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Research Article

Differential Variability and Stability of Cocoa Clones to Varied Rootstock ages of Patch Budding

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Abstract: **The probability that Cocoa genotypes may exhibit different physiological variation when used as scion on the same clonal rootstock of different ages had necessitated the present investigation. Rootstocks were raised from open-pollinated pods of F3Amazon for the ages of one, two and three months. Budwoods from five genotypes were patch budded on the common rootstock of different ages. Growth and development of the budded materials were monitored for four months. The seedling length, girth and the number of leaves per plant of the rootstock at the three ages differed significantly ($P \leq 0.001$). The oldest seedling had the highest significant ($P \leq 0.05$) mean of twelve leaves; 36cm seedling length and 0.51cm stem girth. After budding, DAH, FW and LA exhibited significant ($P \leq 0.01$) genotypic variation. The three ages differed significantly ($P \leq 0.001$) for FW. The only variable with significant ($P \leq 0.05$) clone by age interaction was PDM. Broadsense heritability was quite low (< 23%) in FW, DW and PDM; however, DAS, DAH and LA had 59, 71 and 85% respectively. With respect to PDM, the five clones performed differently in response to the three ages of the root stock. Shukla stability variance and Wricke ecovalence agreed and uniformly identified CRINTc-2 to be most stable genotype across the three ages at budding. The PDM performance of other genotypes is rootstock-age specific. The understanding of the interaction of clones with different rootstock ages in the patch budding or grafting protocols may greatly enhance wider production of cocoa clones.**

Keywords: cocoa, patch budding, genotypes, rootstocks, heritability, stability.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a native of Amazon region of South America. There are over 20 species of the genus. *Cacao* is the most economic species and it is widely cultivated for its bean. It is considered one of the most important perennial crops with an estimated world output of 3.5 million tons in 2006 (ICCO, 2007)[1]. It is predominantly grown in the tropical areas of Central and South America, Asia and Africa[2]. The bulk of cocoa production comes from the tropics of West Africa. Propagation in cocoa is either through sexually generated or clonal seedlings. In West Africa for instance, majority of the cocoa plantation were established from sexually generated seedlings, at least it is easier and cheaper Vos *et al*[3]. Plantations established from clones attain pod production much earlier [4, 5]. Budding and grafting on seedling rootstocks is a common practise for cocoa plantation establishment in smallholder cocoa sectors of the Southeast Asia[5]. Reports from South America and Asia have proved vegetative propagation technique for cocoa plantation establishment to be highly productive. The adoption of clonal seedlings in cocoa plantation establishment in West Africa is yet very poor. Cocoa plantation establishment by vegetative rather than sexual propagation means may enhance youth attraction to cocoa farming and heave cocoa productivity per hectare in West Africa.

The choice of rootstock for vegetative propagation in most horticultural crops is dependent among other factors on: vigour, precocity and yield efficiency[6]. Genotype with large cocoa beans was recommended by Shepherd *et al* [7]. Large bean size enhances vigorous seedling establishment for efficient patch budding and top grafting. In addition to good phenotypic quality of the cocoa bean, the need for good resistance of the genotype(s) to some diseases or pests had been lately encouraged. In Central America and the Caribbean, seeds from cocoa clone which are resistant to *Ceratocystis fimbriata* are used to establish the rootstocks [5]. EET-400 was the root stock choice of Irizarry and Geonaga[8] because it was found to demonstrate consistent yield capacity. The relative tolerance of ICS 60 to heavy soil made it the choicest genotype for rootstocks establishment by Murray and Cope [9]. F3 Amazon is the mostly preferred genotype for cocoa rootstock establishment in Nigeria, owing to its resistance to swollen shoot disease.

Maintenance and reproducibility of any genetic material with unaltered genetic constitution is achievable through vegetative propagation. Losses of genetic materials have been prevented through vegetative propagation of ageing and old genotypes. Patch budding and top grafting on cocoa rootstock had been at much later ages of three, four and five months. The present study was therefore proposed to identify a probable lower rootstock age (with high efficiency) for patch budding and to understand differential variability and stability of different cocoa genotypes (used as scion) to varied rootstock ages. Derived information from this study would be essential for programme planning on germplasm conservation and mass propagation of clones of different genotypes.

MATERIALS AND METHODS

An experiment was designed to understand how three different ages of the cocoa rootstocks affects the growth and development of five different genotypes used as scion. The experimental layout was a factorial in completely randomized design (CRD) of three replicates, containing two factors: the three ages of rootstock development and five genotypes. Rootstocks were raised in pots from open pollinated pods of F3 Amazon in May, 2011 on well sifted top soil. The ages of the rootstocks were 1, 2 and 3 months after planting. The five genotypes from which bud woods were obtained were of different genetic groups and they include N38 (Amelonado), T65/7 (Amazonian), CRINTc-1 (T101/15 x N38 – Amazon-Amelonado), CRINTc-2 (T65/7 x N38 - Amazon-Amelonado) and F3Amazon (a check).

The length, girth and the number of leaves on the rootstocks at the different ages before budding were recorded. The scion of each genotype was patch budded on the rootstocks of different ages. The experimental unit of each treatment was ten budded plants per replicate. The experiment was monitored for four months. Data were obtained from the experimental unit on number of days to sprouting (DAS) and days to hardening (DAH). The leaf area was obtained at three month using the leaf area meter. The experiment was terminated with the destructive sampling of each experimental unit to obtain the fresh and

dry matter weight. Dry matter percentage was estimated as the percentage ratio of the dry matter to the fresh weight and then log transformed.

All the data were subjected to the statistical analysis using the Statistical Analysis Software (SAS), version 9.2, (SAS, 2007). The analysis of variance (ANOVA) was calculated using the PROC GLM procedure in SAS. Broadsense heritability was estimated from the variance component of the ANOVA as the ratio of the genotypic variance to the phenotypic variance, following Adewale *et al* [10]. Percentage dry matter which had significant ($P < 0.05$) genotype by age interaction was further investigated using two stability statistical methods:

Wricke's ecovalence (Wi) of Wricke (1962),

$Wi = (Yij - Yi. - Y.j + Y.)^2$ and

Stability variance (σ_i^2) of Shukla (1972),

$\sigma_i^2 = 1/(g-1)(g-2)(e-2)\ [g(g-1)\sum (Yij - Yi. - Y.j + Y.)^2 - \sum\sum (Yij - Yi. - Y.j + Y.)^2]$

Where; Wi = ecovalence of the i-th cultivar, σ_i^2 = Shukla stability variance of the i-th cultivar

Yij = the observed phenotypic value of the i-th cultivar in the j-th environment, Yi. = mean of i-th cultivar across the entire environment, Y.j = mean of j-th environment, Y. = grand mean, g = number of genotypes or cultivar and e = number of environments.

RESULTS

The component of variance and the means of the three ages of development of the rootstocks before budding are presented in **Table 1.**

Table 1: The components of variance and Means of three developmental Stages of the rootstocks before patch budding

Variance components						
	Leaf Count		Seedling Height (cm)		Stem Girth (cm)	
Items	Age	Error	Age	Error	Age	Error
DF	2	40	2	40	2	40
Mean squares	23.08***	0.82	144.21***	9.17	0.014***	0.0008
CV (%)	8.35		8.96		8.90	
Mean Separation						
Ages of root stocks	Mean		Mean		Mean	
3 Months	12.27		35.85		0.51	
2 Months	11.04		33.36		0.49	
1 Month	10.24		28.46		0.44	
LSD(0.05)	0.765		2.303		0.0263	

The leaf count, seedling height and stem girth differed significantly ($P \leq 0.001$) with respect to the three ages of rootstock. Variation within the sampling unit or coefficient of variation for the three variables is very low, lesser than 10% **(Table-1).** The differences between the means of the three treatment revealed that older rootstocks had significantly ($P < 0.05$) higher leaf count, seedling height and stem girth. Generally, the ascending order of magnitude for the three traits with respect to the three ages was 1 month

< 2 months < 3 months **(Table- 1).** From **Table 2**, significant ($P \leq 0.01$) variation occurred among the five clones with respect to days to hardening (DAH), fresh weight (FW) and leaf area (LA). The five clones reached hardening stage differently. T65/7 and CRINTc-2 reached hardening faster than other clones at a relative period of 90 days after budding. F3Amazon got to hardening latest; about ten days behind T65/7 and CRINTc-2 **(Table-2).** CRINTc-1 had the highest leave area (93.43) and fresh biomass weight (157.79g), N38 had the least for the two parameters (**Table-2**).

Table 2: The components of variance, variability and estimates of broadsense heritability for Some growth and developmental traits of the budded scion

Source of Variation	DF	Mean Squares					
		DAS	DAH	FW	DW	PDM	LA
Clones	4	76.58	172.18**	12.37**	0.84	15.04	4800.49***
Age	2	9.75	21.80	39.41***	2.66	28.74	77.05
Clone x Age	8	10.75	14.93	1.99	1.93	70.62*	324.54
Error	28	33.35	33.94	2.05	1.26	28.44	435.09
CV (%)		11.25	6.12	9.96	22.31	15.26	38.84
HB (%)		58.71	70.89	22.16	12.56	10.53	85.16
Separation of means of the clones							
Clones		DAS	DAH	FW	DW	PDM	LA
CRINTc-1		-	95.33	157.79	-	-	93.43
T65/7		-	90.77	156.51	-	-	52.04
F3_Amazon		-	100.33	137.63	-	-	45.68
CRINTc-2		-	90.88	136.59	-	-	43.05
N38		-	98.66	133.36	-	-	34.34
$LSD_{0.05}$			**8.00**	**13.08**			**20.14**

***CV – Coefficient of variation, HB – Broadsense Heritability, Df – Degree of freedom, DAS – Days to sprouting, DAH – Days to Hardening, FW – Fresh biomass weight (g), DW – Dry weight (g), PDM – Percentage dry matter, LA – Leaf Area.**

The age at budding significantly ($P \leq 0.001$) influenced the FW of the five genotypes. Each of the five clones that were budded on three months old rootstock accumulated higher FW. The interaction between clones and the age at budding significantly ($P \leq 0.05$) influenced the percentage dry matter content. Except for LA which had quite high (38.84%) coefficient of variation (CV), the CV of the other five traits in this study was very low (< 23%). Among the six traits studied, LA had the highest broad sense heritability of 85%, followed by DAH with broad sense heritability of 71%. There were inconsistencies in the response of the five clones to the three different ages at which budding was done with respect to percentage dry matter (PDM). For instance, the highest PDM occurred at the budding age of 2, 1, 3, 2 and 1 month(s) respectively for CRINTC-1, CRINTc-2, F3Amazon, N38 and T65/7 (**Fig.1**).

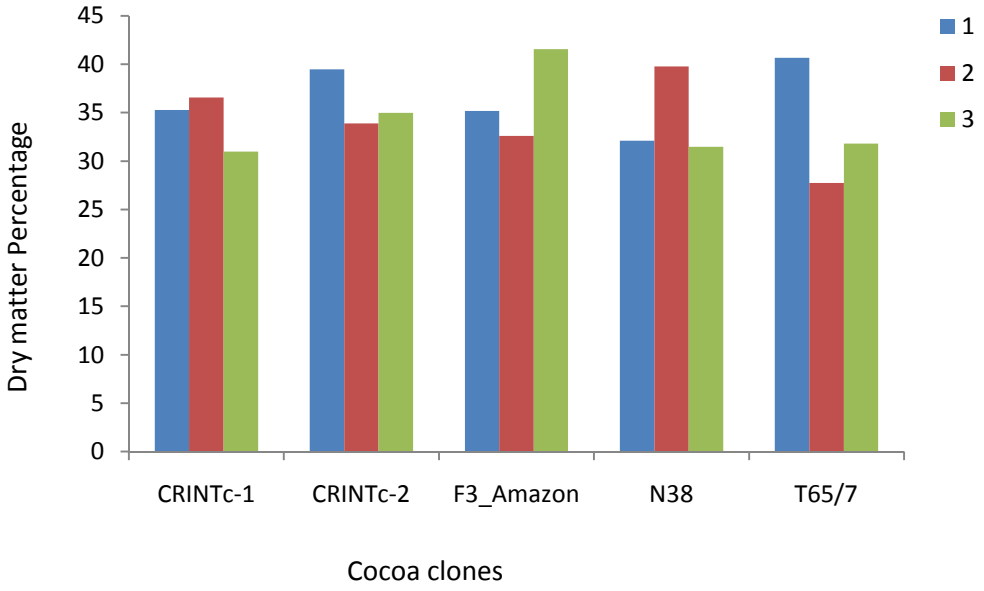

Fig. 1: The varied dry matter percentage of the five clones in response to the three ages of patch budding.

The stability of the inconsistent responses of the different clones to the different ages of budding for PDM which was identified in Figure 1 was further explained in **Table 3.** The stability variance of Shukla for the five cocoa clones ranged between 0.62 (CRINTc-2) and 7.25 (N38). However, the range was between 5.19 (CRINTc-2) and 58.37 (N38) for Wricke ecovalence method. CRINTc-2 and N38 both had the lowest and the highest values for both stability statistics. Therefore, the order of stability rating (by Shukla stability variance and Wricke ecovalence) of the five cocoa clones from the most stable to the least stable was: CRINTc-2, CRINTc-1, F3Amazon, T65/7 then N38 **(Table- 3).** The mean PDM for CRINTc-2 (36.12%) came very close behind F3Amazon which had the highest value of 36.44%. However, owing to the magnitude of the two stability indices, CRINTc-2 was very stable compared to the highly inconsistent F3Amazon (**Table- 3**).

Table- 3: The stability of the dry matter percentage of five cocoa clones to three different ages of root stocks before patch budding.

Clones	Mean across Environments (three rootstock ages)	Stability variance (σi^2)	Wricke Ecovalence (W_i)
CRINTc-1	34.28	1.03	16.39
CRINTc-2	36.12	-0.62	5.19
F3_Amazon	36.44	6.34	52.23
N38	34.46	7.25	58.37
T65/7	33.39	6.92	56.13

DISCUSSION

The use of F3Amazon as the common rootstock was in consonance with the recommendation of Eskes and Lachenaud[4]. According to them, the use of the same genotype as a rootstock (common environment) for the test of many genotypes as scion would lead to significant genetic variation. The result in this study seems to reveal the relative importance of age in physiological development of plants. Older rootstocks

quantitatively had higher leave number, longer seedling and wider stem girth. It is equally noteworthy to infer that the rate of budding success and subsequent progressive development of the scion would among other factors be influenced by the age of the rootstock at budding. An investigation may be proposed to identify other genotypes with better budding efficiency than F3Amazon. Within the present germplasm, it may not be impossible to identify genotype(s) with better budding receptivity and higher yield efficiency[4.]

Our result on genotypic variation observed among the five clones in this study concors with the reports on variability in morphological and physiological traits of cocoa[11], clonal yield and pod index[8] and bean traits[12]. Hardening is a physiological stage in the development of the budded scion when the plants are assumed to have matured enough to be transplanted. Hardening stage is characterized by full maturation of about 70% of the leaves on the developing clone. Genotypes with lower number of days to reaching hardening such as T65/7 and CRINTc-2 would be most preferable for early field establishment.

It was observed in this study that the leaf area had a positive link with the fresh biomass yield. Shoot biomass had been remarked by many authors [13,14,] as a dependent variable on many physiological traits, most especially the leaf area. The quantitative proportion of genetic in the phenotypic expression (i.e. broad sense heritability) of LA and DAH by the five clones is high and very informative for subsequent selection programme. In subsequent breeding programme therefore, the choice of CRINTc-1 at the expense of others because of the higher leaf area after budding may be justified. CRINTc-2 and T65/7 would equally have higher selection preference above other clones owing to the attainment of hardening at relatively shorter number of days.

Our result further revealed that the five clones have their most preferred rootstock age for the highest percentage dry matter. Except for F3Amazon (the check) in this study, budding success for high percentage dry matter can be achieved when rootstocks are within the ages of one to two months. One to two months are much earlier age for patch budding compared with the five months used by Irizarry and Geonaga [8]. Our result identified the possibility of having patch budding success on F3Amazon rootstocks at a lower age of one to two months. Successful budding at earlier ages of root stocks would shorten the time frame of raising clones at commercial scale for plantation establishment.

Negative value in the stability variance of Shukla is insignificant in stability determination; the magnitude of the value is what counts[15]. Therefore, Shukla stability variance and Wricke ecovalence both identified CRINTc-2 as the most stable clone for the three ages, because its value for both statistics was nearest to zero compare to the values obtained for other clones. This implies that budding can be carried out with high efficiency at any of the three tested ages of rootstock development using CRINTc-2 as a scion. The two statistics i.e. Shukla stability variance (σi^2) and Wricke's Ecovalence (W_i) had a perfect agreement in the order of classifying the five clones. Many authors [16, 17, 18,] have also observed very high correlation between the two methods of stability measurements. The identified stability in the two hybrids (i.e. CRINTc-1 and CRINTc-2) seems to unravel another significant quality in them. They can be patch budded with high efficiency on the rootstocks of F3Amazon at the age range of one to two months. This is a physiological potential[19] in the hybrids. While we suggest a repeat of this kind of investigation for many other cocoa genotypes, we further recommend an extensive investigation of stock-scion combination for many genotypes of cocoa for better yield efficiency in cocoa.

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