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Cocoa vegetative propagation in Nigeria: a search for other suitable rootstock clones

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Abstract

The most suitable rootstock (from reports) for clonal cocoa propagation in West Africa has been F_3 Amazon. A contest of this assertion would be necessary owing to the availability of many cocoa genetic resources in West Africa. Hence, the present research investigated the suitability of five other cocoa clones along with F_3 Amazon as rootstock for three hybrid cocoa clones as scions. The factorial in Completely Randomized Design experiment had three replications. Pod and bean characters (Pod length, Pod girth, Pod weight, Pod thickness, Number of beans per pod and Weight of wet beans per pod) of the six clones were significantly ($P < 0.01$) different, moreover, some juvenile vegetative characteristics measured at intervals equally differed significantly ($P < 0.01$) too. Top grafting survival ranged between 15% (F_3 Amazon and CRIN Tc2) and 94% (WACRI and CRIN Tc1). Rootstock receptivity for the three scions ranged between 33% (F_3 Amazon) and 73% (N38). Number of active nodes after top grafting was between one and three. CRIN Tc1 had the highest (75%) compatibility with the six clones. Furthermore, significant ($P < 0.05$) differences equally existed among the eighteen union combinations for three periodic intervals of leaf counts. In this experiment, three other clones (WACRI_S1, N38 and T101/15) had higher receptivity for union and regeneration of CRIN Tc1, CRIN Tc2 and CRIN Tc3 compared to F_3 Amazon. This result approves the efficiency of other clones as better rootstock for top grafting in cocoa and formally informs that they could be alternative rootstocks (where they are available) for cocoa vegetative propagation instead of F_3 Amazon.

Keywords: cocoa seedling, clonal seedlings, CRIN Tc, vegetative propagation, WACRI-series

Introduction

The duly fermented and dried cocoa bean is the principal raw material for chocolate. World annual cocoa bean production in 2011-2012 is approximately 3.6 Million metric tonnes (CacaoNet, 2012)). Major producers includes Cote d'ivoire, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador and Malaysia (Schwan and Wheals, 2004; ICCO, 2009; Afoakwa, 2010). It is noteworthy that two-third of the world's cocoa beans production comes from West Africa (Anon, 2005). The bean size is one of the most important yield components in cocoa (Soria, 1978). While a pod can house up to 40 cocoa beans, variation in bean size is a reflection of the race and cultivar group (Souza and Dias, 2004).

The development of superior hybrids has made a significant contribution to cocoa productivity (Dias et al., 2003). Cocoa hybrids as reported by Dias et al., (1998) showed wide adaptability with reduced year effect on agronomic and yield performances compared to the unselected local cultivars. The procedure of hybrid generation in cocoa has been through the art of selective choice of known parent materials with proven genetic qualities and hence hand pollination among them. Moreover, the programme of making parental stocks available has been through vegetative replication and multiplication of proven genetic resources. Among the various technical options, grafting and rooted cutting techniques has been used to some degree in development of cocoa clones (Maximova *et al.*, 2005).

Literature has reported some notable rootstock cocoa clones: EET-400 according to Irizarry and Geonaga (2000) was preferred because it was found to demonstrate consistent high yield capacity, ICS 60 because it had relative tolerance to heavy soil (Murray and Cope, 1955), F3 Amazon owing to its resistance/tolerance to swollen shoot disease (Aikpokpodion et al., 2003) and high seedling establishment efficiency etc. Irrespective of genotype, Shepherd et al. (1981) hinted that large beans produced vigorous seedlings that can be budded for higher vegetative propagation success. The choice of root stock for vegetative propagation in most horticultural crops is dependent (among many factors) on vigour of stock and scion, precosity, yield, yield efficiency, etc. (Daymond et al., 2002).

The rootstock may also confer resistance to pests or diseases on the scion (Pang, 2004). Vegetative propagation systems provide a means to capture the additive genetic gain (Maximova *et al.*, 2005). Vigour and yield is highly correlated (Adebisi *et al.*, 2010; Ambika *et al.*, 2014). Bean size is a physical quantitative character which varies among genotypes. While the morphometric trait is important in breeding for yield, the bean size is also an indicator for good vigour in seedlings. Thus identifying the simple link between the two parameters is worth investigating. Moreover, since the rootstock influences the propagation success, identifying rootstock genotype(s) with high seedling vigour and compatibility/receptivity with scion(s) could provide a positive change in asexual propagation of cacao via top grafting. This study is a follow up to the need for a quest as suggested by Adewale *et al.* (2013a) to assess the compatibility efficiency other genotypes as root stock clones rather than F3 Amazon. The study therefore seeks to understand the physiological response of different genotypes (used as rootstock and scion) to top grafting protocols.

Materials and Methods

Uniformly ripe open pollinated pods were obtained from five genotypes (T65/7, T101/15, N38, T16/17, WACRI Series 1 and F3 Amazon) in five blocks within plantations at the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. Metric information on the pods and the beans were recorded. Data collected on the pods were:

pod length, pod girth (diameter), pod thickness, number of beans/pod while the data on bean metric values include: wet bean weight, bean length, width and thickness. Beans from each genotype were sown in polythene bags in three replications, each replication containing a plot size of fifteen polythene bags sown with beans from each rootstock genotype. Records were taken on the seedlings for 12 weeks after germination on days to emergence, height of the cotyledon at germination, number of leaves and stem girth during in the nursery. The experimental layout in the nursery was a completely randomized design (CRD) of three replications containing the six genotypes as treatments. The sampling unit was fifteen seedlings for each genotype in a replication. Top grafting was carried out following the protocol in Gomes and Sodre (2015) on each seedlings in the three replications using the semi-hard budwood collected from the fan branches of CRINTc1, CRINTc2 and CRINTc3 collected at the Hybrid Trial plot, CRIN, Ibadan, Nigeria. Top grafting of the three scions on the six rootstock clones gave rise to a 6 x 3 factorial combinations for the stock-scion union experimentation. The treatment combination was treated as a factorial in CRD. Each scion budwood had three nodes intact on the stock after top grafting. Growth and development of the scions on the stocks were monitored for another twelve weeks and data on the number of leaves after grafting were recorded at two week interval. Variation for pod, bean and vegetative characteristics among the genotypes were verified by analysis of variance (ANOVA) in SAS (version 9.3, 2011) using PROC GLM. The percentage survival of top grafting was subjected to ANOVA in SAS (version 9.3, 2011) and descriptive statistics. Means of the treatments were separated using Duncan Multiple Range Test at P = 0.05.

Results

Table 1 revealed the baseline information on the characteristics of the pod and bean of the six genotypes whose bean were raised for root stock seedling. Very significant ($P \leq 0.01$) variation existed among the six genotypes for all the pod and bean characteristics except bean thickness and height of the cotyledon after germination (Table 1). Coefficient of variation among the characteristics measured ranged between 4.12% (BL) to 36.12% (PT); only pod weight and thickness had > 30% coefficient of variation. The mean separation of the of the pod and bean characteristics which showed significant variation in Table 1 was presented in Table 2. Pods of F3 Amazon and WACRI series 1 had the highest length, width and weight. Moreover, they had the highest number of beans and wet bean weight too (Table 2). WACRI series 1 was the genotype with the longest and widest bean of 2.29cm and 1.24c respectively (Table 2).

The number of leaves measured at two weeks interval for seven intervals is presented in Table 3. Significant ($P \leq 0.05$) variation existed among the six genotypes for the number of leaves at 2, 4 and the 14th week after planting. F3 Amazon had the lead value for the number of leaves at the 2nd week after planting while T65/7 had the highest value at the 4th and the 14th week after planting (Table 3). Significant ($P \leq 0.001$) variation existed among the six genotypes for stem girth at weeks 2, 4, 8, 10 and 14 (Table 4). F3 Amazon consistently had higher values than others for this characteristic. N38 had the lowest values for stem girth across the seven intervals of measurement (Table 4). From Table 5, highly significant ($P \leq 0.001$) variation among the genotypes was observed at each interval of plant height measurement. F3 Amazon and N38 consistently had the highest and the least values for the characters at the seven intervals of measurements.

Survival of scion on the stock varied significantly in Figure 1. The stock-scion compatibility with the highest survival percentage was WACRI-CRIN Tc1 (Fig. 1). Generally, among the three scions, CRIN Tc1 showed the highest compatibility with each of the six clones used as stocks. N38 had the best compatibility with the three scions; the mean survival percentage of the three scions on N38 was 73% (Fig. 1). Except for T101/15, the compatibility of CRIN Tc3 with the other stocks was much lower compare to CRIN Tc1 and CRIN Tc2. Growth and development process of the different stock-scion unions is presented in Table 6. At 21days after top grafting, only the union between WACRI_S1 and CRIN Tc3 retained the three initial nodes. Within the same time, the active nodes in the union between N38 and CRIN Tc2, T16/17 and CRIN Tc2 and T65/7 and CRIN Tc3 had decreased to 1.8 (Table 6). Among the six clones used as rootstocks, N38 mostly and consistently supported the three scions to produce the highest number of leaves (3.06±0.70, 3.93±0.07 and 7.90±0.31) at 4, 8 and 12 weeks after top grafting (Table 6). Support for growth and development (as measured in the number of leaves) on the scion by WACRI_S1 and T101/15 was equally significant but specific. In the 8th and 12th weeks, WACRI_S1 mostly supported CRIN Tc1 to produce 5 and 8 leaves, while T101/15 enhanced the number of leaves in CRIN Tc2 by 5 and 9 respectively (Table 6). Generally, the support by F3 Amazon for number of leaves rated fourth behind N38, WACRI_S1 and T101/15.

Discussion

Vegetative propagation has an important role where it is necessary or desirable to reproduce trees true to type (Wood, 1987). Top grafting in which a scion stick containing several buds is appropriately inserted into a top split (top grafting) of a rootstock is essentially used for establishing seed garden and some experimental work aimed at conservation of germplasm and raising of clonal planting material. N'goran and Eskes (2006) noted that the use of top grafting has been applied in most countries in order to obtain higher success rate in clonal planting material generation.

Tefera *et al.* (2017) rating of coefficient of variation by magnitude were: 0-10% - low, 10-20% - moderate and >20% - high. This descriptive statistic measures the within-sample variation. When the estimate is low or moderate, high uniformity exist among the sample from which data is generated and that implies that such sample and data are reliable. High within sample variation is a significant occurrence in tree crops. We in this study and Adewale *et al.* (2013b) observe high coefficient of variation in pod and bean characteristics. Pods develop along the tree trunks and on primary, secondary, tertiary, etc. branches of the cacao. Access to assimilate would not be equal for all developing pods on any of these different parts. Therefore, variation in pod characteristics from the same tree abound and may be well linked to the point on the tree where pods are attached to develop.

The high number of beans with corresponding heavier weight obtained from bigger pods in this study seem to infer that bigger pods have larger hollow to house many and heavier cocoa beans. The wider space within the pod may have physically enhanced bean development in number and weight. Moreover, bigger pods equally had higher pod thickness. The husk is a very important component for highly valued traditional antiseptic soap in the Yoruba culture of Western Nigeria. Moreover, there is a technology for converting the pod husk to organically useful and environmentally friendly compost to improve soil fertility (Ogunlade, personal communication, 2014). The scale of the awareness of the above uses seems to be low; otherwise industrial involvement ought to have being handling the "wastes" in cocoa plantations. Critically, large heaps of pod husk are still very evident in farmers' plot, the same has annually hosted enormous pathogenic inocula for disease infestation which annually accounts for yield loss up 20% to according to DuVal *et al.* (2017) despite thorough management schemes. A study to understand the percentage contribution of inocula from pod husk heaps in cocoa farms may be necessary.

Outcome from such study may generate a policy development on how to improve cocoa production through the process of managing the waste by improved industrial utilization of the heaps of pod husk.

The growth and development indicators of the seedlings of the six cocoa genotypes employed as rootstock in the study reflected genotypic variations. Plant height, stem girth and number of leaves are essential plant vigour-related characteristics. For the time length of measuring the growth and development of the seedlings, F3 Amazon consistently had the highest plant height and the widest stem girth while N38 had the least vigour. Apart from the choice of the genotype based on its resistance/tolerance to swollen shoot (Aikpokpodion et al., 2003), its further qualification may have been linked to its high performance for vigour-related characteristics in the nursery, as observed in the present study.

The response of the rootstock and the scion clones differed significantly. Compatibility of CRIN Tc3 with the six rootstock clones was much lower compared to CRIN Tc1 and CRIN Tc2. Adewale *et al.* (2013a) noticed and reported a physiological potential in CRINTc1 and CRINTc2. For patch budding, the two hybrids showed very high compatibility efficiency with eight weeks old F3 Amazon root stock. Generation of clones from these elite genetic materials (hybrids) (i.e. CRIN Tc1, CRIN Tc2 and CRIN Tc3) may be challenging with differing proportion of success. Improved cloning through top grafting can be enhanced by the choice of specific rootstock clone with the best compatible support; for instance, T101/15 mostly supported the regeneration of CRIN Tc3. To achieve a high degree success in top grafting, therefore, specific stock and scion genotypes with high compatibility efficiency would need to be identified: Dadzie *et al.* (2014) and Gomes and Sodre (2015) asserted this on Kola (*Cola nitida*) and Cacao vegetative propagation respectively.

Moreover, just as Dadzie *et al.* (2014) reported for Kola, stock-scion compatibility of the six root stock genotypes with CRIN Tc1, CRIN Tc2 and CRIN Tc3 was irrespective of the individual vigour of the six root stock genotypes. WACRI_S1 and T101/15 were the clones which mostly supported the retention of the active nodes on the scion after 21 days. N38 which showed the lowest vigour was the most supportive root stock genotype for the rejuvenation of the three scion clones. This seems to depict that vigour may not be the only characteristic for enhanced stock-scion union in tree propagation by asexual means.

Typically, While Vazifeshenas *et al.* (2009) on their work on pomegranate reported that the rootstock controls grafting success; they further hinted that the process of scion growth is not fully understood. Some authors (Kamboj and Quinlan, 1997; Son and Kuden, 2003) attributed stock-scion interaction, compatibility and success to phyto-hormone roles. Simons (1986) had speculated successful union to changes in the hydraulic resistance and the flow of stem-sap across the union point. The intimate contact of cambial tissues at the union point according to Gomes and Sodre (2015) favours the development of functional connection within the xylem and phloem tissues. Physiologically however, process of cell recognition, callus formation, as well as vascular and parenchyma tissue differentiations are critical process in graft development (Hartmann et al., 2002).

Growth and development characteristics of the scion on the stock continued linearly on the six rootstock clones, but the linear process differed significantly among the six clones. WACRI_S1, N38 and T101/15 were far ahead of F3 Amazon in their support for top grafting in this study. Hence, where they are available, they are more efficient substitute for F3 Amazon as effective root stock clones.

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Tables and Figure

Table 1: Variance and descriptive statistics of the pod and bean characteristics of the six cocoa genotypes

		Mean square									
SOV	DF	PL	PW	PT	PG	NBPP	WBWPP	BL	BW	BT	HCG
Rep	4	2.52	244.83	0.09	0.35	28.37	523.53	0.08	0.02*	0.04	0.06
Genotypes	5	24.41***	47101.88**	0.13	3.16***	460.75***	4807.58**	0.06**	0.02**	0.04	0.34
Error	20	2.85	9364.63	0.05	0.58	27.68	102.33	0.01	0.003	0.04	0.24
Mean	-	14.32cm	284.18g	0.64cm	7.28cm	39.26	76.24g	2.02mm	1.12mm	0.69mm	3.03cm
CV (%)	-	11.8	34.05	36.12	10.52	13.4	13.26	4.12	5.03	27.09	16.36

*, **, ***; Significant at 0.05, 0.01, 0.001 probability level respectively

SOV = Source of variation, DF = Degree of freedom, PL = Pod length, PW = Pod weight, PT = Pod Thickness, PG = Pod girth, NBPP = Number of beans, WBWPP = Wet bean weight, BL = Bean length, BW = Bean weight, BT = Bean Thickness, HCG = Height of cotyledon at germination.

Table 2: Means of the metric measurements on pod and beans of the six cocoa genotypes

Genotypes	PL (cm)	PW (g)	PG (cm)	NBPP	WBWPP(g)	BL(cm)	BW(cm)
F3 Amazon	16.68a	371.00a	8.32a	47.60ab	106.80ab	1.96bc	1.22a
N38	13.54b	348.90a	7.46ab	42.50bc	89.94b	2.06b	1.08b
WACRI_S1	16.94a	374.70a	8.00a	51.00a	113.20a	2.29a	1.24a
T101/15	13.34b	263.70ab	6.88bc	37.24dc	47.00c	2.00bc	1.08b
T16/17	11.66b	140.10c	6.18c	26.20e	42.70c	1.94c	1.03b
T65/7	13.74b	206.70bc	6.88bc	31.00de	57.80c	1.91c	1.06b

*Means with the same letter are not significantly different at P = 0.05 and mean comparison is along each column

PL = Pod length, PW = Pod weight, PT = Pod Thickness, PG = Pod girth NBPP = Number of beans, WBWPP = Wet bean weight, BL = Bean length, BW = Bean width

Table 3: Variation in means of number of leaves at different weeks after planting of the six cocoa genotypes

Weeks After Planting	2	4	6	8	10	12	14
Genotype Variance	2.68*	7.58***	1.99	4.77	4.32	7.48	12.87**
F3 Amazon	5.20a	7.20ab	8.47a	10.93a	11.73a	12.27a	12.40b
N38	4.00c	5.80c	7.73a	9.80a	10.73a	10.93a	12.93b
T101/15	4.40bc	6.40bc	7.53a	9.73a	10.67a	11.33a	12.87b
T16/17	4.47bc	7.20ab	8.13a	10.33a	11.67a	12.73a	14.33a
T65/7	4.93ab	7.60a	8.20a	10.67a	11.67a	12.33a	14.60a
WACRI_S1	4.53bc	6.13c	7.67a	9.53a	10.73a	11.40a	12.67b
Grand Mean	4.59	6.72	7.96	10.17	11.20	11.83	13.30
CV (%)	17.86	17.19	15.63	15.05	14.21	15.24	14.13

*, **, *** - Shows significance at 0.05, 0.01 and 0.001 level of probability respectively. Means with the same letter are not significantly different at P = 0.05 and mean comparison is along each column.

Table 4: Variation in means of stem girth at different weeks after planting of the six cocoa genotypes

Weeks After Planting	2	4	6	8	10	12	14
Genotype Variance	0.003***	0.001***	0.24	0.006***	0.008***	0.005	0.011***
F3 Amazon	0.20a	0.22a	0.30a	0.36a	0.43a	0.48a	0.55a
N38	0.16c	0.20b	0.60a	0.30c	0.36c	0.45a	0.49c
T101/15	0.18b	0.20b	0.27a	0.32bc	0.41ab	0.47a	0.54ab
T16/17	0.20a	0.21b	0.30a	0.33ab	0.40b	0.48a	0.51bc
T65/7	0.20a	0.20b	0.29a	0.34ab	0.40b	0.45a	0.49c
WACRI_S1	0.20a	0.20b	0.29a	0.32bc	0.41ab	0.49a	0.51bc
Grand Mean	0.19	0.21	0.34	0.33	0.40	0.47	0.52
CV (%)	12.58	7.90	111.15	12.14	8.69	9.95	9.85

*, **, *** - Shows significance at 0.05, 0.01 and 0.001 level of probability respectively. Means with the same letter are not significantly different at P = 0.05 and mean comparison is along each column.

Table 5: Variation in means of plant height at different weeks after planting of the six cocoa genotypes

Weeks After Planting	2	4	6	8	10	12	14
Genotype Variance	48.82***	55.62***	60.31***	86.89***	95.65***	82.08***	84.23***
F3 Amazon	17.88a	19.87a	22.30a	26.90a	29.27a	30.80a	33.46a
N38	12.73d	14.13c	16.23c	19.70b	22.15c	23.73c	26.53b
T101/15	16.14ab	17.77ab	19.90ab	24.07ab	27.70ab	28.47ab	31.77a
T16/17	13.95cd	16.17bc	18.33bc	21.97b	24.20bc	26.30bc	29.37ab
T65/7	14.71bc	16.60b	18.57bc	22.80ab	25.17bc	27.50abc	30.83ab
WACRI_S1	15.75bc	17.80ab	19.47abc	22.20b	25.70abc	27.43abc	31.37ab
Grand Mean	15.19	17.05	19.13	22.93	25.69	23.37	30.55
CV(%)	6.73	7.77	8.89	10.44	7.79	7.96	8.82

*, **, *** - Shows significance at 0.05, 0.01 and 0.001 level of probability respectively. Means with the same letter are not significantly different at P = 0.05 and mean comparison is along each column.

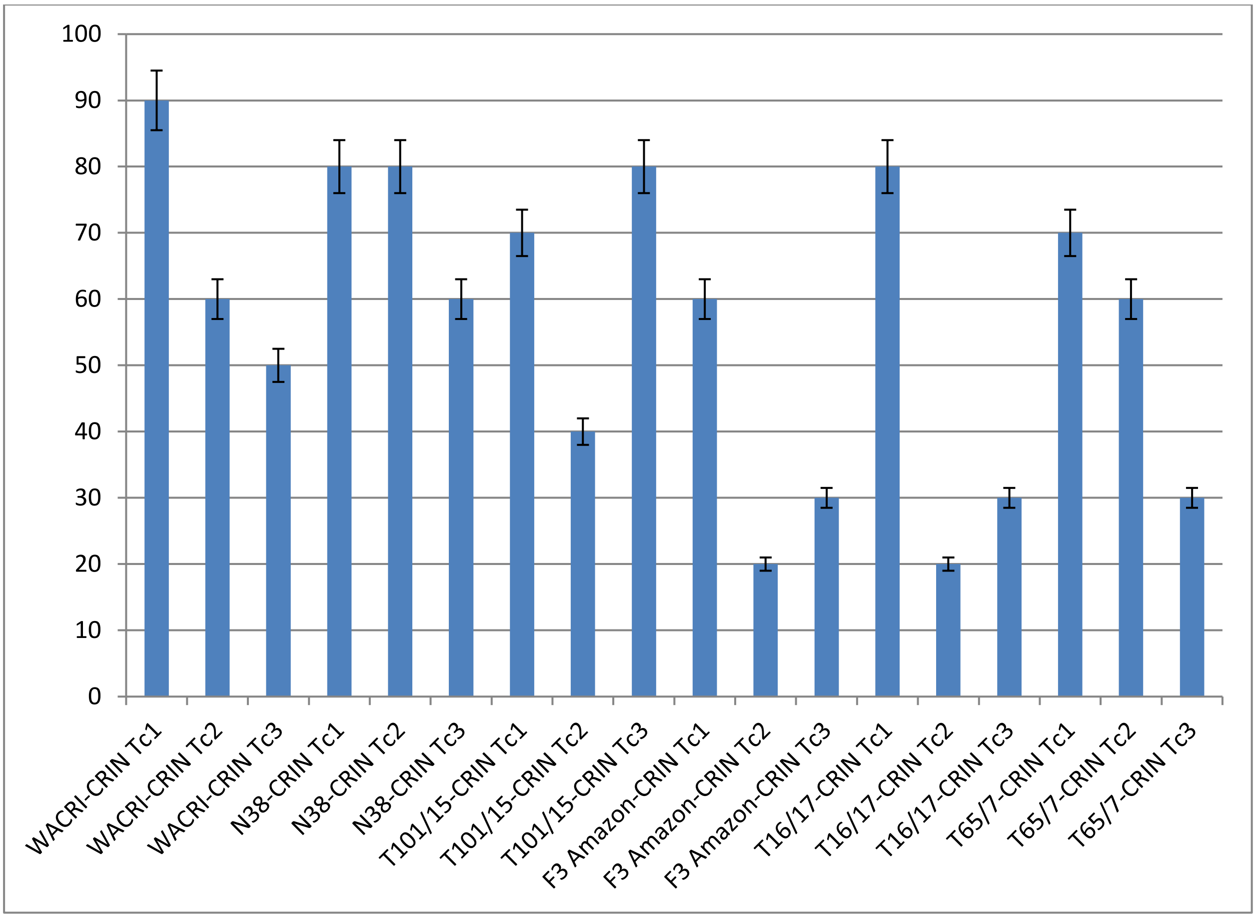

Figure 1: Variation in the survival percentage of the scions on the different stocks.

Table 6: Means for number of active nodes and number of leaves on the scions after top grafting on six different cocoa clones

Number of Active nodes at 3 weeks after grafting					Number of Leaves at 4 Weeks after grafting			
	Scion				Scion			
Stock	CRIN Tc1	CRIN Tc2	CRIN Tc3	Mean±SE	CRIN Tc1	CRIN Tc2	CRIN Tc3	Mean±SE
WACRI_S1	2.8	2.2	3	2.66±0.24	1.4	1.6	1.8	1.60±0.11
N38	2.4	1.8	2.8	2.33±0.29	4.4	2	2.8	3.06±0.70
T101/15	2.6	2.8	2.4	2.60±0.12	3	1.6	1.2	1.93±0.55
F3 Amazon	2.2	2	2	2.06±0.07	3.4	0.2	1.8	1.80±0.92
T16/17	2	1.8	2	1.93±0.07	0.8	0.4	0.2	0.46±0.18
T65/7	2	2.4	1.8	2.06±0.18	1	1.4	0.2	0.86±0.35
Mean±SE	2.33±0.13	2.16±0.16	2.33±0.20		2.33±0.60	1.20±0.30	1.33±0.41	
Number of Leaves at 8 Weeks after grafting					Number of Leaves at 12 Weeks after grafting			
	Scion				Scion			
Stock	CRIN Tc1	CRIN Tc2	CRIN Tc3	Mean±SE	CRIN Tc1	CRIN Tc2	CRIN Tc3	Mean±SE
WACRI_S1	5.3	2	4.6	3.96±1.00	8.5	5.6	7.2	7.10±0.83
N38	4	3.8	4	3.93±0.07	8.5	7.5	7.7	7.90±0.31
T101/15	4	5.4	3.5	4.30±0.57	6.3	8.6	7.3	7.40±0.66
F3 Amazon	4	2.1	3.8	3.30±0.60	7.3	4.3	6.3	5.96±0.88
T16/17	2.2	3.4	2.33	2.64±0.38	5.8	6.6	5	5.80±0.46
T65/7	3.6	4.4	2	3.33±0.71	6.2	6.8	3.5	5.50±1.01
Mean±SE	3.85±0.45	3.52±0.54	3.37±0.41		7.10±0.51	6.56±0.61	6.16±0.66	