ROOTING CLASSIFICATION AND CULTURE RESPONSES OF SOME CACAO HYBRIDS[1] /

*O. A. ODEGBARO**
*N. O. ADEDIPE**

Resumen

Un número grande de híbridos de cacao fue clasificado en **difícil, intermedio** *y* **fácil,** *según su habilidad en enraizar. Diferencias significantes (P = 0.05) se observaron en la producción de raíces de clases de cacao aparentemente similares. Los porcentajes medios de injertos enraizados oscilan de 28.5 a 61.5 entre los híbridos de Amazonas* F_2; *de 22.6 a 79.5 entre los híbridos del Amazonas* F_3, *y de 26.1 a 63.9 entre los híbridos Elite de la CRIN. Se descubrió que en muchos casos clones de un mismo genotipo entre varios híbridos cayeron en un mismo promedio de enraizamiento. También se llevó a cabo una investigación sobre la producción de raíces de algunos de los híbridos en relación a algunas prácticas culturales. Se descubrió que la presencia de solamente una hoja por injerto redujo significativamente la producción de raíces (P = 0.01) comparado con un alto número de hojas por injerto. Se observó que dos hojas por injerto fueron adecuadas para plántulas a enraizar. La reducción en el tamaño de las hojas a la mitad no tiene efecto adverso en el enraizamiento y la práctica aún aumenta la supervivencia de los injertos bajo las láminas del polietileno usado para la producción de raíces de los injertos.*

Sin embargo, reducir a un octavo del tamaño normal de la hoja disminuye significativamente la producción de raíces (P = 0.001).

Los injertos enraizaron mejor de 7 a 8 semanas después de iniciado el crecimiento de nuevas hojas en un tallo cortado.

Introduction

In Nigeria, cacao is still propagated mainly by seedlings raised in government nurseries from pods of high yielding trees distributed by the Cocoa Research Institute of Nigeria (CRIN). Freeman (5) described the methods of raising seedlings in the nursery. Everard (4) had earlier observed that sowing seeds at permanent sites, which was the practice before nurseries were introduced, was risky as rodents dig up and eat the germinating seeds. However, the propagation of cacao by rooted cuttings holds the key to revolutionising cacao production in Nigeria, as elsewhere.

The propagation of cacao by cuttings started to gain impetus in 1955 when Archibald (1) gave a full description of the technique. He showed that the preservation of desirable characteristics through propagation by cuttings was accompanied by other advantages when compared with propagation by seedlings. The cacao plants derived from cuttings come into bearing earlier than seedling trees; and due to the short habit of growth, the pods are easier to harvest. The reduced height also facilitates total coverage of the trees and pods with pesticides.

In recent years, the demand for vegetatively propagated cacao planting material has been increas-

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* Asst. Director (Agric. Sciences), Federal Ministry of Science and Technology, PMB 12793, Lagos (formerly of Cocoa Research Institute of Nigeria, Ibadan); and Profesor of Plant Physiology, University of Ibadan, Ibadan, Nigeria, respectively.

ing. The fall in yields as a result of pests, diseases and physiological decline has led to the desirability, or even the necessity, of finding new areas for cacao plantations in addition to an extensive rehabilitation of existing cacao areas. It is desirable that maximum area should be planted with high-yielding clonal selections rather than with seedlings of uncertain performance. New hybrids are being developed (2) whose parents have to be multiplied vegetatively. The rooting potential and behaviour of selected hybrids have to be thoroughly worked out before they can be included in polyclonal hybrid seed gardens for providing clonal materials for distribution to farmers for new plantings and for the rehabilitation of old unproductive plantations (10).

One major problem in the production of cacao cuttings is the existence of difficult rooters among the various selected hybrids and the seasonality of cutting productivity and rooting response (9). The studies reported here were undertaken to classify some important cacao hybrids into rooting classes in order to identify potentially good ones for propagation by cuttings. Some experiments are also reported on cultural responses of some of the hybrids during propagation by rooted cuttings.

Materials and methods

Some cacao hybrids which had been selected for high yields and disease resistance, and others selected for ease of establishment, were used. These included 15 F_2 Amazon hybrids planted in 1949, 41 F_3 Amazon hybrids planted in 1950 and 9 CRIN elite ease-of-establishment hybrids planted in 1964. The parental identification of the various hybrids is given in Tables 1 to 3. The parentage defines the actual parents in the original countries from which the materials were introduced into Nigeria. Full details about the origin of the various hybrids are described by Odegbaro (9).

The trees were planted at a spacing of 3 m x 3 m. They were properly maintained over the years by spraying regularly with insecticides and fungicides and by carrying out routine maintenance pruning. The canopy was completely closed and weeding requirements had been very minimal and mostly limited to places where overhead shade trees had fallen, damaging the continuity of the canopy. The trees were subjected to intensive yield recording over the years, and a selection based on yield records was being made for further breeding work.

The standard procedure of rooting cuttings, which has become routine for cutting production at CRIN, with necessary experimental modifications, was employed. Cuttings were collected between 0730 and 0800 h, kept in polyethylene bags and brought as soon as possible to the rooting shed for further treatment before they were set for rooting. Generally, cuttings were taken from semi-hardened, recently matured flushes with green leaves. In the rooting shed, all but the upper four leaves of the cutting were removed with a sharp knife, while a new stem surface was exposed by cutting the lower end and immediately dipping it in distilled water contained in a large plastic bowl. The stems were 15 to 20 cm long.

Some of the cuttings were treated with indole-3-butyric acid rooting hormone (IBA). The concentrated dip method was used, the concentrated solution containing 0.50% of the hormone in 50% ethanol. The cuttings of one treatment were tied together with their bases flush to the same level and were dipped to within 1 to 2 cm of their bases. After about 2 minutes, the cuttings were removed from the solution and the bundles loosened. Excess solution was shaken off and the cuttings were set in the rooting medium.

The rooting medium was composed of sawdust and top soil in black polyethylene bags. The bags were filled in such a way as to contain sawdust in a central core within the top soil and a 5 cm layer at the top of the soil. The cutting was set within the sawdust core. The bags were arranged in a randomised complete block, thoroughly watered and covered with transparent polyethylene sheets as in the method of McKelvie (8). The plants were watered daily to keep the humidity under the polyethylene sheets as near 100% as possible. The cuttings were scored for rooting after 30 days. For the classification of the various hybrids into rooting classes, 3 or 6 replicates of 20 cuttings each were randomly selected from all parts of the canopy and were set in randomised complete blocks. The experiment was carried out 3 times and the overall mean values were used for statistical analysis.

Results

Classification of cacao hybrids into rooting classes

The IBA treatment masked the division between easy rooters and difficult rooters and therefore only the results of cuttings treated with distilled water were used to classify the hybrids into rooting classes. Analysis of variance showed that variations among the hybrids were statistically significant ($P = 0.001$) and therefore the Duncan's Multiple Range Test was used to compare the means. The results are presented in Tables 1 to 3.

In Table 1, the first four hybrids were classified as easy rooters because they were significantly better than the next six (intermediate). The last five were significantly different from the rest and they were classified as difficult rooters

Among the 41 F_3 Amazon hybrids tested (Table 2), the first 14 scoring between 61 2% and 79 5% rooting were significantly better than the rest and were grouped as easy rooters. The next 15, scoring between 47 9% and 59 1%, , were grouped as intermediate rooters The rest, scoring between 45 0% and 22.6%, , feel into the difficult rooting range

Among the CRIN elite hybrids (Table 3) P47 was significantly better than all others. The next best were P49 and P39, which could be grouped with P47 as easy rooters P78, P89, P59 and P77 were the poorest and they fell among the difficult rooters P53 and P68 were intermediate

A reference to the genotype and parentage of the hybrids in Tables 1 to 3 indicates that in many cases hybrids of the same genotype fell into the same rooting range amont the F_2 and F_3 Amazon hybrids

Rooting responses to cultural practices

Leafless cacao cuttings do not normally produce roots, even under treatment with root-inducing hormones It is, however, the practice to reduce the number of leaves and the leaf area on a cutting without adversely affecting root formation To test the effect of number of leaves per cutting on rooting response, 480 cuttings were taken randomly from trees in the F_3 Amazon plot The first 240 were treated with Indole-butyric acid (IBA), and the remaining 240 were treated with distilled water. Ten cuttings were set per replicate in six replicates of cuttings with four leaves, two leaves and one leaf.

The results of the rooting response after 30 days are shown in Table 4. There was a significant difference in the mean percentage of cuttings rooted ($P = 0.01$) when more than one leaf was left on each cutting There were also significant differences in the mean number of roots per cutting and in root length ($P = 0.05$) when more than one leaf was left on each cutting Possession of more than three leaves, however, did not confer any great advantage in rooting response.

Table 1. Classification of F_2 Amazon hybrids into rooting classes.

Hybrid identification number	Genotype	Parentage	Mean % rooting	Statistical significance*
W4/407	T60/887	Pa35 x Na32	61 5	ab
W4/162	T36/763	Pa35 x Na32	57 3	bc
W4/297	T65/283	Pa7 x IMc47	57 1	bc
W4/296	T63/289	Pa7 x IMc47	54 0	bc
W4/127	T79/1139	Na32 x Pa7	46 9	cd
W4/450	T12/1233	Sca 12	46 1	cd
W4/628	T79/501	Na32 x Pa7	43 1	de
W4/630	T79/501	Na32 x Pa7	43 1	de
W4/627	T79/501	Na32 x Pa7	43 0	de
W4/622	T79/501	Na32 x Pa7	42 0	ef
W4/624	T79/501	Na32 x Pa7	32.9	gh
W4/687	T65/326	Pa7 x IMc47	32 8	gh
W4/692	T65/326	Pa7 x IMc47	32 0	gh
W4/633	T79/501	Na32 x Pa7	29.2	hi
W4/681	T65/326	Pa7 x IMc47	28 5	hi

* Means with the same letters are not significantly different from one another Means with different letters are significantly different ($P = 0\ 05$). Each figure is the mean of 3 replicates

KEY:

61.5% to 54 0% = Easy rooters
46 9% to 42 0% = Intermediate rooters.
32.9% to 28.5% = Difficult rooters

To test how the cultural practice of reducing the size of the leaves on the cuttings would affect rooting response 480 cuttings were randomly selected from a population of F_3 Amazon trees and the number of leaves on each cutting was reduced to three, the number found in the earlier trial to be optimum for rooting. The cuttings were divided into four groups; the first group had the size of each leaf reduced to about one-eighth, the second group had it reduced to about one-quarter, the third group to one-half and the last group was planted without leaf size reduction

Table 2. Classification of F_3 Amazon hybrids into rooting classes.

Hybrid identification number	Genotype	Parentage	Mean % rooting	Statistical significance
W5/689	T87/1241	IMc60 x Na34	79 5	a
W5/681	T87/1241	IMc60 x Na34	78 1	a
W5/479	T60/888	Pa7 x Na32	68.9	ab
W5/591	T72/1436	Na32 x IMc60	68 9	ab
W5/702	T87/1244	IMc60 x Na34	67 7	ab
W5/676	T87/1329	IMc60 x Na34	65 0	ab
W5/566	T79/414	Na32 x Pa7	64 6	ab
W5/347	T63/967	Pa35 x Na32	64 2	ab
W5/581	T72/1436	Na32 x IMc60	64 0	ab
W5/396	T63/968	Pa35 x Na32	63 9	ab
W5/461	T60/88	Pa7 x Na32	63 7	ab
W5/587	T72/1436	Na32 x IMc60	62 9	ab
W5/385	T63/968	Pa35 x Na32	61.3	ab
W5/391	T63/968	Pa35 x Na32	61 2	ab
W5/184	T63/733	Pa35 x Na32	59 1	bc
W5/37	T85/876	IMc60 x Na34	59.0	bc
W5/392	T63/968	Pa35 x Na32	58.9	bc
W5/403	T63/764	Pa35 x Na32	58.9	bc
W5/677	T87/1329	IMc60 x Na32	56 0	bc
W5/405	T63/968	Pa35 x Na32	55.0	bc
W5/23	T63/884	Pa35 x Na32	52 8	bc
W5/699	T87/1244	IMc60 x Na34	52 1	bc
W5/574	T72/1436	Na32 x IMc60	51.4	bc
W5/387	T63/968	Pa35 x Na32	51.0	bc
W5/32	T85/876	IMc60 x Na34	50 9	bc
W5/344	T63/967	Pa35 x Na32	50.8	bc
W5/16	T63/884	Pa35 x Na32	49.8	bc
W5/253	T63/967	Pa35 x Na32	49 8	bc
W5/398	T63/764	Pa35 x Na32	47.9	cd
W5/700	T87/1244	IMc60 x Na34	45.0	de
W5/165	T63/884	Pa35 x Na32	44 0	de
W5/213	T65/327	Pa7 x IMc47	43 1	de
W5/9	T63/884	Pa35 x Na32	41 1	ef
W5/698	T87/1244	IMc60 x Na34	40.2	ef
W5/47	T85/876	IMc60 x Na34	38 2	fg
W5/202	T65/327	Pa7 x IMc47	38.2	fg
W5/75	T79/502	Na32 x Pa7	34 2	gh
W5/345	T63/966	Pa35 x Na32	32 0	gh
W5/692	T87/1244	IMc60 x Na34	27.2	hi
W5/15	T63/884	Pa35 x Na32	26 4	hi
W5/390	T63/968	Pa35 x Na32	22 6	hi

Each figure is the mean of 3 replicates

KEY:

79 5% to 61.2% = Easy rooters.
59.1% to 47.9% = Intermediate rooters
45 0% to 22 6% = Difficult rooters.

The results presented in Table 5 show that when the leaf size was reduced to one-eighth, there was a significant adverse effect on both rooting response (P = 0.001) and root length and the number of roots produced (P = 0.05)

Cuttings for cacao propagation are taken in relation to the growth of flushes, and it is commonly stated that cuttings should be taken from recently hardened flush. This statement indicated that the exact time to take cuttings for rooting is not precisely defined and the purpose of the following experiment was to determine this.

Branches of mature hardened flushes were tagged and observed daily for the onset of bud bursting for the initiation of flushing. Shoot elongation was measured from a mark made with India ink placed 10 cm from the end of the shoot tip of each of 10 tagged branches. The length of the shoot was measured weekly from the India ink mark. The results are shown in Figure 1. Because the growth cycles of the shoots were asynchronous, the data were grouped around the initiation of flushing marked by F in the figure. The experiment was done four times with 10 branches tagged on each occasion.

Those points to either side of the point F were the means at weekly intervals before and after the initiation of growth

Growth was rapid for the first two to three weeks from the initiation of flushing, after which the rate of growth slowed down. No more growth in length was observed after the sixth week. Cuttings of new flushes were taken for rooting weekly from the third week after initiation of flushing. At this time, the leaves were fully expanded and the flushes were still pink in colour. No cutting rooted at the third and fourth weeks.

The cuttings died after a few days of setting. A few cuttings rooted at five weeks. The best rooting occurred after seven and eight weeks. At the ninth week, the rooting percentage fell with the initiation of new flushes

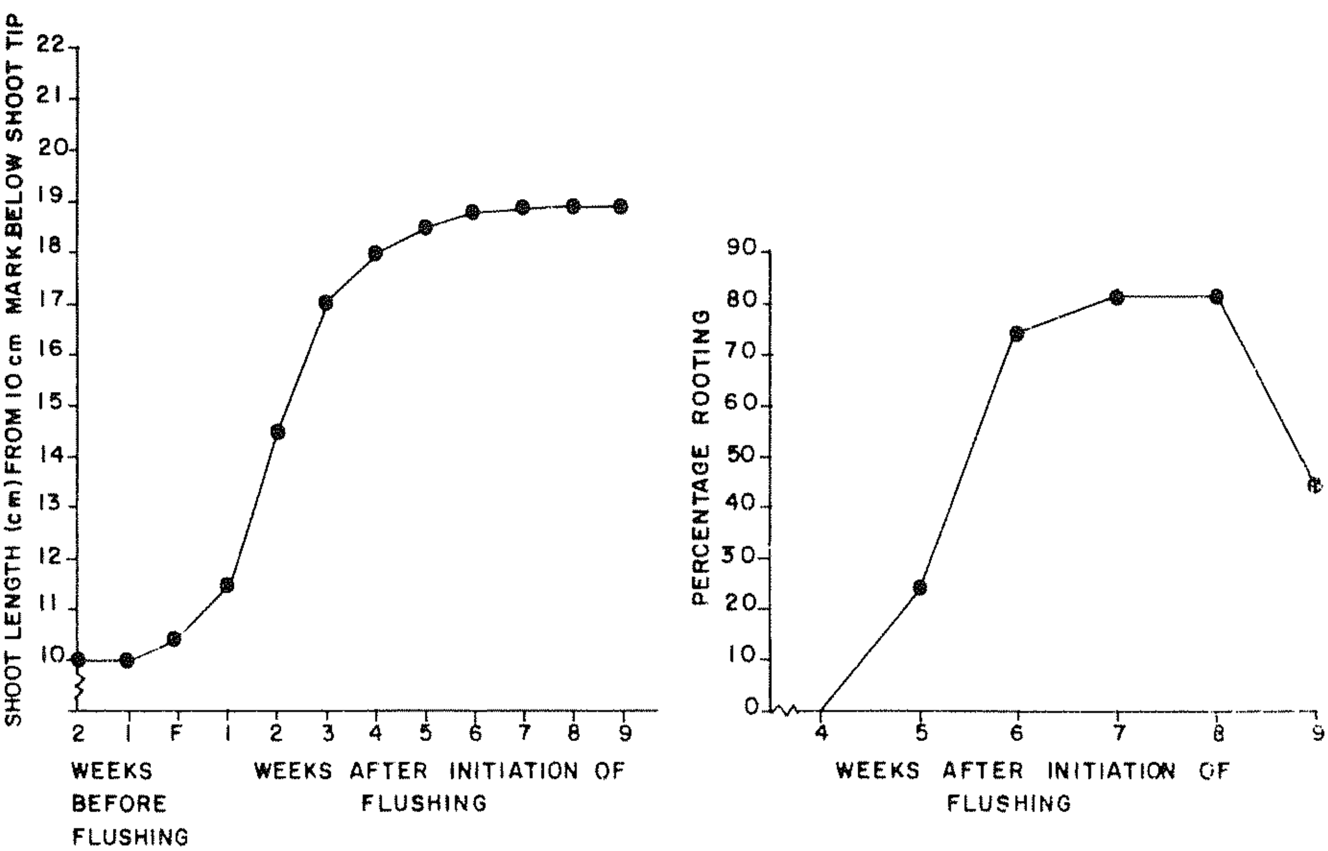

Fig. 1 Shoot elongation and rooting response at weekly intervals after initiation of flushing in F3 Amazon cacao.

Table 3. **Classification of CRIN elite hybrids into rooting classes.**

Hybrid identification number	Parentage	Mean % rooting	Statistical significance
P47	ICS1 x Na32	63.9	a
P49	ICS1 x Na32	53 3	b
P39	C77 x C64	47.5	b
P53	T7/12 x Na32	38.1	c
P68	T65/7 x Na32	35.8	c
P77	T85/5 x Pa32	33 6	cd
P59	T17/11 x Na32	29 2	cd
P89	Na32 x Na32	28 1	cd
P78	T85/45 x Na32	26 1	d

Each figure in the mean of 6 replicates

KEY:

63 9% to 47 5% = Easy rooters.
38 1% to 35 8% = Intermediate rooters
33 6% to 26 1% = Difficult rooters

Discussion

The preservation of desirable characteristics through vegetative propagation and other advantages makes cacao propagation by cuttings and important tool in cacao rehabilitation, re-establishment and new plantings This calls for the most economic rooting of the best cultivars for continuous nursery operation However, Evans (3) reported from the West Indies that cacao clones differ in the ease with which they are rooted, the slow rooting ones producing few or no roots under conditions which are satisfactory for the easy rooting clones. It thus appears that the first step in cacao propagation by rooted cuttings is to determine the rooting behaviour of the cacao cultivars selected for various desirable characteristics

The data in Tables 1 to 3 show that large differences occurred in the rooting response of apparently similar cacao types. Among the F_2 Amazon hybrids, rooting response ranged from 61 5% to 28.5% . Among the F_3 Amazon, it ranged from 79 5% to 22 6% and among the CRIN elites from 63.9% to 26.1% The effect of the hormones used was to reduce these wide gaps and so mask the division into easy and difficult rooters. Hartman and Kester (6) reported that great differences exist among species and among clones in the rooting ability of cuttings taken from then. In their opinion, it is often difficult to predict whether or not cuttings of a certain clone will root easily; empirical trials with each clone were necessary With cacao, Archibald (1) also reported that considerable variation in rooting behaviour was shown between cuttings from different clones, different trees of the same clone, different parts of the same tree and different parts of the same shoot. He ascribed the variation to internal factors

All processes occurring in plants are subject to the dual control of the genetic complement of the plant and the environmental factors to which the plant is subjected Whenever the same process is studied for two different varieties of the same plant, the effect of the genetic make-up on the physiology of the plant comes under consideration. The differences in physiology between varieties are as much a reflection of their genetic differences as their more immediate apparent differences in morphology Although in many respects, morphological manifestations of genetic differences are more obvious than physiological manifestations, the latter can be much more important, e g. varieties which may be nearly indistinguishable morphologically may differ markedly in their physiology.

These physiological differences can also be exploited, in addition to the genetic differences, for improving the performance of the plant. In the studies reported here, when the various hybrids were divided into their rooting classes, it was found that, in many cases, clones of the same genotype fell into the same rooting classes (Tables 1 to 3). This indicates that the genetic background of clones can be important in their rooting response and it could therefore be possible to breed or select for high rooting response in cacao.

Unlike many temperate plants, leafless cacao cuttings do not normally produce roots. In the investigations reported, it was found that two leaves per cutting were adequate for good rooting (Table 4), although up to four leaves my be retained as insurance against loss of leaves through physiological hazards and/or pest attack. When the leaf area was reduced to as little as one-eighth, there was highly significant reduction in percentage of rooting (Table 5). This confirms the essentiality of the leaves for the rooting process. Hunter *et al.* (7) found that the presence of a leaf in the absence of light was sufficient for root initiation in cacao, but the survival of the cutting depended on light. The latter suggests that continuation of photosynthesis in the rooting bin is essential for the growth of roots. Starch tests on cuttings showed that starch disappeared from the bases of the cuttings fairly rapidly. In cuttings from which the leaves were removed, only slight traces of starch could be found, while those on which the leaves were retained showed abundant starch. It is thus obvious that two very important factors determining the rooting and the survival of the cacao cuttings are the quantity of carbohydrates in the cuttings at the time of striking and the efficiency of the leaf in manufacturing carbohydrates. This was shown for chrysanthemum cuttings by Samananda *et al.* (12). It remains to be seen if their suggestions for combined applications of hormones will improve rooting further in cacao.

The striking of cuttings in relation to flushing has received very little attention from researchers. The age of flush is important. Swarbrick (13) obtained the best rooting of *Cola* spp. when he used three-month old wood from fully hardened flush. Pyke (11) could obtain only callus formation from hardwood cuttings of cacao, and he concluded that cacao cuttings should be taken from soft or semi-soft materials from a recently matured flush. There has been a need to define more precisely the degree of softness of wood and maturity of the flush that will produce rooting results. The data of Figure 1 show that the precise time lies between seven and eight weeks after the initiation of flushing.

Summary

A large number of cacao hybrids were classified into **difficult, intermediate** and **easy** rooters. Significant differences (P = 0.05) were found in the rooting responses of apparently similar cacao types. Mean percentage of cuttings rooted ranged from 28.5 to 61.5 among the F_2 Amazon hybrids; from 22.6 to 79.5 among the F_3 Amazon hybrids and from 26.1 to 63.9 among the CRIN elite hybrids. It was found that in many cases clones of the same genotype among the various hybrids fell into the same rooting range.

An investigation of rooting response of some of the hybrids to some cultural practices was also carried out. It was found that the presence of only one leaf per cutting significantly reduced rooting (P = 0.01) when compared with a higher number of leaves per cutting. Two leaves per cutting were found to be adequate for shoot rooting. Reduction in the size of leaves by half was found to have no adverse effect on rooting and the practice even enhanced the survival of the cuttings under the polyethylene sheets.

Table 4. Effect of number of leaves per cutting on rooting response of randomly selected F_3 Amazon trees: mean percent rooting, mean number of roots per cutting and mean root length 30 days after setting.

Number of leaves per cutting	Mean percent of cuttings rooted		Mean root number per cutting		Mean root length per cutting (cm)	
	DW	IBA	DW	IBA	DW	IBA
1	46.7**	58.4**	2.2*	3.9*	1.4*	3.0*
2	65.3	80.7	4.0	5.4	2.7	3.7
3	66.9	82.6	4.3	5.6	2.9	3.8
4	64.5	79.5	4.0	5.9	3.1	3.5

Each figure is the mean of 6 replicates.

* Significantly different at P = 0.05

** Significantly different at P = 0.01

DW = Cuttings treated with distilled water

IBA = Cuttings treated with Indole-butyric acid used for rooting the cuttings. However, reducing the size to one-eighth of the normal leaf size significantly reduced rooting (P = 0.001). Cuttings rooted best at seven to eight weeks after the initiation of flushing

Table 5. Effect of reduction in leaf size on rooting response of randomly selected F_3 Amazon hybrids.

Leaf size	Mean percent of cuttings rooted		Mean root number per cutting		Mean root length per cutting (cm)	
	DW	IBA	DW	IBA	DW	IBA
One–eighth	9.8***	14.5***	1.2*	1.3*	1.0*	1.5*
One–quarter	60.4	63	2.2	2.5	2.1	2.9
Half	61.5	72.3	2.8	2.7	2.6	3.4
Full	68.9	80.2	3.4	3.5	2.9	3.5

Each figure is the mean of 6 replicates.

* Significant at P = 0.05.

*** Significant at P = 0.001.

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