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Effect of growth regulators and environment on biochemical changes and rooting of cuttings in cocoa (*Theobroma cacao* L.)

V Jegadeeswari, K Arunkumar and B Dharmalingam

Abstract
An investigation was conducted to study the effect of growth regulators and environment on biochemical changes and rooting of cutting of cuttings in cocoa. In this present investigation also C:N ratio was higher in long cuttings in both plagiotropic shoots (10.15) and orthotropic shoots (9.73) which have proven to record high percentage of sprouting and rooting than two node cuttings. Cuttings treated with IBA 10000 ppm registered high phenol content in plagiotropic (1.93 mg/g) as well as orthotropic shoots (1.76 mg/g) than other treatment. The peroxidase content was slightly higher in IBA 7500 ppm treated cuttings in both plagiotropic (1.15 change in the OD 430 nm / min / g) and orthotropic shoots (0.58 change in the OD 430 nm / min / g). Thus, the best treatment combination was found to be that of plagiotropic long cuttings treated with IBA 7500 ppm. But some more limiting factors need to be assessed for getting higher success percentage and make this a method for mass multiplication of cocoa through cuttings as a feasible venture.

Keywords: IBA, plagiotropic shoots, orthotropic shoots

Introduction

Cocoa (*Theobroma cacao* L.) belonging to the family ‘Malvaceae’ (Alverson *et al.*, 1999) [1] is one of the world’s most important perennial crop, explored exclusively for chocolate manufacturing. It is third important beverage crop in the world, next to tea and coffee. In India, its cultivation started in 1970s and at present is grown around 71,370 ha with a total production of 15,130 metric tonnes and the productivity of 4.7 mt/ha (NHB, 2014) [10]. Cocoa plants are grown under the shade of arecanut and coconut plantations in South India (Shama Bhat, 1988) [15]. It is extensively cultivated in Kerala, Karnataka, Andhra Pradesh and Tamil Nadu, with Kerala ranking first in cocoa cultivation. In Tamil Nadu, the total area accounts for 24,000 ha, with an annual production 1,100 metric tonnes accounting a productivity of 21.8 mt/ha (NHB, 2014) [10].

Cocoa is highly cross pollinated crop, largely multiplied through seed and seedlings are used as the planting material (N’ Goran *et al.*, 2000) [9], the seedling progenies exhibit higher variability. Earlier work done in other countries and in India show that grafting, budding and stem cutting are the various methods of clonal propagation followed in cocoa (Kailasam *et al.*, 1964) [8].

This work is mainly focused on to study the effect of growth regulators and environment on biochemical changes and rooting of cuttings in cocoa.

Materials and Methods

The study was undertaken at Department of Spices and Plantation crops, TNAU, Coimbatore and Cocoa nursery, Anaimalai during the year 2014-15. The propagation structure like Mc Kelvies polythene sheet tunnel (mini poly tunnel) and mist chamber and two type of cuttings like two node and long cuttings are used. The source of cuttings like plagiotropic shoots and orthotropic shoots and rooting media like garden mixture (1part of sand, 2 parts of red earth and 1 part of farm yard manure) are used. The growth regulators like Indole Butyric Acid (IBA – 5,000 ppm; 7,500 ppm and 10, 000 ppm) are used. The experiment was laid out in the mist chamber at TNAU, Coimbatore and in mini polytunnel, Cocoa nursery, Anaimalai with Factorial Completely Randomized Design (FCRD). There were 12 treatments. Each treatment was replicated twice; each treatment consisted of 100 cuttings.

The treatments for rooting of two node and long cutting collected from plagiotropic and Orthotropic shoots at Cocoa nursery, Anaimalai and Mist chamber, TNAU are as given in Table 1.

Table 1: Treatment details

Treatment	Source of cutting	IBA concentrations	Type of cuttings
T_1	Plagiotropic shoots	5000 ppm (G_1)	Two node (N_1)
T_2			Long cutting (N_2)
T_3		7500 ppm (G_2)	Two node (N_1)
T_4			Long cutting (N_2)
T_5		10000 ppm (G_3)	Two node (N_1)
T_6			Long cutting (N_2)
T_7	Orthotropic shoots	5000 ppm (G_1)	Two node (N_1)
T_8			Long cutting (N_2)
T_9		7500 ppm (G_2)	Two node (N_1)
T_{10}			Long cutting (N_2)
T_{11}		10000 ppm (G_3)	Two node (N_1)
T_{12}			Long cutting (N_2)

The biochemical parameters like total carbohydrate, total nitrogen, carbohydrate- nitrogen ratio, total phenols and peroxidase activity was recorded. The total carbohydrate at the base of the cuttings were estimated by the method of Somogyi (1952) [16] and expressed in percentage on fresh weight basis. The nitrogen content at the base of the cuttings on dry weight basis was estimated by Micro kjeldal method (Humphries, 1956) [6] and expressed in percentage. The ratio of carbohydrates to nitrogen (C/N) was calculated based on analysis of total carbohydrate and total nitrogen present at the time of root initiation. Folin-Ciocalteau reagent method was used for estimating the total phenol (Bray and Thorpe, 1954) [3]. The result was expressed as milligram equivalent for pyrocatechol per gram of the sample. Peroxidase activity was determined according to the procedure given by Perur (1962) [12] and was expressed as change in the OD 430 nm/ min/ g.

Results and Discussion

Total carbohydrate content

Total carbohydrate content analyzed in the stem portion of the cuttings at 120th days. Total carbohydrate content in plagiotropic shoot cuttings exhibited significant differences among the environments, different growth regulator concentrations and type of cuttings. (Table 2a).

Among the environments, cuttings kept under mini poly tunnel (E_1) registered higher carbohydrate content (14.37%) followed by the cuttings kept under automated mist chamber (E_2) (6.45%). Among the growth regulator concentrations, cuttings treated with IBA 7500 ppm (G_2) registered maximum carbohydrate content (15.14%) followed by the cuttings treated with IBA 10000 ppm (G_3) (9.14%) and cuttings treated with IBA 5000 ppm (G_1) (6.96%). Among the type of cuttings, two node cuttings (N_1) recorded maximum carbohydrate content (11.10%) than long cuttings (N_2) (9.72%).

In two way interaction, E × G, G × N and E × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under mini poly tunnel (E_1G_2) registered the maximum carbohydrate content (16.00%) followed by the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) which recorded 14.28%.

In G × N interaction, long cuttings treated with IBA 7500 ppm (G_2N_2) recorded maximum carbohydrate content (15.75%) followed by the two node cuttings treated with IBA 7500 ppm (G_2N_1) (14.53%).

In E × N interaction, maximum carbohydrate content was observed in long cuttings kept under mini poly tunnel (E_1N_2) which registered 14.57% followed by two node kept under mini poly tunnel (E_1N_1) (14.17%).

The three way interaction also showed high significance. In that, maximum carbohydrate content (16.90%) was recorded in long cuttings treated with IBA 7500 ppm and kept under mini poly tunnel ($E_1G_2N_2$) and followed by the two node cuttings treated with IBA 7500 ppm and kept under the same condition ($E_1G_2N_1$) which recorded 15.10%.

In the case of orthotropic shoot cuttings, the effect of environments, growth regulator concentrations and type of cuttings showed high significance (Table 2b).

Among the environments, cuttings kept under automated mist chamber (E_2) registered higher carbohydrate content (9.45%) followed by the cuttings kept under mini poly tunnel (E_1) (6.37%). Among the growth regulator concentrations, cuttings treated with IBA 7500 ppm (G_2) registered maximum carbohydrate content (11.60%) followed by the cuttings treated with IBA 10000 ppm (G_3) (9.49%). Among the type of cuttings, long cuttings (N_2) recorded maximum carbohydrate content (10.91%) than two node cuttings (N_1) (4.92%).

In two way interaction, E × G, G × N and E × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) registered the maximum carbohydrate content (15.52%) followed by the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3) which recorded 12.83%.

In E × N interaction, maximum carbohydrate content was observed in long cuttings kept under mini poly tunnel (E_1N_2) (12.75%) followed by two node cuttings kept under automated mist chamber (E_2N_1) which registered 9.83%.

The three way interaction also showed high significance. In that, maximum carbohydrate content (17.19%) was recorded in two node cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_1$) and followed by long cuttings treated with IBA 7500 ppm and kept under mini poly tunnel ($E_1G_2N_2$) which recorded 15.36%.

Table 2a: Effect of environment, type of cuttings and growth regulators on total carbohydrates (%) of planting in plagiotropic shoot cuttings of cocoa

		Plagiotropic shoots						Mean
		N_1			N_2			
E_1	G_1	14.63			13.19			13.91
	G_2	15.10			16.90			16.00
	G_3	12.78			13.61			13.19
Mean		14.17 (22.07)			14.57 (22.39)			14.37 (22.23)
E_2	G_1	0.00			0.00			0.00
	G_2	13.96			14.60			14.28
	G_3	10.14			0.00			5.07
Mean		8.03 (13.70)			4.87 (7.88)			6.45 (10.79)
$\overline{X}$		11.10 (17.88)			9.72 (15.13)			10.41 (16.51)
		G_1: 7.32 (11.53)	G_2: 4.53 (22.40)	G_3: 11.46 (19.72)	G_1: 6.60 (10.93)	G_2:15.75 (23.36)	G_3: 6.81 (11.11)	
		G_1- 6.96 (11.23)		G_2- 15.14 (22.88)		G_3- 9.14 (15.42)		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.47	0.57	0.47	0.81	0.81	0.66	1.15
CD (P=0.05)		1.02**	1.25**	1.02**	1.77**	1.77**	1.44**	2.50**
NS - non significant, * - Significant, ** - highly significant								
E_1 - Mini poly tunnel E_2 - Automated mist chamber			G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm			N_1 - Two node cutting N_2 - Long cutting		

Table 2b: Effect of environment, type of cuttings and growth regulators on total carbohydrates (%) of planting in orthotropic shoot cuttings of cocoa

		Orthotropic shoots						Mean
		N_1			N_2			
E_1	G_1	0.00			10.64			5.32
	G_2	0.00			15.36			7.68
	G_3	0.00			12.25			6.13
Mean		0.00 (0.58)			12.75 (20.86)			6.37 (10.72)
E_2	G_1	0.00			0.00			0.00
	G_2	17.19			13.84			15.52
	G_3	12.29			13.38			12.83
Mean		9.83 (15.20)			9.07 (14.62)			9.45 (14.91)
$\overline{X}$		4.92 (7.89)			10.91 (17.74)			7.92 (12.82)
		G_1: 0.00 (0.58)	G_2: 8.60 (12.54)	G_3: 6.15 (10.55)	G_1: 5.32 (9.81)	G_2:14.60 (22.45)	G_3: 2.82 (20.96)	
		G_1- 2.66 (5.20)		G_2- 11.60 (17.50)		G_3- 9.49 (15.76)		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.28	0.34	0.28	0.48	NS	0.39	0.68
CD (P=0.05)		0.61**	0.74**	0.61**	0.61**	-	0.86**	1.48**
NS - non significant, * - Significant, ** - highly significant								
E_1 - Mini poly tunnel E_2 - Automated mist chamber			G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm			N_1 - Two node cutting N_2 - Long cutting		

Total nitrogen content

Number of days for sprout initiation in plagiotropic shoot cuttings was significantly affected by environments, growth regulator concentrations and type of cuttings (Table 3a).

Among the environments, cuttings kept under automated mist chamber (E_2) registered low amount of nitrogen content (0.46%) while the cuttings kept under mini poly tunnel (E_1) registered more amount of nitrogen content (1.29%). Among the growth regulator concentrations, cuttings treated with IBA 10000 ppm (G_3) registered minimum amount of nitrogen content (0.78%) while cuttings treated with IBA 7500 ppm (G_2) registered maximum amount of nitrogen content (0.83%). Among the type of cuttings, long cuttings (N_2) recorded low amount of nitrogen content (0.82%) than two node cuttings (N_1) (0.93%).

The two way interaction between the factors such as E × G, G × N and E × N did show high significance. Among the E × G interaction, the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3) registered 0.53% of nitrogen content followed by the cuttings treated with IBA 7500 ppm and kept under mini poly tunnel (E_1G_2) registered 0.82% of nitrogen content and the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) which recorded 0.83% and more amount of nitrogen content (2.04%) was observed in the cuttings treated with IBA 5000 ppm and kept under mini poly tunnel (E_1G_1).

In case of G × N interaction, long cuttings treated with IBA 10000 ppm (G_3N_2) registered low amount of nitrogen content (0.49%) while more amount of nitrogen content (1.20%) was observed in long cuttings treated with IBA 7500 ppm (G_1N_2).

In E × N interaction, long cuttings kept under automated mist chamber (E_2N_2) registered minimum amount of nitrogen content (0.25%) and maximum was observed in long cuttings kept under mini poly tunnel (E_1N_2) (1.39%).

The three way interaction also showed high significance. In that, minimum amount of nitrogen content was recorded in long cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_2$) registered 0.76% followed by long cuttings treated with IBA 7500 ppm and kept under mini poly tunnel ($E_1G_2N_2$) which registered 0.79% and two node cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_1$) which registered 0.91% while more amount of nitrogen content (2.40%) was noted in long cuttings treated with IBA 5000 ppm and kept under mini poly tunnel ($E_1G_1N_2$).

In the case of orthotropic shoot cuttings, the effect of environments, growth regulator concentrations and type of cuttings showed high significance (Table 3b).

Among the environments, cuttings kept under mini poly tunnel (E_1) registered low amount of nitrogen content (0.64%) while automated cuttings kept under mist chamber (E_2) recorded more amount of nitrogen content (0.74%). Among the different growth regulator concentrations, cuttings treated with IBA 5000 ppm (G_1) recorded minimum amount of nitrogen content (0.46%) while the cuttings treated with IBA 10000 ppm (G_3) registered maximum amount of nitrogen content (0.85%). Among the type of cuttings, two node cuttings (N_1) recorded low amount of nitrogen content (0.37%) than long cuttings (N_2) (1.02%).

The two way interaction between the factors such as E × G, G × N and E × N did show high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under mini poly tunnel (E_1G_2) registered 0.46% of nitrogen content followed by the cuttings treated with IBA 10000 ppm and kept under mini poly tunnel (E_1G_3) which recorded 0.56% and more amount of nitrogen content (1.14%) was observed in the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3).

In case of G × N interaction, two node cuttings treated with IBA 7500 ppm (G_2N_1) registered low amount of nitrogen content (0.50%) where more amount of nitrogen content (1.09%) was observed in long cuttings treated with IBA 10000 ppm (G_3N_2).

In E × N interaction, two node cuttings kept under automated mist chamber (E_2N_1) registered minimum amount of nitrogen content (0.73%) and maximum was observed in long cuttings kept under mini poly tunnel (E_1N_2) (1.29%).

The three way interaction also showed high significance. In that, minimum amount of nitrogen content was recorded in long cuttings treated with IBA 7500 ppm and kept under mini poly tunnel ($E_1G_2N_2$) registered 0.93% followed by the two node cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_1$) which registered 0.99% while more amount of nitrogen content (1.82%) was noted in long cuttings treated with IBA 5000 ppm and kept under mini poly tunnel ($E_1G_1N_2$).

Table 3a: Effect of environment, type of cuttings and growth regulators on total nitrogen (%) of planting in plagiotropic shoot cuttings of cocoa

<table>
<tr><th colspan="2" rowspan="2"></th><th colspan="6">Plagiotropic shoots</th><th rowspan="2">Mean</th></tr>
<tr><th colspan="3">N_1</th><th colspan="3">N_2</th></tr>
<tr><td rowspan="3">E_1</td><td>G_1</td><td colspan="3">1.67</td><td colspan="3">2.40</td><td>2.04</td></tr>
<tr><td>G_2</td><td colspan="3">0.85</td><td colspan="3">0.79</td><td>0.82</td></tr>
<tr><td>G_3</td><td colspan="3">1.06</td><td colspan="3">0.98</td><td>1.02</td></tr>
<tr><td colspan="2">Mean</td><td colspan="3">1.19 (6.10)</td><td colspan="3">1.39 (6.55)</td><td>1.29 (6.32)</td></tr>
<tr><td rowspan="3">E_2</td><td>G_1</td><td colspan="3">0.00</td><td colspan="3">0.00</td><td>0.00</td></tr>
<tr><td>G_2</td><td colspan="3">0.91</td><td colspan="3">0.76</td><td>0.83</td></tr>
<tr><td>G_3</td><td colspan="3">1.07</td><td colspan="3">0.00</td><td>0.53</td></tr>
<tr><td colspan="2">Mean</td><td colspan="3">0.66 (3.99)</td><td colspan="3">0.25 (2.06)</td><td>0.46 (3.02)</td></tr>
<tr><td colspan="2" rowspan="3">$\overline{X}$</td><td colspan="3">0.93
(5.04)</td><td colspan="3">0.82
(4.30)</td><td rowspan="3">0.86
(4.67)</td></tr>
<tr><td>G_1: 0.84
(4.00)</td><td>G_2: 0.88
(5.21)</td><td>G_3: 1.07
(5.91)</td><td>G_1: 1.20
(4.73)</td><td>G_2:0.78
(5.04)</td><td>G_3: 0.49
(3.13)</td></tr>
<tr><td colspan="2">G_1- 1.02
(4.37)</td><td colspan="2">G_2- 0.83
(5.13)</td><td colspan="2">G_3- 0.78
(4.52)</td></tr>
<tr><td colspan="2">Interactions</td><td>E</td><td>G</td><td>N</td><td>E×G</td><td>G×N</td><td>E×N</td><td>E×G×N</td></tr>
<tr><td colspan="2">SE(d)</td><td>0.18</td><td>0.21</td><td>0.18</td><td>0.30</td><td>0.30</td><td>0.25</td><td>0.43</td></tr>
<tr><td colspan="2">CD (P=0.05)</td><td>0.38^{**}</td><td>0.46^{**}</td><td>0.38^{**}</td><td>0.66^{**}</td><td>0.66^{**}</td><td>0.54^{**}</td><td>0.93^{**}</td></tr>
<tr><td colspan="9">NS - non significant, * - Significant, ** - highly significant</td></tr>
<tr><td colspan="3">E_1 - Mini poly tunnel
E_2 - Automated mist chamber</td><td colspan="2">G_1 - IBA 5000ppm
G_2 - IBA 7500ppm
G_3 - IBA 10000ppm</td><td colspan="4">N_1 - Two node cutting
N_2 - Long cutting</td></tr>
</table>

Table 3b: Effect of environment, type of cuttings and growth regulators on total nitrogen (%) of planting in orthotropic shoot cuttings of cocoa

		Orthotropic shoots		Mean
		N_1	N_2	
E_1	G_1	0.00	1.82	0.91
	G_2	0.00	0.93	0.46
	G_3	0.00	1.12	0.56
Mean		0.00 (0.58)	1.29 (6.44)	0.64 (3.51)
E_2	G_1	0.00	0.00	0.00
	G_2	0.99	1.18	1.08
	G_3	1.21	1.06	1.14
Mean		0.73 (4.20)	0.75 (4.24)	0.74 (4.22)
$\overline{X}$		0.37 (2.39)	1.02 (5.34)	0.70 (3.87)

	G_1: 0.00 (0.58)	G_2: 0.50 (3.15)	G_3: 0.61 (3.45)	G_1: 0.91 (4.17)	G_2: 1.06 (5.87)	G_3: 1.09 (5.98)	
	G_1- 0.46 (2.38)		G_2- 0.78 (4.51)		G_3- 0.85 (4.71)		
Interactions	E	G	N	E×G	G×N	E×N	E×G×N
SE(d)	0.11	0.13	1.11	1.18	0.18	0.15	0.26
CD (P=0.05)	0.23**	0.28**	0.23**	0.40**	0.40**	0.32**	0.56**
NS - non significant, * - Significant, ** - highly significant							
E_1 - Mini poly tunnel E_2 - Automated mist chamber			G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm		N_1 - Two node cutting N_2 - Long cutting		

Carbohydrate - Nitrogen ratio

C:N ratio in plagiotropic shoot cuttings exhibited significant differences among the environments and growth regulator concentrations and (Table 4a).

Among the environments, cuttings kept under mini poly tunnel (E_1) registered higher C:N ratio (13.40) followed by the cuttings kept under automated mist chamber (E_2) (7.41). Among the growth regulator concentrations, cuttings treated with IBA 7500 ppm (G_2) registered maximum C:N ratio (18.55) followed by the cuttings treated with IBA 10000 ppm (G_3) (8.99) and IBA at 5000 ppm (G_1) (3.67).

In two way interaction, E × G and G × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under mini poly tunnel (E_1G_2) registered the maximum C:N ratio (19.67) followed by the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) which recorded 17.47.

In G × N interaction, long cuttings treated with IBA 7500 ppm (G_2N_2) recorded maximum C:N ratio (20.46) followed by the two node cuttings treated with IBA 7500 ppm (G_2N_1) (16.63).

The three way interaction also showed high significance. In that, maximum C:N ratio (21.45) was recorded in long cuttings treated with IBA 7500 ppm and kept under mini poly tunnel ($E_1G_2N_2$) and followed by long cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_2$) which recorded 19.47.

In the case of orthotropic shoot cuttings, the effect of environments, growth regulator concentrations and type of cuttings showed high significance (Table 4b).

Among the environments, cuttings kept under automated mist chamber (E_2) recorded higher C:N ratio (8.71) followed by the cuttings kept under mini poly tunnel (E_1) (5.64). Among the growth regulator concentrations, cuttings treated with IBA 7500 ppm (G_2) registered maximum C:N ratio (11.55) followed by the cuttings treated with IBA at 10000 ppm (G_3) (8.50). Among the type of cuttings, long cuttings (N_2) recorded maximum C:N ratio (9.73) than two node cuttings (N_1) (4.62).

In two way interaction, E × G and E × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) registered the maximum C:N ratio (14.69) followed by the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3) which recorded 11.43.

In E × N interaction, maximum C:N ratio was observed in long cuttings kept under mini poly tunnel (E_1N_2`) (11.28) followed by the two node cuttings kept under automated mist chamber (E_2N_1) which registered 9.24.

The three way interaction also showed high significance. In that, maximum C:N ratio (17.51) was recorded in two node cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_1$) and followed by the long cuttings treated with IBA 7500 ppm and kept under mini poly tunnel ($E_1G_2N_2$) which recorded 16.82.

Table 4a: Effect of environment, type of cuttings and growth regulators on C/N ratio of planting in plagiotropic shoot cuttings of cocoa

		Plagiotropic shoots						**Mean**
		N_1			**N_2**			
E_1	G_1	8.86			5.82			7.34
	G_2	17.88			21.45			19.67
	G_3	12.28			14.14			13.21
Mean		13.00			13.80			13.40
E_2	G_1	0.00			0.00			0.00
	G_2	15.48			19.47			17.47
	G_3	9.53			0.00			4.76
Mean		8.34			6.49			7.41
$\overline{X}$		10.65			10.15			10.40
		G_1: 4.43	G_2: 6.63	G_3: 10.90	G_1: 2.91	G_2: 0.46	G_3: 7.07	
		G_1- 3.67		G_2- 18.55		G_3- 8.99		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.89	1.09	NS	1.53	1.53	NS	2.17
CD (P=0.05)		1.93**	2.36**	-	3.34*	3.34**	-	4.73**
NS - non significant, * - Significant, ** - highly significant								
E_1 - Mini poly tunnel E_2 - Automated mist chamber				G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm		N_1 - Two node cutting N_2 - Long cutting		

Table 4b: Effect of environment, type of cuttings and growth regulators on C/N ratio of planting in orthotropic shoot cuttings of cocoa

		Orthotropic shoots						Mean
		N_1			N_2			
E_1	G_1	0.00			5.88			2.94
	G_2	0.00			16.82			8.41
	G_3	0.00			11.15			5.57
Mean		0.00			11.28			5.64
E_2	G_1	0.00			0.00			0.00
	G_2	17.51			11.88			14.69
	G_3	10.21			12.66			11.43
Mean		9.24			8.18			8.71
$\overline{X}$		4.62			9.73			7.17
		G_1: 0.00	G_2: 8.75	G_3: 5.10	G_1: 2.94	G_2: 4.35	G_3: 1.91	
		G_1- 1.47		G_2- 11.55		G_3- 8.50		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.65	0.80	0.65	1.13	NS	0.92	1.60
CD (P=0.05)		1.42^{**}	1.74^{**}	1.42^{**}	2.46^{**}	-	2.01^{**}	3.48^{**}
NS - non significant, * - Significant, ** - highly significant								
E_1 - Mini poly tunnel E_2 - Automated mist chamber			G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm		N_1 - Two node cutting N_2 - Long cutting			

Total phenol content

Total phenol content in plagiotropic shoot cuttings exhibited significant differences among the environments, growth regulator concentrations and type of cuttings. (Table 5a).

Among the environments, cuttings kept under mini poly tunnel (E_1) recorded higher phenol content (1.65 mg/g) followed by the cuttings kept under automated mist chamber (E_2) (0.91 mg/g). Among the growth regulator concentrations, cuttings treated with IBA 10000 ppm (G_3) registered maximum phenol content (1.93 mg/g) followed by the cuttings treated with IBA 7500 ppm (G_2) (1.17 mg/g) and cuttings treated with IBA 5000 ppm (G_1) (0.74 mg/g). Among the type of cuttings, two node cuttings (N_1) recorded maximum phenol content (11.73 mg/g) than long cuttings (N_2) (0.83 mg/g).

In two way interaction, E × G and G × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 10000 ppm and kept under mini poly tunnel (E_1G_3) registered the maximum phenol content (2.38 mg/g) followed by the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3) which recorded 1.48 mg/g and the cuttings treated with IBA 5000 ppm and kept under mini poly tunnel (E_1G_1) which recorded 1.47 mg/g.

In G × N interaction, two node cuttings treated with IBA 10000 ppm (G_3N_1) recorded maximum phenol content (3.13 mg/g) followed by two node cuttings treated with IBA 7500 ppm (G_2N_1) (1.26 mg/g).

The three way interaction also showed high significance. In that, maximum phenol content (3.28 mg/g) was recorded in two node cuttings treated with IBA 10000 ppm and kept under mini poly tunnel ($E_1G_3N_1$) and followed by the two node cuttings treated with IBA 10000 ppm and kept under automated mist chamber ($E_2G_3N_1$) which recorded 2.97 mg/g.

In the case of orthotropic shoot cuttings, the effect of environments, growth regulator concentrations and type of cuttings showed high significance (Table 5b).

Among the environments, cuttings kept under automated mist chamber (E_2) registered higher phenol content (1.51 mg/g) followed by the cuttings kept under mini poly tunnel (E_1) (0.89 mg/g). Among the growth regulator concentrations, cuttings treated with IBA 10000 ppm (G_3) registered maximum phenol content (1.76 mg/g) followed by the cuttings treated with IBA 7500 ppm (G_2) (1.22 mg/g). Among the type of cuttings, long cuttings (N_2) recorded maximum phenol content (1.71 mg/g) than two node cuttings (N_1) (0.69 mg/g).

In two way interaction, E × G and E × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3) registered the maximum phenol content (2.70 mg/g) followed by the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) which recorded 1.84 mg/g.

In E × N interaction, maximum phenol content was observed in long cuttings kept under mini poly tunnel (E_1N_2) (1.78 mg/g) followed by the long cuttings kept under automated mist chamber (E_2N_2) which registered 1.64 mg/g.

The three way interaction also showed high significance. In that, maximum phenol content (2.89 mg/g) was recorded in long cuttings treated with IBA 10000 ppm and kept under automated mist chamber ($E_2G_3N_2$) followed by the two node cuttings treated with IBA 10000 ppm and kept under automated mist chamber ($E_2G_3N_1$) which recorded 2.52 mg/g and long cuttings treated with IBA 5000 ppm and kept under mini ploy tunnel ($E_1G_1N_1$) which recorded 2.49 mg/g.

Table 5a: Effect of environment, type of cuttings and growth regulators on total phenols (mg/g) of planting in plagiotropic shoot cuttings of cocoa

		Plagiotropic shoots		Mean
		N_1	N_2	
E_1	G_1	1.62	1.32	1.47
	G_2	1.14	1.04	1.09
	G_3	3.28	1.48	2.38
Mean		2.01	1.28	1.65
E_2	G_1	0.00	0.00	0.00
	G_2	1.38	1.12	1.25
	G_3	2.97	0.00	1.48

Mean	1.45			0.37			0.91
$\overline{X}$	1.73			0.83			1.28
	G1: 0.81	G2: 1.26	G3: 3.13	G1: 0.66	G2:1.08	G3: 0.74	
	G1- 0.74		G2- 1.17		G3- 1.93		
Interactions	E	G	N	E×G	G×N	E×N	E×G×N
SE(d)	0.08	0.10	0.08	0.14	0.14	NS	0.20
CD (P=0.05)	0.18**	0.21**	0.18**	0.31**	0.31**	-	0.44**
NS - non significant, * - Significant, ** - highly significant							
E1 - Mini poly tunnel E2 - Automated mist chamber			G1 - IBA 5000ppm G2 - IBA 7500ppm G3 - IBA 10000ppm		N1 - Two node cutting N2 - Long cutting		

Table 5b: Effect of environment, type of cuttings and growth regulators on total phenols (mg/g) of planting in orthotropic shoot cuttings of cocoa

		Orthotropic shoots						**Mean**
		N1			**N2**			
E1	G1	0.00			2.49			1.25
	G2	0.00			1.21			0.61
	G3	0.00			1.63			0.82
Mean		0.00			1.78			0.89
E2	G1	0.00			0.00			0.00
	G2	1.64			2.04			1.84
	G3	2.52			2.89			2.70
Mean		1.38			1.64			1.51
$\overline{X}$		0.69			1.71			1.20
		G1: 0.00	G2: 0.82	G3: 1.26	G1: 1.25	G2: 1.63	G3: 2.26	
		G1- 0.62		G2- 1.22		G3-1.76		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.09	0.11	0.09	0.15	NS	0.13	0.22
CD (P=0.05)		0.19**	0.24**	0.19**	0.34**	-	0.28**	0.48**
NS - non significant, * - Significant, ** - highly significant								
E1 - Mini poly tunnel E2 - Automated mist chamber				G1 - IBA 5000ppm G2 - IBA 7500ppm G3 - IBA 10000ppm		N1 - Two node cutting N2 - Long cutting		

Peroxidase activity

Peroxidase activity in plagiotropic shoot cuttings exhibited significant differences among the environments and growth regulator concentrations (Table 6a).

Among the environments, cuttings kept under mini poly tunnel (E_1) registered higher peroxidase activity (0.70 changes in the OD 430 nm / min / g) followed by the cuttings kept under automated mist chamber (E_2) (0.55 changes in the OD 430 nm / min / g). Among the growth regulator concentrations, cuttings treated with IBA 7500 ppm (G_2) registered maximum peroxidase activity (1.15 changes in the OD 430 nm / min / g) followed by the cuttings treated with IBA 10000 ppm (G_3) (0.55 changes in the OD 430 nm / min / g) and cuttings treated with IBA 5000 ppm (G_1) (0.18 changes in the OD 430 nm / min / g).

In two way interaction, E × G, G × N and E × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) which registered the maximum peroxidase activity 1.28 changes in the OD 430 nm / min / g followed by the cuttings treated with IBA 7500 ppm and kept under mini poly tunnel (E_1G_2) (1.01 changes in the OD 430 nm / min / g)

In G × N interaction, long cuttings treated with IBA 7500 ppm (G_2N_2) recorded maximum peroxidase activity (1.26 changes in the OD 430 nm / min / g) followed by the two node cuttings treated with IBA 7500 ppm (G_2N_1) (1.04 changes in the OD 430 nm / min / g).

In E × N interaction, maximum peroxidase activity was observed in long cuttings kept under mini poly tunnel (E_1N_2) (0.80 changes in the OD 430 nm / min / g) followed by the two node cuttings kept under automated mist chamber (E_2N_1) which registered 0.63 changes in the OD 430 nm / min / g.

The three way interaction also showed high significance. In that, maximum peroxidase activity (1.43 changes in the OD 430 nm / min / g) was recorded in long cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_2$) and followed by two node cuttings treated with IBA 7500 ppm and kept under automated mist chamber ($E_2G_2N_1$) which recorded 1.14 changes in the OD 430 nm / min / g.

In the case of orthotropic shoot cuttings, the effect of environments, growth regulator concentrations and type of cuttings showed high significance (Table 6b).

Among the environments, cuttings kept under automated mist chamber (E_2) registered higher peroxidase activity (0.46 changes in the OD 430 nm / min / g) followed by cuttings kept under mini poly tunnel (E_1) (0.29 changes in the OD 430 nm / min / g). Among the growth regulator concentrations used, cuttings treated with IBA 7500 ppm (G_2) registered maximum peroxidase activity (0.58 changes in the OD 430 nm / min / g) followed by the cuttings treated with IBA 10000 ppm (G_3) (0.42 changes in the OD 430 nm / min / g). Among the type of cuttings, long cuttings (N_2) recorded maximum peroxidase activity (0.54 changes in the OD 430 nm / min / g) than two node cuttings (N_1) (0.22 changes in the OD 430 nm / min / g).

In two way interaction, E × G and E × N showed high significance. Among the E × G interaction, the cuttings treated with IBA 7500 ppm and kept under automated mist chamber (E_2G_2) registered the maximum peroxidase activity (0.79 changes in the OD 430 nm / min / g) followed by the cuttings treated with IBA 10000 ppm and kept under automated mist chamber (E_2G_3) which recorded 0.60 changes in the OD 430 nm / min / g.

In E × N interaction, maximum peroxidase activity was observed in long cuttings kept under mini poly tunnel (E_1N_2) (0.59 changes in the OD 430 nm / min / g) followed by the long cuttings kept under automated mist chamber (E_2N_2) which registered 0.48 changes in the OD 430 nm / min / g and the two node cuttings kept under automated mist chamber (E_2N_1) which registered 0.45 changes in the OD 430 nm / min / g.

Table 6a: Effect of environment, type of cuttings and growth regulators on peroxidase (change in the OD 430 nm / min / g) of planting in plagiotropic shoot cuttings of cocoa

		Plagiotropic shoots						**Mean**
		N_1			**N_2**			
E_1	G_1	0.28			0.43			0.35
	G_2	0.93			1.09			1.01
	G_3	0.58			0.88			0.73
Mean		0.60			0.80			0.70
E_2	G_1	0.00			0.00			0.00
	G_2	1.14			1.43			1.28
	G_3	0.74			0.00			0.37
Mean		0.63			0.48			0.55
$\overline{X}$		0.61			0.64			0.63
		G_1: 0.14	G_2: 1.04	G_3: 0.66	G_1: 0.22	G_2: 1.26	G_3: 0.44	
		G_1- 0.18		G_2- 1.15		G_3- 0.55		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.06	0.08	NS	0.11	0.11	0.09	0.15
CD (P=0.05)		0.14*	0.17**	-	0.24**	0.24*	0.19*	0.33**

NS - non significant, * - Significant, ** - highly significant

E_1 - Mini poly tunnel E_2 - Automated mist chamber	G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm	N_1 - Two node cutting N_2 - Long cutting

Table 6b: Effect of environment, type of cuttings and growth regulators on peroxidase (change in the OD 430 nm / min / g) of planting in orthotropic shoot cuttings of cocoa

		Orthotropic shoots						**Mean**
		N_1			**N_2**			
E_1	G_1	0.00			0.56			0.28
	G_2	0.00			0.73			0.36
	G_3	0.00			0.48			0.24
Mean		0.00			0.59			0.29
E_2	G_1	0.00			0.00			0.00
	G_2	0.84			0.74			0.79
	G_3	0.51			0.70			0.60
Mean		0.45			0.48			0.46
$\overline{X}$		0.22			0.54			0.38
		G_1: 0.00	G_2: 0.42	G_3: 0.25	G_1: 0.28	G_2: 0.74	G_3: 0.59	
		G_1- 0.14		G_2- 0.58		G_3- 0.42		
Interactions		E	G	N	E×G	G×N	E×N	E×G×N
SE(d)		0.04	0.05	0.04	0.08	NS	0.06	NS
CD (P=0.05)		0.10**	0.12**	0.10**	0.17**	-	0.14**	-

NS - non significant, * - Significant, ** - highly significant

E_1 - Mini poly tunnel E_2 - Automated mist chamber	G_1 - IBA 5000ppm G_2 - IBA 7500ppm G_3 - IBA 10000ppm	N_1 - Two node cutting N_2 - Long cutting

The biochemical basis for root formation implies that there is root promoting and root inhibiting substances produced in plants and their interaction is thought to be involved in rooting. It was also reported that biochemical factor, other than auxin were controlling rooting (Hartmann *et al.*, 2002) [5]. The levels of total carbohydrates and starch in their cuttings are positively related with the rooting but not through one cause-effect relationship (Delrio *et al*., 1991 [4], Rahman *et al*., 2002) [14]. Reduced nitrogen content in stock plants is known to increase the root formation in cuttings (Pearse, 1943) [11]. C:N ratio have long been used to estimate influences of nitrogen and carbohydrates on rooting. It is generally held that high C:N ratio favours rooting (Jackson, 1993) [7]. In this present investigation also C:N ratio was higher in long cuttings which have proven to record high percentage of rooting and sprouting than two node cuttings. Growth regulator treatment with IBA 7500 ppm recorded high peroxidase activity and hence high rooting can be seen in this treatment. Changes in peroxidase activities have been proposed as molecular marker for rooting in *Ricinodendron heudelotii* (Beffa *et al.*, 1990) [2]. Phenols also play a major role in rooting process and they act as a main substrate for peroxidases and prevents peroxidase catalyzed oxidation of auxin (Racchi *et al.*, 2001) [13].

Conclusion

Final report of this study is the best treatment combination was found to be that of plagiotropic long cuttings treated with IBA 7500 ppm. But some more limiting factors need to be assessed for getting higher success percentage and make this a method for mass multiplication of cocoa through cuttings as a feasible venture.

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