Short Communication

Evaluation of cocoa varieties for shoot induction under *in vitro* condition

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Abstract

In vitro propagation in cocoa is a potential tool for the rapid multiplication of true to type plants. Single noded cuttings of 2-3 cm collected from the budded plants maintained in polyhouse were used as explants. The experiment was done in five varieties: CCRP 2, CCRP 6, CCRP 8, CCRP 15 and Scavina. The best surface sterilization of explants was achieved using Mancozeb 0.2 per cent for 30 minutes followed by Cefotxim 0.1% for 10 minutes and then ethyl alcohol 70 per cent for 3 minutes and mercuric chloride 0.1 per cent for 5 minutes. Axillary bud sprouting was induced in basal Woody Plant Media (WPM) fortified with 2-isopentinyl adenine (2ip) and Indole-3-Acetic Acid (IAA). The period of single shoot induction from leaf axils was lowest (8.41) in variety CCRP 15 in media WPM+2ip 1ppm+IAA 0.02ppm. Among the varieties, highest multiple shoot induction percentage (93.75%) was recorded in variety Scavina.

Keywords: Cocoa, Indole Acetic Acid, *In vitro*, Propagation, Woody Plant Medium, 2 isopentanyl adenine.

Cocoa (*Theobroma cacao* L.) is a beverage crop of huge economic significance. In recent years, as cocoa cultivation is being taken up by farmers, the demand for planting material is increasing. Conventionally it is propagated through seeds and other vegetative methods like cutting, grafting and budding. But due to the limited availability of polyclonal gardens for seed collection and intensive labour requirement for the vegetative propagation methods, *in vitro* propagation can be considered as an alternative method of propagation that facilitates rapid multiplication of true to type plants within a short period of time. It allows proper screening of plants for various biotic and abiotic stresses. It also facilitates conservation and distribution of cocoa germplasm. Hence, the present study was undertaken in the tissue culture laboratory of Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2016-2018 with the objective to develop a protocol for *in vitro* regeneration and also to determine the variation in morphogenesis and regeneration among elite cocoa clones. The experiment was done in five varieties: CCRP 2, CCRP 6, CCRP 8, CCRP 15 and Scavina (Table 1). The media used in the present study was basal Woody Plant Media (WPM) with combination of 2ip (1-4 ppm) and IAA (0.02-0.08 ppm) for culture establishment, and combination of 2ip (1-4ppm), IAA (0.02-0.08 ppm) and $AgNO_3$ (5 ppm) for multiple shoot induction. The explants were single noded cuttings of 2-3 cm collected from the budded plants.

The best surface sterilization of nodal segments was achieved by washing in Teepol followed by shaking in 0.2% Mancozeb for 30 min and then with Streptocycline 0.1% for 10 min outside the laminar air flow chamber. Inside the laminar air flow, explants were treated with ethyl alcohol 70% followed by mercuric chloride 0.1% for 5 min. This treatment showed the highest survival percentage of cultures with minimum microbial contamination.

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Table 1. Details of cocoa varieties used for the study

Cocoa varieties	Characters
CCRP 2	Variety evolved through single plant selection from local population. Trees are high yielding with smooth green pods
CCRP 6	Variety evolved through single parent selection from exotic collection (IMC 10)
CCRP 8	Hybrid between CCRP 1 and CCRP 7. High yielding and produces medium sized pods
CCRP 15	Newly released hybrid from KAU. Parentage – G VI 55 X Criollo
Scavina	Exotic and a high tissue culture responding genotype

A similar treatment was done by Devi (2011) using 70% ethyl alcohol for 3 min followed by 0.1% mercuric chloride for 5 min for nodal segments of *Saraca asoca,* which resulted in maximum contamination free cultures. Bonga (1982) revealed the importance of using alcohol alone or in combination with other chemicals for surface sterilization of explant tissues. The surface sterilization of explants was followed by inoculation in media fortified with growth regulators. The culture tubes were incubated in culture rooms at 24±2°C at a relative humidity of 60–70% under white fluorescent lamps provided for artificial illumination.

The experiment was done in factorial CRD with two factors, *viz.,* media and varieties. Each treatment was replicated two times with six culture tubes for each replication.

WPM fortified with growth regulators like 2ip and IAA was found be the best for shoot induction in all varieties (Figure 1). WPM supplemented with kinetin 1 mg/L and IAA 0.02 mg/L showed successful bud sprout and leaf expansion from pre-existing meristems (Mallika et al*.,* 1992). The period of shoot induction was significantly influenced by the media and was lowest (8.41) when WPM+2ip 1ppm+IAA 0.02ppm was used. Period of shoot induction showed significant difference among different varieties also. Lowest period of shoot induction was noted in variety CCRP 2 (7.10) and was statistically on par with variety Scavina (8.37). Interaction effect of media and varieties varied significantly. In respect to the differential response of variety with different media, the period of shoot induction was lowest in variety CCRP 15 in the media WPM+2ip 1ppm+IAA 0.02ppm (6.33). However, this was statistically on par with CE_1V_1 (7.33), CE_1V_2 (7.25), CE_1V_4 (6.90), CE_2V_1 (7.5), CE_2V_2 (6.95), CE_2V_5 (8.65), CE_3V_1 (7.00), CE_3V_3 (8.75), CE_3V_5 (8.65) and CE_4V_1 (6.58) (Table 2). Bindu (1997) had stated that the minimum number of days for bud break was 7.33 in WPM supplemented with 2ip, adenine sulphate, silver nitrate, cycocel and phloroglucinol.

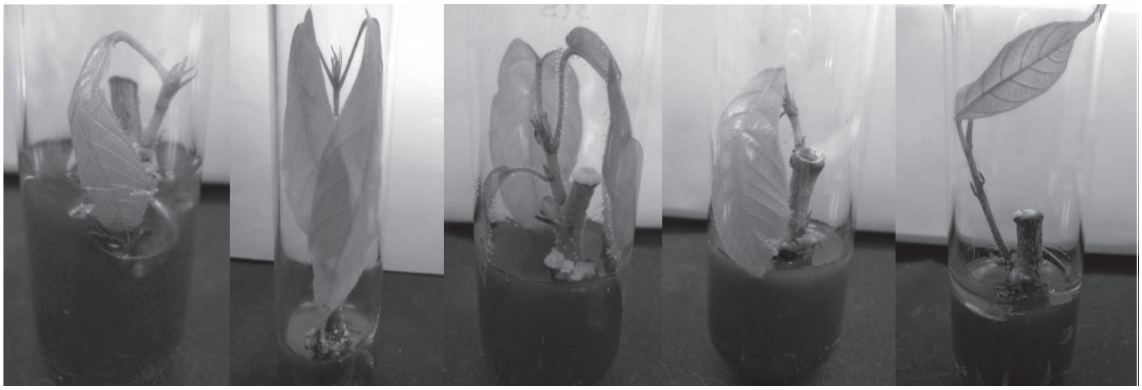

Figure 1. Single shoot produced from leaf axils of different cocoa varieties
CE_1 - WPM+2ip 1ppm+IAA 0.02ppm
CE_2 - WPM+2ip 2ppm+IAA 0.04ppm
CE_3 - WPM+2ip 3ppm+IAA 0.06ppm
CE_4 - WPM+2ip 4ppm+IAA 0.08ppm

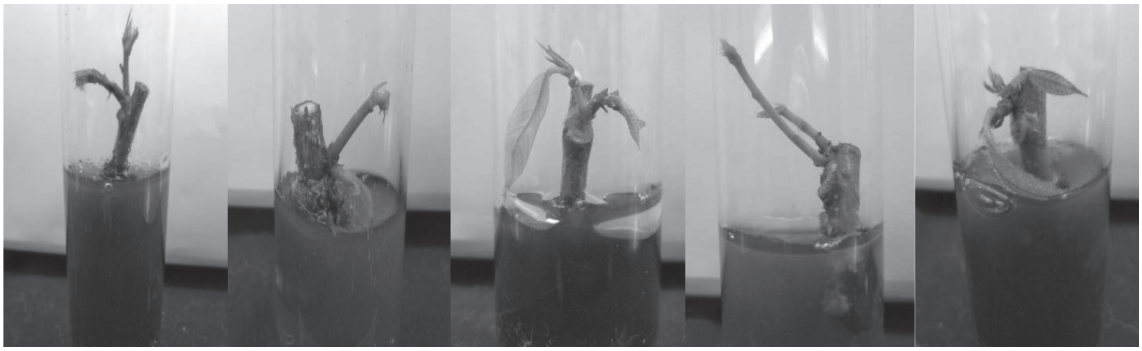

Figure 2. Multiple shoots produced from leaf axils of different cocoa varieties
CE_1 - WPM+2ip 1ppm+IAA 0.02ppm CE_3 - WPM+2ip 3ppm+IAA 0.06ppm
CE_2 - WPM+2ip 2ppm+IAA 0.04ppm CE_4 - WPM+2ip 4ppm+IAA 0.08ppm

In the present study, WPM with different concentrations of 2ip and IAA along with $AgNO_3$ was used for multiple shoot induction in the cocoa varieties (Figure 2). Giridhar et al. (2005) confirmed that in *Decaleptis hamiltonii*, the best multiplication under *in vitro* condition was obtained with 2ip as compared to other sources of cytokinin like kinetin, BAP and thidiazuron. Addition of silver nitrate reduced the callus formation at the base of cocoa shoots. According to Mallika et al. (1992), callus formation can be encountered in any explant of cocoa when inoculated in medium without growth regulators. Callusing at the base of explants led to stunted growth and later, drying of shoots occured. Shoot induction percentage varied significantly among media. Highest shoot induction percentage was recorded in WPM+2ip 4ppm+IAA 0.08ppm+ $AgNO_3$ 5ppm (91.67 %) which was followed by WPM+2ip 3ppm+IAA 0.06ppm+ $AgNO_3$ 5ppm (86.67 %). The shoot induction percentage varied significantly among the different varieties. The highest shoot induction percentage was recorded in Scavina (93.75 %), followed by CCRP 2 (85.42 %), and the lowest shoot induction percentage was reported in CCRP 15 (72.92 %). There was significant variation in shoot induction percentage with respect to different media and varieties. Shoot induction percentage varied from 66.67 % to 100 %. The shoot induction was recorded as cent per cent in MS_1V_5, MS_2V_5, MS_3V_3, and MS_4V_1. This was statistically on par with MS_1V_1 (91.67 %), MS_1V_2 (83.33 %), MS_3V_3 (91.67 %), MS_3V_5 (91.67 %), MS_4V_2 (91.67 %), MS_4V_3 (91.67 %) and MS_4V_4 (91.67 %). The lowest shoot induction percentage was 66.67 % in MS_2V_1, MS_2V_2 and MS_2V_4 (Table 3).

The clonal propagation of superior genotypes of cocoa through *in vitro* method is considered as a thrust area of cocoa biotechnology. By the study a

Table 2. Effect of culture establishment media, varieties and their interactions on period of shoot induction.

	CCRP 2	CCRP 6	CCRP 8	CCRP 15	Scavina	Mean (Media)
CE_1	7.33	7.25	10.75	6.90	9.83	8.41
CE_2	7.50	6.95	15.47	9.67	8.65	9.65
CE_3	7.00	12.05	8.75	14.00	8.65	10.09
CE_4	6.58	10.65	9.30	16.58	6.33	9.89
Mean (Varieties)	7.10	9.23	11.07	11.79	8.37	

CD (CE) – N/A CD (V) – N/A CD (CE x V) – N/A
CE_1 - WPM+2ip 1ppm+IAA 0.02ppm CE_3 - WPM+2ip 3ppm+IAA 0.06ppm
CE_2 - WPM+2ip 2ppm+IAA 0.04ppm CE_4 - WPM+2ip 4ppm+IAA 0.08ppm

Table 3. Effect of culture establishment media, varieties and their interactions on multiple shoot induction percentage.

	CCRP 2	CCRP 6	CCRP 8	CCRP 15	Scavina	Mean (Media)
MS_1	9.62(91.67)	9.19(83.33)	7.14(50.00)	7.14(50.00)	10.05(100.00)	8.63(75.00)
MS_2	8.23(66.67)	8.23(66.67)	9.18(83.33)	8.23(66.67)	10.05(100.00)	8.79(76.67)
MS_3	9.18(83.33)	9.18(83.33)	9.62(91.67)	9.18(83.33)	9.62(91.67)	9.36(86.67)
MS_4	10.05(100.00)	9.62(91.67)	9.62(91.67)	9.62(91.67)	9.18(83.33)	9.68(91.67)
Mean (Varieties)	9.27(85.42)	9.05(81.25)	8.89(79.17)	8.54(72.92)	9.74(93.75)	

$\sqrt{x}$ + 0.5 transformed values, original values are given in parenthesis

CD (CE) – N/A CD (V) – N/A CD (CE x V) – N/A

MS_1 - WPM+2ip 1ppm+IAA 0.02ppm+$AgNO_3$ 5ppm MS_3- WPM+2ip 3ppm+IAA 0.06ppm+$AgNO_3$ 5ppm

MS_2 - WPM+2ip 2ppm+IAA 0.04ppm+$AgNO_3$ 5ppm MS_4 - WPM+2ip 4ppm+IAA 0.08ppm+$AgNO_3$ 5ppm

defined protocol for shoot induction in five elite cocoa clones with maximum multiple shoot induction percentage was developed. Variety specific media for shoot induction ensures production of maximum number of *in vitro* shoots which can be transferred to rooting media to get maximum tissue culture cocoa plants. Moreover, contamination of cultures often occurs during *in vitro* propagation of cocoa which could be successfully controlled through best surface sterilization method used in the study.

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