrids with T86/2 as maternal parent produced positive heterosis for all the studied traits except pod weight.

Conclusions

One of the major goals of cocoa breeding is to increase the number of beans per pod and the bean weight, so any line with a positive GCA value for these traits is considered desirable in a cocoa breeding program. Availability of heritable genetic variation in a germplasm provides a reliable basis in breeding programs. The understanding of their general combining abilities suggests unmistakable guide to selection. Cross combinations with additive gene effect and superior heterotic performance for pod and bean trait will be a hybrid of significant preference. The justification of non-recommendation of open-pollinated pods to farmers for cultivation is clinched on the indefinite pedigree status and unknown productive potentials of such pods. Continuous intraspecific hybridization within available germplasm of cocoa ought to remain a routine breeding program for step-wise advances of cocoa genetic improvement.

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