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Genotype and within-pod bean position microenvironment effect on seed choice for raising cocoa (*Theobroma cacao* L.) seedlings

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Abstract: The probable role of within-pod microenvironment on seed sizes, seedling vigour and biomass yield of four cocoa genotypes was investigated for two years. The respective main, sub and sub-sub plots in the split-split plot experimental design were years, genotypes and within-pod bean positions. Data were taken on cocoa bean length, width and thickness after each pod was opened. Four weekly periodic data were obtained for plant height (PH), stem girth (SG) and number of leaves (NOL); root and shoot biomass yield were also recorded. Analysis of variance revealed significant ($p \leq 0.05$) bean position, genotypes, years and some interaction effect on the studied traits. Means of the levels of the three factors differed significantly ($p \leq 0.05$). Proximal, middle and distal positions were distinct within-pod microenvironments. The pod middle cavity housed the longest, widest and heaviest beans. Trend analysis of the growing sequences of NOL, PH and SG by the four genotypes differed with bean locations. For bean length, GGE biplot respectively identified CRIN Tc1, CRIN Tc2 and CRIN Tc3 as the best genotype for middle, proximal and the distal positions. The intra-locular space within the pod enhanced differential seed development and maturation; this was evident in the seedling vigour.

Key words: bean position; cocoa; micro-environments; GGE biplot; seedling vigour

Genotip in mikrookolje glede na položaj semena znotraj ploda vplivata na izbor semen kakavovca (*Theobroma cacao* L.) za vzgojo sadik

Izvleček: Vplivi mikrookolja znotraj plodne glavice na velikost semen, vigor sadik in pridelek biomase so bili preučevani pri štirih genotipih kakavovca v dveh zaporednih rastnih sezonah. Poskus je bil zasnovan na glavnih ploskvah in treh vrstah podploskev kot poskus z deljenkami glede na leto poskusa, genotipe in položaj semen znotraj glavice. Izmerjeni so bili dolžina, širina in višina semen kakavovca potem, ko so se plodovi odprli. Na štiri tedne so bili izmerjeni višina rastlin (PH), obseg debla (SG) in število listov (NOL); izmerjeni sta bili tudi biomasa korenin in poganjkov. Analiza variance je pokazala značilno povezavo ($p \leq 0{,}05$) med položajem semen v plodu, genotipom, letom poskusa in nekatere interakcije med preučevanimi znaki. Poprečki vrednosti znakov semen glede na položaj v plodu so se razlikovali statistično značilno ($p \leq 0{,}05$). Proksimalna, srednja in distalna pozicija v plodu so imele značilna mikrookolja. Osrednja votlina ploda je imela najdaljša, najširša in najtežja semena. Analiza trendov je pokazala naraščajoče vrednosti znakov NOL, PH in SG za vse štiri genotipe in položaj v plodu. Za dolžino semen so bili na osnovi GGE biplota identificirani genotipi CRIN Tc1, CRIN Tc2 in CRIN Tc3 kot najboljši za srednji, proksimalni in distalni položaj v plodu. Intralokularni prostor v plodu je vzpodbudil diferencialni razvoj semen in njihovo zorenje, kar je bilo očitno tudi na vigorju sejank.

Ključne besede: položaj semen; kakavovec; mikrookolje; GGE biplot; vigor sejank

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1 INTRODUCTION

Cocoa (*Theobroma cacao* L.), a native crop of South America is well adapted to and flourishes productively in the rainforest ecology of West and Central Africa. The region accounts for the largest proportion of global production, especially from Cote d'Ivoire, Ghana, Cameroon and Nigeria. The economic product (i.e. the beans) whose number ranges between 20 to 60 per pod (Ortiz, 2016) are basic raw material for the production of chocolate (Motamayor et al., 2008; Amma et al., 2011).

Among the four major cocoa producing member states in the West and Central Africa, production in Nigeria is at the last place. This has grossly being attributed to low yield from most farmers' field. Mathew et al. (2012) identified the use of low quality seed for raising seedlings, low emergence and poor seedling vigour as some of the factors responsible for low productivity within the plantation. In plantation cropping, seedlings obtained from the nursery influences establishment in the field and hence the productivity in the orchard (Baiyeri, 2006). Therefore, cocoa beans meant for propagation to raise seedlings are expected to have completed their structural and functional development within the pod (the fruit) before they are plucked for use in raising seedlings (Opoku-Ameyaw et al., 2010).

The customary practice for cocoa plantation establishment in West and Central Africa has been based on use of seeds in order to generate planting material (Adewale et al., 2016). The observed norm among the farmers has been indiscriminate use of all seeds within the pod irrespective of bean size differences and the location within the pod where they are housed. Seed sizes of genotypes is not only a result of the genetic structure but also some other contributory factors. Seed growth and development is dependent on the biomass investment from the mother plant, such that seeds with high resources have bigger size. Moreover, the endosperm content determines seed sizes which differs significantly within the same fruit (Susko and Lovett-Doust, 2000; Khan et al., 2014). Why should there be significant variation in the seed sizes from the same developmental locations? Khan et al. (2014) identified: within-pod resource quantity, fertilization gradients and neighbor effect to be among some of the probable factors responsible for within-pod seed size variation. Nakamura (1988) identified proximity of the ovules to the stylar end as another important factor in *Phaseolus vulgaris* L.; noting that seeds closer to the style (proximal) end were significantly better in size than those nearer to the receptacle (the distal) end.

Giles (1990) strongly remarked that the within-plant variance cannot be interpreted as anything other than a random environmental variance effect; it is equally obvious that the variation in the sizes of seeds from the same pod is predominantly due to within-pod microenvironment. The fruit (pod) is a controlled environment but within it, there is environmental deviation (Singh and Pokhriyah, 2001) or intra-locular variation due to differential variation in space along the length from one end to the other. The unequal intra-ovary volume/space along the pod length affects and determines the phenotypic development of the seeds they host in various locations along the length.

The fruits provide the environmental space for the seeds and protects the seeds from unfavorable biotic and abiotic condition. However, Bennett et al. (2011) further hinted that the function of the pod to the seeds is far beyond safeguarding them to maturity as an environment, it equally regulates seed growth and maturation. The location/apartment where different seeds appears within the fruit has a role to play in the resource distribution and sharing scheme within the pods (Lee, 1988). In cocoa, Ibikunle (1967) noted that seeds which developed in the expanded (i.e. middle) part of the cocoa pod produced bigger-sized beans and hence seedlings with better vigour.

Poor seedling survival at nursery and reduced population of established seedlings on the field are among the attending problem of using all healthy beans from every portions within the cocoa pod. The knowledge of cocoa seedling performance based on positional location within the pod is a needed quest, hence the present investigation, so that beans with no promising vigour will be excluded from use in the propagation scheme. Reports from seed quality test for field establishment of maize (Cruz- Garcia et al., 1995; Moreno-Martinez et al., 1998), barley (Copeland and McDonald, 2001) and rapeseed (Ghassemi-Golezani et al., 2010) noted that low quality seeds produces low vigour seedlings with very poor field establishment. The present study was therefore proposed because there is rare information on the typical role of bean position in the pod on the structural development of the beans. Attempts by Iremiren et al. (2007) and Hammed et al. (2013) were on a single genotype each, hence, genotype and within-pod environment interaction on seedling vigour could not be highlighted in their investigations.

Consideration of the housing environment of the cocoa bean as a link to the expression of seedling vigour has not been well attempted in cocoa, hence the need for the present investigation. Highlighting the significance of the bean position within the pod, its relevance to determining bean size and hence, the seedling vigour is worthwhile for identification of portion(s) within the fruit where the most suitable beans capable of supporting good seedling vigour for optimum field establishment is/

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are located. Therefore, the two years replicated experiment which employed four different cocoa genotypes has the following objectives: to identify the role of bean positions within the pod and its interaction with genotypes in the determination of growth and developmental traits of cocoa seedlings.

2 MATERIALS AND METHODS

With an interest to understand the relative differential performances of cocoa seeds, usually called beans in the different localized positions within the fruit capsules called pods of different cocoa genotypes and the possible link of the same to seedling vigour and development; a research was conducted for two consecutive years at the Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Ibadan, Nigeria. Pods for the experiment were obtained from the hybrid trial plot established in 1999 at the institute. Four physiologically matured cocoa pods were harvested per genotype during the main season (October-November) in three replications in 2014 and 2015. The four hybrids genotypes (CRIN 2011) used for the study were: CRIN Tc1 (T65/7 × N38), CRIN Tc2 (T101/15 × N38), CRIN Tc3 (P7 × PA150), and CRIN Tc4 (T56/7 × T57/22). Each fruit was longitudinally opened and beans were partitioned based on their nearness to the two ends of the pod and the middle as: the proximal (toward the stylar tip), middle (the expanded portion of the fruit) and distal (part closest to the receptacle). An image of cocoa pod delineating the three sections within the pod is shown in Plate 1.

Beans within each class were cleaned with sawdust to remove the mucilaginous pulp. Twenty beans were sampled for each of the three positions per genotype and metric measurements on length, width and thickness were taken on the cleaned beans using the venier caliper following Omokhafe and Alika (2004) and Kaushik et al. (2007). Individual mass of the sampled beans for the three positions of each genotypes were also measured and recorded.

The three classified groups of beans per genotype were pre-germinated for 72 hours before they were sown into the polythene bags in the nursery. This was repeated for two years. The experimental design employed was split-split plot design with years, genotypes and bean positions as main, sub and sub-sub plot factors respectively. The number of replications used was three.

Among the measurements taken on the seedlings after germination were: number of leaves per plant, plant height and stem girth. Data on the morphological characteristics continued from 2nd weeks after seedling emergence to the sixth month. Destructive sampling was done for the sampling unit after the termination of the experiment to obtain the fresh and dry root, shoot and total biomass yield. The data were subjected to analysis of variance (ANOVA) and means of the different main effects were separated by Tukey's honestly significant differences. The association between the bean indices and the harvested biomass after destructive sampling were tested by correlation analysis. All analysis were carried out in SAS (version 9.4, 2011). Furthermore, trend analysis was done using R Development Core Team (2013) to understand the sequence of response of the growth data taken at intervals.

Traits with significant genotype by bean position interactions from the ANOVA were further partitioned using the "which won where" option in the GGE biplot in GEA-R (Pacheco et al., 2016). From the component of the ANOVA, genetic estimates were calculated for phenotypic and genotypic coefficients of variation (PCV

Plate 1: The three cross-sections of an opened cocoa pod housing cocoa beans

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and GCV) following the method of Singh and Chaudhay (1999):

$PCV = (\sigma^2_p / X)^{½}$……...........…………………. .Eq. 1

$GCV = (\sigma^2_g / X)^{½}$……..…Eq. 2

Where σ^2_p, σ^2_g and X are phenotypic and genotypic variances and grand mean respectively.

Broad sense heritability (Hbs) was expressed as the percentage of the genotypic variance to the phenotypic variance for split-split plot design as described by Bokmeyer et al. (2009), cited in Clark and Watkins (2012) and modified as follows:

$Hbs = \sigma^2_g / \sigma^2_g + \sigma^2_{gy/y} + \sigma^2_{gp/p} + \sigma^2_{gr(y)/rp} + \sigma^2_{gyp/yp +} \sigma^2_{e/ryp}$ ……
……...Eq. 3

Repeatability (r_c) was estimated following Ortiz and Ng (2000), as follows:

$r_{c =} \sigma^2_g/(\sigma^2_y + \sigma^2_{gy})$ ….....................…………….Eq. 4

3 RESULTS

Table 1 shows the significant (p < 0.05) differences in the bean metric traits for years, varieties, within-pod

Table 1: Variance components for the bean metric traits, correlations among them and their mean performances based on years, varieties and bean position

Sources of Variation	DF	BL (cm)	BW (cm)	BT (cm)	BM. (g)
Replications	2	0.04	0.03	0.02	0.08
Years	1	4.86***	0.03	1.34***	5.08***
Error (a)	2	0.05	0.03	0.04	0.05
Varieties	3	3.36***	2.89***	2.24***	8.42***
Years*Varieties	3	0.98***	0.52***	0.42***	3.69***
Error (b)	12	0.04	0.03	0.03*	0.06
Bean Positions	2	1.35***	0.62***	0.56***	1.23***
Years* Bean Positions	2	0.87***	0.06	0.04	0.58***
Varieties* Bean Positions	6	0.27***	0.07**	0.36***	0.45***
Years*Varieties* Bean Positions	6	0.35***	0.04	0.15***	0.57***
Error (c)	32	0.05	0.02	0.02	0.08
	Correlations among the three bean metric traits with bean mass				
Bean mass	0.96ns	0.99*	0.99**	-	
	Mean separation of the three main effects				
Years	2014	2.11b	1.14a	0.65b	1.68a
	2015	2.31a	1.13a	0.76a	1.48b
Varieties	CRIN Tc 1	2.37a	1.27a	0.83a	1.89a
	CRIN Tc 2	2.34a	1.26a	0.81a	1.71b
	CRIN Tc 3	2.07b	0.95c	0.53c	1.33c
	CRIN Tc 4	2.06b	1.06b	0.66b	1.39c
Bean Positions	Proximal	2.13b	1.11b	0.75a	1.53b
	Middle	2.31a	1.20a	0.64b	1.68a
	Distal	2.19b	1.08b	0.73a	1.53b

† BL - Bean Length, BW - Bean Width, BT - Bean Thickness, BM. - Bean mass.
*, ** and *** - Significance at p = 0.05, 0.01 and 0.001
Mean comparison is along the column for year, varieties and bean position; means with the same alphabet are not significantly different from each other

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bean positions, years by varieties, years by bean positions and varieties by bean position interactions. Bean length and its thickness differed significantly ($p \leq 0.05$) among the two years and higher significant values occurred in 2015. However, bean mass was significantly ($p \leq 0.05$) higher (1.68 g) in 2014 compared to 1.48 g in 2015 (Table 1). Beans length, width and thickness were highest for CRIN Tc1 and 2, but minimum values for the same were obtained in CRIN Tc3. The four metric traits (length, width, thickness and mass) of the beans varied significantly ($p < 0.05$) with positions where the beans were located within the pod. Beans in the middle position had significant ($p < 0.05$) longer, wider and heavier beans mass when compared to beans at the proximal and distal positions (Table 1). Furthermore in Table 1, individual bean mass had strong to very strong and significant ($p \leq 0.01$) correlation with bean width (r = 0.99) and thickness (r = 0.99).

Table 2 shows different sources of variation in the analysis of variance and the mean values of some traits at the termination of the experiment for the beans from the three within-pod environments for the four genotypes in the two years. The fresh shoot mass of the cocoa seedlings was not significantly enhanced in this study (Table

Table 2: Variance components for the seedling biomass traits, correlations among them and their mean performances based on years, varieties and bean position

Sources of Variation	DF	SFM	SDM	RFM	RDM
Replications	2	4.2	2.47	1.24	1.02***
Years	1	6.73	25.32***	0.31	1.89***
Error (a)	2	10.03	0.07	14.42	0.04
Varieties	3	31.32*	1.98*	2.85*	0.49***
Years*Varieties	3	7.47	0.23	3.68*	0.01
Error (b)	12	8.27	0.18	1.07	0.04
Bean Positions	2	11.05	1.74	4.91**	0.46***
Years* Bean Positions	2	14.72	0.45	3.68*	0.01
Varieties* Bean Positions	6	6.29	0.44	2.362	0.12
Years*Varieties* Bean Positions	6	11.34	0.69	1.35	0.06
Error (c)	32	6.02	0.64	1.04	0.06
	Correlations among the fresh and dried biomass of the shoot and root				
Shoot Dry Mass	0.99**	-	0.88ns	0.95ns	
	Mean separation of the three main effects				
Years	2014	9.90a	2.51b	4.69a	0.79b
	2015	10.51a	3.69a	4.56a	1.12a
Varieties	CRIN Tc 1	10.87ab	3.56a	5.20a	1.19a
	CRIN Tc 2	8.73b	2.77b	4.37b	0.93b
	CRIN Tc 3	9.54ab	2.98ab	4.36b	0.89b
	CRIN Tc 4	11.67a	3.09ab	4.58b	0.81b
Bean Positions	Proximal	9.91a	2.99a	4.23b	0.86b
	Middle	10.98a	3.41a	5.12a	1.11a
	Distal	9.72a	2.90a	4.53ab	0.89b

† SFM – Shoot fresh mass, SDM – Shoot dry mass, RFM – Root fresh mass, RDM – Root dry mass*, ** and *** - Significance at p = 0.05, 0.01 and 0.001
Mean comparison is along the column for year, varieties and bean position; means with the same alphabet are not significantly different from each other

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Table 3: Mean performances of the vegetative growth traits across different years varieties and bean positions in the pod

	NOL 6WAS	NOL 10WAS	NOL 14WAS	NOL 18WAS	NOL 22WAS	PH 6WAS	PH 10WAS	PH 14WAS	PH 18WAS	PH 22WAS	SG 6WAS	SG 10WAS	SG 14WAS	SG 18WAS	SG 22WAS
	Year														
2014	4.75b	6.89a	8.80a	10.68a	13.22a	16.43a	19.63a	21.82a	25.67a	27.43a	0.40a	0.58a	0.63b	0.67a	0.92a
2015	5.23a	7.27a	8.53a	9.66b	11.86a	14.89b	18.36b	21.82a	23.41b	27.36a	0.22b	0.45b	0.66a	0.74a	0.82a
	Varieties														
CRIN Tc 1	5.16ab	7.23ab	8.52b	9.99ab	12.32a	16.25a	20.84a	23.77a	25.92a	28.18a	0.31a	0.45a	0.66a	0.75a	0.89a
CRIN Tc 2	4.53c	6.35c	7.81b	9.37b	12.09a	14.65b	17.40b	19.56b	22.95b	26.71b	0.28a	0.57a	0.64a	0.74a	0.81a
CRIN Tc 3	4.70bc	6.88bc	8.60b	10.29ab	12.56a	14.52b	16.88b	19.30b	22.58b	25.56b	0.33a	0.61a	0.70a	0.76a	0.90a
CRIN Tc 4	5.56a	7.87a	9.72a	11.03a	13.19a	17.21a	20.86a	23.70a	26.69a	29.11a	0.33a	0.53a	0.56a	0.68a	0.89a
	Cocoa Beans Position														
Proximal	4.86a	6.80a	8.44a	9.86ab	12.34ab	15.71ab	18.96ab	21.5b	24.22b	27.52ab	0.34a	0.59a	0.65a	0.83a	0.98a
Middle	5.13a	7.31a	9.13a	10.89a	13.42a	16.36a	19.78a	22.78a	25.83a	28.65a	0.34a	0.53a	0.59a	0.65a	0.82a
Distal	4.97a	7.14a	8.42a	9.75b	11.85b	14.91b	18.24b	20.46b	23.56b	26.00b	0.25a	0.51a	0.71a	0.81a	0.81a

NOL-Number of leaves, PH- Plant height, SG-Stem Girth, WAS- weeks after sowing
Mean comparison is along the column for years, varieties and bean positions; means with the same alphabet are not significantly different

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Table 4: Some genetic estimates of some variables

Traits	PCV	GCV	GCV:PCV	Heritability (%)	Repeatability
Bean length	134.63	123.30	91.59	83.88	0.77
Bean width	167.87	159.92	95.26	90.75	0.85
Bean thickness	192.70	178.89	92.83	86.17	0.84
Bean mass	258.05	230.85	89.46	80.03	0.70
Shoot fresh mass	198.76	175.23	88.16	77.73	0.81
Shoot dry mass	88.21	79.92	90.60	82.08	0.90
Root fresh mass	112.68	78.46	69.63	48.48	0.44
Root dry mass	75.88	71.44	94.15	88.64	0.98
NOL6WAS	95.90	87.95	91.71	84.11	0.82
NOL10WAS	107.46	101.40	94.37	89.05	0.92
NOL14WAS	119.43	113.76	95.25	90.73	0.97
NOL18WAS	104.27	91.69	87.93	77.33	0.76
NOL22WAS	96.80	56.48	58.35	34.04	0.29
PH6WAS	153.71	139.67	90.87	82.57	0.73
PH10WAS	216.38	209.40	96.77	93.65	0.91
PH14WAS	241.21	227.25	94.21	88.76	0.83
PH18WAS	189.17	177.50	93.83	88.05	0.82
PH22WAS	166.93	127.03	76.10	57.91	0.50
SG6WAS	53.21	17.96	33.75	11.39	0.11
SG10WAS	94.86	58.25	61.41	37.71	0.41
SG14WAS	126.20	88.47	70.10	49.14	0.47
SG18WAS	56.98	29.07	51.01	26.02	0.23
SG22WAS	56.67	18.57	32.77	10.74	0.10

NOL-Number of leaves, PH- Plant height, SG-Stem girth taken at four weeks interval from the 6th to the 22nd weeks after planting

2), however, the dry shoot mass shown in 2014 had a lead and significant ($p \leq 0.05$) value of 3.69 g compare to 2.51 g in 2015; the trend was same for the dry root mass (Table 2). The middle positional beans produced the highest significant ($p \leq 0.05$) mean for root fresh and dry mass. Neither year nor the three beans position inside the cocoa pod affected the shoot fresh mass. However, among the varieties, the highest fresh and dry root mass value was observed in CRIN Tc1. Moreover, shoot fresh and dry mass had a strong (0.99) and significant ($p \leq 0.01$) correlation (Table 2).

From the means in Table 3, significantly ($p \leq 0.05$) higher mean was obtained for NOL6 weeks after sowing (WAS) and SG14WAS in 2015. However, higher significant ($p \leq 0.05$) values were obtained for NOL18WAS, PH6WAS, PH10WAS, PH18WAS, SG6WAS and SG10WAS in 2014 (Table 3). Among the four genotypes, CRIN Tc1 and CRIN Tc4 had the significantly ($p \leq 0.05$) higher means for number of leaves and plant heights at 6th to the 22nd WAS (Table 3). Beans originating from the middle of the pod produced significantly ($p \leq 0.05$) the highest number of leaves (18 and 22 WAS) and plant height (6th to 22nd WAS) in Table 3.

For all the variables studied in this experiment, the phenotypic coefficient of variation were higher than the genotypic coefficient of variation (Table 4). The proportion of the genetic component in the phenotypic coefficient of variation ranged between 32.77 (SG22WAS) to 96.77 (PH10WAS). Stem girth 22 WAS which had the lowest GCV: PCV, equally had the lowest broad sense heritability (10.74) and repeatability (0.10). The highest (90.75) broad sense heritability occurred in bean width while the highest (0.98) repeatability was recorded for root dry mass (Table 4).

Table 5 unveiled the specific pattern of variability and sequence of response of each of the four genotypes to three vegetative and agronomic variables. The sources of variation from the table includes: the total treatment, each of the two factors within the treatment (i.e. intervals and bean position) and variability based

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Table 5: Trend analysis of the growth traits measured at intervals in correspondence to the three bean positions within the pod for four cocoa varieties

		CRIN Tc 1			CRIN Tc 2		
Sources of Variation	DF	NOL	PH	SG	NOL	PH	SG
		Mean Squares			Mean Squares		
Treatments	14	7.27***	18.14***	0.11	6.99**	17. 49***	0.06
Interval (In)	4	24.54***	58.51***	0.24*	23.84***	55.55***	0.11
In-Linear	1	66.86***	190.71***	0.35*	67.02***	176.61***	0.18
In-Quadratic	1	1.70**	7. 45**	0.15	7.17*	0.55	0.03
In-Cubic	1	1.15*	2.08	0.05	5.7*	0.03	0.01
In-quantic	1	0.05	0.20	0.01	1.89	0.67	0.14
Bean Position(BP)	2	1.17*	8.13**	0.06	0.58	10.35***	0.03
BP-Linear	1	0. 48	0.29	0.10	0.60	18.66***	0.05
BP-Quadratic	1	1.87**	15.98***	0.03	0.57	12.02*	0.01
Error	8	0.15	0. 46	0.05	0.17	0.24	0.04
		CRIN Tc 3			CRIN Tc 4		
Sources of Variation	DF	NOL	PH	SG	NOL	PH	SG
		Mean Squares			Mean Squares		
Treatments	14	9.13**	19.17**	0.04	7.22**	20.05***	0.03***
Interval(In)	4	28.09***	62.76***	0.04	24.26**	63.12***	0.11***
In-Linear	1	83.66***	189.68***	0.03	75.19***	206. 46***	0.28***
In-Quadratic	1	0.29	2. 46	0.02	0.03	1.09	0.12***
In-Cubic	1	0.54	0.14	0.04	0.31	0.56	0.004**
In-quantic	1	0.001	0.34	0.001	0.04	0.17	0.003*
Bean Position(BP)	2	5. 49*	3.15	0.01	1.31**	10.35**	0.0003
BP-Linear	1	2.96*	0.98	0.002	7.38*	3.23	0.00007
BP-Quadratic	1	7.99**	5.32	0.02	7.56*	17. 49**	0.0006
Error	8	0.55	1.39	0.04	0.17	0.93	0.0003

Note. DF - Degree of freedom, *,**,and*** - significance at p- 0.05, 0.01 and 0.001, NOL - Number of leaves, PH -Plant height; SG - Stem girth

on different forms of trend for each of the two factors. Highly significant ($p \leq 0.01$) variabilities were noted for the fifteen treatment combinations and the five intervals of data measurements for number of leaves, plant height and stem girth of the four genotypes. However, there was significant ($p \leq 0.05$) treatments effect on stem girth for CRIN Tc4 while interval effect was equally significant ($p \leq 0.05$) for CRIN Tc1 and 4 for stem girth (Table 5). With respect to bean position as a source of variation, its effect was significantly ($p \leq 0.05$) notable for number of leaves and plant height for CRIN Tc1 and 4; CRIN Tc2 and 3 showed respective significance ($p \leq 0.05$) for plant height and number of leaves (Table 5). Number of leaves showed linear ($p \leq 0.001$), quadratic ($p \leq 0.01$) and cubic ($p \leq 0.05$) response for CRIN Tc1 and CRIN Tc2. Moreover, trend for plant height was linear ($p \leq 0.001$) and quadratic ($p \leq 0.001$) for CRIN Tc1 but only linear for CRIN Tc2 while the trend response for stem girth was only linear ($p \leq 0.05$) for CRIN Tc1 (Table 5). Within the same table, CRIN Tc3 and CRIN Tc4 showed only significant ($p \leq 0.05$) linear response for number of leaves and plant height. Specifically, the stem girth displayed significant ($p \leq 0.05$) linear to quantic responses for CRIN Tc4 (Table 5). Still from Table 5, the trend response with respect to bean position for plant height was both linear ($p \leq 0.05$) and quadratic ($p \leq 0.05$) in CRIN Tc2, number of leaves and plant height displayed quadratic ($p \leq 0.05$) response in CRIN Tc1 and CRIN Tc4. However, for CRIN Tc3 and CRIN Tc4, the number of leaves exhibited both linear ($p \leq 0.05$) and quadratic ($p \leq 0.05$) responses.

Factors 1 and 2 (Figure 1) cumulatively explained the total variance. The proximal, the middle and the distal position were three distinct within-pod environments (each appearing in different sector) for cocoa bean length

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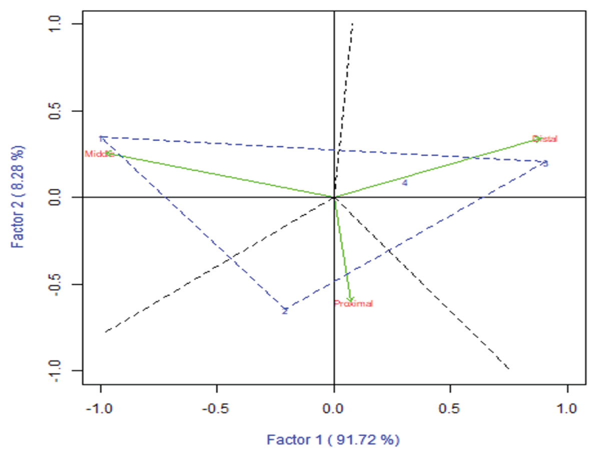

Figure 1: Bean position by varieties interaction display for cocoa bean length
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

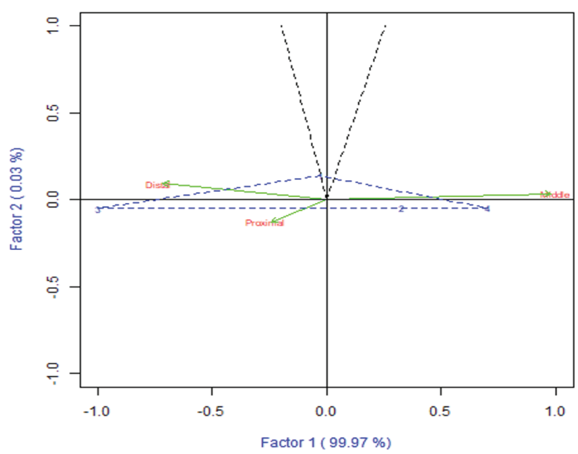

Figure 2: Bean position by varieties interaction displayed for cocoa bean thickness
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

determination. Each of the three distinct within-pod microenvironments identified different best performing genotype; such that CRIN Tc1, CRIN Tc2 and CRIN Tc3 respectively had the best bean length in the middle, proximal and the distal portion within the pod (Figure 1). Only two sectors were prominent in Figure 2 and the three within-pod positions differentially dispersed within the major sector. However, CRIN Tc1, CRIN Tc3 and CRIN Tc4 were respectively the vertex genotype for distal, proximal and middle within-pod microenvironment respectively for cocoa bean thickness determination.

Figure 3 displayed the bean position by varieties interaction for cocoa bean width. The three within-pod locations were distinct for bean width determination. The trapezia polygon had four sectors which identified CRIN Tc1 as the vertex genotype for beans at the middle cavity, CRIN Tc3 for the distal and CRIN Tc4 for the proximal within-pod location (Figure 3). The polygonal display of the bean position by varieties interaction for cocoa bean mass was a triangle with three sectors (Figure 4). The proximal and the distal within-pod microenvironments were accommodated in one mega environment while the middle portion of the pod environment was alone in another sector as another mega environment. The vertex genotype for both the proximal and distal within-pod environment was CRIN Tc3, but the sector which captured the middle environment had CRIN Tc4 as the vertex genotype (Figure 4).

3 DISCUSSION

The performances of the four bean metric measurements and seedling vegetative traits were affected by the

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year effect. This seem to reveal that the variables are not stable but very plastic as they significantly responded to changes in the wider environment of yearly climatic variation. The four studied genotypes and the three bean positions equally distinguished themselves on the four beans metric and other vegetative traits. This further substantiate that character expression is dependent on the environment, the genotype and the interaction of both (Crossa et al., 1991; Mortazavian and Azizinia, 2014). Moreover, by this study, the two different years (2014 and 2015), the four cocoa varieties (CRIN Tc1, CRIN Tc2, CRIN Tc3 and CRIN Tc4) and the three bean positions (proximal, middle and distal) were unique treatments in the experiments.

The environment within the ovary is not consistently uniform, hence, compartments within the seed-developing space do impacts and determines the physical and physiological traits of the seed (Illipronti et al., 2000). The spacious hollow at the middle cavity of the cocoa pod may have supported the recorded bean features of longer length, wider width and heavier mass of the beans which developed in the area. It is clear from our result that the length and width of the bean rather than the thickness were noted to be more important in bean mass determination. We equally noted that beans from the middle position supported increased biomass yield of roots and shoots for the four cocoa genotypes. In *Cryptocarya alba* (Molina) Looser, large sized seeds were associated with larger shoots, roots and number of leaves (Chacon et al., 1998). The reduced value for the same traits at the proximal and distal ends could be due to some constriction of the hollow within the pods. However, beans at the two ends were significantly thicker than those in the middle of the cavity. This seems to infer that adequate space is very necessary for efficient seed development in the ovary.

The tapering structure of the pod at proximal and distal ends may be very key in the reduction on the sizes of the seeds located in and towards the two ends. So, the available space within the pod (which varies along its length) could be a determinant of the seed sizes and hence mass of beans in the cocoa pods. This result is in consonance with the report of Iremiren et al. (2007) and Hammed et al. (2013) on their work with a genotype called F3 Amazon. Following the descriptive pod and pod apex shape by Phillip-Mora et al. (2013), the pod shape of the four genotypes used in this study was more similar to 'Angoleta' with the pod apex ranging between acute to obtuse. However, for whichever shape the cocoa pod has, the middle part (which is usually raised or expanded) seem to provide wider space for bean development compare to the two tapering ends. The identified significant within-pod variation which leads to three group of beans from the same pod observed in this study may not be true for 'Calabacillo' pod shaped cocoa (Phillip-Mora et al., 2013) which are usually ball-like round. Contrary to the report of Perin et al., (2002) on melon (*Cucumis melo* L.) where stems are the organs mostly affected by seed size, our result negates their assertion, the significant differences in the metric traits on the beans did not affect cocoa seedlings stem girth as it does to other vegetative traits.

Genotypes are genetic entity with specific characteristics which makes them different from another one. Bekele et al (2006) had much earlier noted that there exist considerable genetic variation in fruit size, shape and bean size of cocoa. The four cocoa genotypes used in this study differed in sizes of their beans and the impact of each of the bean traits on vegetative and biomass yield

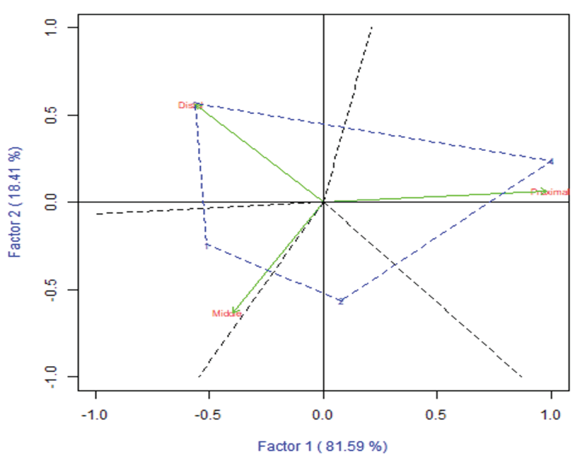

Figure 3: Bean position by varieties interaction display for cocoa bean width
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

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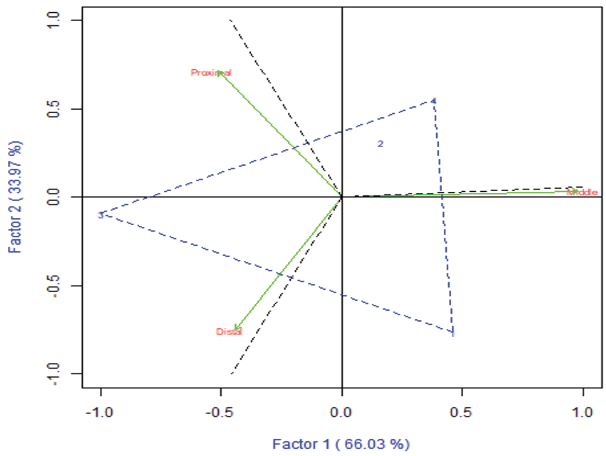

Figure 4: Bean position by varieties interaction display for cocoa bean mass
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

of the four cocoa genotypes; this information is a useful resource on which selection programme on wet bean sizes can thrive.

Our research outcome conforms to the expected norm that the phenotypic variance component are always higher than the genotypic component. However, variables which shows very small deviation are remarked to be reliable traits (Adewale et al., 2010), because the proportion which accrued to environmental variation is small while the quantity of variation for the genetic portion is high. The observed high genetic components in the phenotypic expression of the four bean metric traits, number of leaves and plant height measured at different interval in this study is remarkable. High and positive correlation existed between broad sense heritability and repeatability, revealing that traits with high broad sense heritability will have high repeatability. It is noteworthy that traits with high repeatability may have low response to environmental variation, hence the corresponding high broadsense heritability could be due to additive gene action.

Our research considered two notable environments: the within-pod environment (a micro environment) and the year (a macro environment), both which affected the expression of the bean metric traits and vegetative characteristics differently. GGE biplot identified the proximal, middle and distal positions within the pod environments for cocoa bean length, width, thickness and mass to be very unique. Hammed et al. (2013) who observed this three distinct divisions along the cocoa pod length, like us, did remark that the beans in the middle were longer, wider, thicker and heavier than other beans from the two extreme ends of the pod. For the three metric measurements on the bean, the GGE biplot clearly distinguish bean sizes, noting that the length, width and thickness of beans differ in respect to the positions where they are located along the inner cavity of the pod length. This therefore infers that the environment where beans are located during development primarily determines the phenotypic expression of its length, width, thickness and mass. The significant differences for preference of the four genotypes for the various within-pod location is a reflection of genotypic variation.

From this study therefore, mass of individual beans did not differ at proximal and distal positions, hence the zoning of the two microenvironments as a single mega-environment by GGE biplot for the trait. This further denotes that the mass of beans from the two ends of the cocoa pod do not differ from each other; meaning that the variation between both for mass of individual bean was not significant enough (Yan and Kang, 2003). Furthermore, the relevance of the individual bean mass of the two positions (proximal and distal) within the cocoa pod is the same. The long-standing recommendation of utilizing Cocoa beans from the middle cavity rather than the two ends (Ibikunle, 1967; Iremiren et al., 2007; Hammed et al., 2013) may have stemmed from the conspicuous variation in bean size and mass among beans from the same pod coupled with the linear correspondence between heavier seeds and high seedling vigour (Enayatgholizadeh et al., 2011).

4 CONCLUSION

Our work clearly revealed that the three inner locations (proximal, middle and distal) along cocoa pods length are prominent in distinguishing cocoa bean sizes; identifying the cocoa beans in the middle of the pod to be of the highest quality for all the metric measurements

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including bean mass. We suggest consideration of the influence of pod position on the cacao tree in subsequent related work to assess its probable influence on bean sizes relative to within pod positions. Where adequate bean may not be available for seedling generation, seeds from the two ends of the pod may not be discarded as seedlings from them improved in their growth with active photosynthesis after establishment. However, selection of and usage of beans in the middle of the pod could lead to the production of good, uniform and vigorous seedlings that will support higher cocoa productivity.

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