Journal of Agripreneurship and Sustainable Development (JASD)
Volume 2, Number 1, June, 2019
ISSN (Print): 2651-6144; ISSN (Online): 2651-6365

USE OF INDOLE-3-BUTYRIC ACID (IBA) AND NANOFORMULATED HORMONE IN MARCOTTING OF MALAYSIAN HYBRID OF CACAO (*THEOBROMA CACAO* L.)

[1]Jibrin A. D. and [2]Domingo E. A.
[1]Department of Crop Production, Faculty of Agriculture and Agricultural Technology,
Abubakar Tafawa Balewa University PMB0248, Bauchi, Nigeria.
[2]Institute of Crops Science, College of Agriculture and Food Science, University of the
Philippines, Los Baños, College, Laguna 4031, Philippines.
Corresponding Author's E-mail: fialdawji@gmail.com **Tel.:** +234 803 4579 808

ABSTRACT

Experiments were conducted at Agricultural Experiment Station, Lagalag, Tiaong, Quezon (13.8^0 North latitude, 121.40^0 East longitude, 35 m.a.s.l) to examine rooting response of marcotted Malaysian hybrid of cacao (Theobroma cacao L.) stems as influenced by Indole-3-butyric acid (IBA) at 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 ppm, nanoformulated hormone at 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 ppm and distilled water. Experiments were arranged in Randomized Complete Block Design (RCBD) replicated three times. Results showed IBA and nanoformulated hormone concentrations were significant on all root parameters. 900 ppm IBA and 250 ppm nanoformulated hormone developed callus in 5 days, produced highest percent rooting (80 and 100 %), root density (4.00 and 5.00), roots dry matter (4.80 and 3.67 g plant^{-1}), root length (7.77 and 12.17 cm), number of roots (8.33 and 15.67), number of lateral roots (15.67 and 16.67), specific root length (1.60 and 3.40 cm g^{-1}) and root diameter (0.40 cm), respectively. Increasing concentrations of IBA tends to increase rooting responses of marcots and decrease rooting characteristics at every increasing level of nanoformulated hormone. Proximate analysis showed significant changes in nitrogen and carbohydrate concentrations before and after experiments. Cacao can be vegetatively propagated via marcotting in combination with either IBA or nanoformulated hormone for increase supply and year round availability of planting materials which is limited due to the long gestation period of cacao.

Keywords: Callus, Development, Gestation, Influence, Rooting.

INTRODUCTION

Cacao (*Theobroma cacao* **L.**) has high potential to contribute to the world economy (ICCO, 2015). However, cacao yield and production has been dwindling across the regions of its production, principally due to its long gestation period among other factors. Marcotting is a fast method of propagation more especially when auxin is used to induce more uniform rooting (Bareja, 2013). Marcotting method of propagation is being applied in propagating trees, shrubs, bamboo and herbaceous plants and tree crops such as cacao (*Theobroma cacao* L), citrus (*Citrus spp*.), guava (*Psidium guajava* L.), coffee (*Coffea spp*), tamarind (*Tamarindus indica* L.), mangosteen (*Garcinia mangostana* **L.**) and jackfruit (*Artocarpus heterophyllus* L.). Marcotting has been widely used in the domestication of indigenous fruit trees, in capturing the attributes of elite trees within genetically diverse wild populations and to avoid the long slow process of tree breeding (Asare, 2013). Auxins promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base of lemon plant and auxins interacts with other cytokinin in rooting of plant stems ((Hartmann *et al.,* 2002), types of plant material such as buds and scions and environmental conditions (Fett-Neto *et al.,* 2001) to effect rooting. Generally, auxins are applied to marcots in order to increase root initiation, percentage of rooting, number and quality of rooting and uniformity of rooting.

Nanoformulated hormone has been developed in Nanobiotechnology Laboratory of Biotechnology, University of the Philippines Los Baños, College, Laguna. Its use to induce more uniform rooting in cacao has not been investigated and varietal response to this hormone is not yet known. Marcotting of high performing cacao variety and its response to rooting hormone is one among the strategies that can be used to address the long gestation period of cacao. The growth, development and architecture of marcotted plant roots are regulated by factors such as kind of hormones, carbohydrate, nitrogen levels, time, and age of stem, media used, and available moisture in the plant and care of the marcots. The influence of marcotting, Indole-3-butyric acid (IBA) and nanoformulated hormone on root development of marcotted Malaysian hybrid cacao plant stems have not been fully investigated. Many cacao farmers both in the Philippines and Nigeria have limited information on the use of IBA, nanoformulated hormone and marcotting on cacao plant stems. It is because of this inadequate information that this research was conducted. In general, the objective of the study is to examine the root development of marcotted Malaysian hybrid cacao stems as influenced by IBA and nanoformulated hormone, specifically, to optimize the concentrations of IBA and nanoformulated hormone to induce rooting and promote root development in Malaysian hybrid cacao marcots.

MATERIALS AND METHODS

The experiment was conducted at the cacao farm of the Department of Agriculture Experiment Station, Lagalag, Tiaong, Quezon located at 13.8°North latitude, 121.40°East longitude with elevation of 35 m. a. s. l in August, 2017. Twenty-four (24) 5-10 years old cacao trees of uniform height and canopy diameter were selected separately for the two Experiments. In these trees, mature current seasons shoots having 4-6 mature leaves, brown stem with 1-1.5 cm in diameter and 29-33 cm long were selected; these shoots were tagged for marcotting. Each shoot was appropriately tagged and assigned a treatment. Using a sharp grafting knife, a ring of bark about 1-1.5 cm long preferably between nodes was removed; cambial layer was carefully scraped off. After all the stems were ringed, IBA treatments namely: T_1 = 0 ppm, T_2 = 100 ppm, T_3 = 200 ppm, T_4 = 300 ppm, T_5 = 400 ppm, T_6 = 500 ppm, T_7 = 600 ppm, T_8 = 700 ppm, T_9 = 800 ppm and T_{10} = 900 ppm were separately applied by soaking a piece of cotton into the IBA solutions, squeezing out three drops at the upper cut end of the stem and putting the cotton around the ringed portion. Nanoformulated hormone treatments namely: T_1 = 0 ppm, T_2 = 50 ppm, T_3 = 100 ppm, T_4 = 150 ppm, T_5 = 200 ppm, T_6 = 250 ppm, T_7 = 300 ppm, T_8 = 350 ppm, T_9 = 400 ppm and T_{10} = 450 ppm were separately applied at the upper cut ends of the ringed portion using a mini water paint brush. The nanoformulated hormone was brushed around the upper cut end of the stem. The nanoformulated hormone was sourced from the Nanotechnology Laboratory of the Institute of Biotechnology and Molecular Biology of University of the Philippines Los Baños, Laguna. A stock solution of 1200 ppm was readied from which nine concentrations namely: 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm, 400 ppm and 450 ppm solution were prepared. Commercial Indole -3-butyric acid (IBA) was used to prepare 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm and 900 ppm solutions. IBA was initially dissolved in sodium hydroxide and gradually in distilled water. Distilled water was used for control treatment.

After treatment, the ringed portion was covered with sterilized coconut coir dust which served as rooting medium and secured with a sheet of polythene plastic film (approx. 15 x 30 cm). Both ends of the plastic film were tied with plastic straw. Coir dust analyzed according to AOAC (2016) procedures on the dry basis has the following chemical properties: pH 5.63, conductivity 0.213 mS/cm, moisture 89.92%, total Kjeldahl nitrogen 0.44%, and carbohydrate 4.47%.Two shoots per treatment per replication were sampled for carbohydrate and nitrogen analysis (Proximate analysis) before and after the rooting experiment, all samples were analyzed according to AOAC (2016). The experiment was terminated soon after the root became visible through the plastic 45 days after marcotting. The experiment was arranged in a Randomized Complete Block Design (RCBD) and replicated three times. Ten marcots were assigned per treatment per replication. Average temperature ranged from 29°C to 30°C; humidity, 80-90%; sunshine duration, 261-337.3 min; rainfall, 0.6-1.9 mm; and wind speed, 1.01-1.3 kph at 1 M height. Data were collected on percent rooting (%), number of roots, root diameter (cm), root density, number of lateral roots, Dry matter of roots (g plant^{-1}), root length (cm) and specific root length (cm g^{-1}). All data were transformed to their log values before analysis using the Analysis of variance of RCBD as described by STAR (Statistical Tool for Agricultural Research). Tukey's HSD (Honest Significant Difference) was used to separate the significantly differences among the treatment means.

RESULTS AND DISCUSSION

Days to Callus Development

In general, it was also observed that the higher the concentration of IBA, the shorter is the period of callus development Figure 1 and 2. Treating marcots with IBA and nanoformulated hormone accelerated callus formation. Callus developed in 5 days in marcots treated with IBA 900 ppm and 800 ppm earlier than those treated with 0.00ppm, 100 ppm, 200 ppm, 300 ppm and 700 ppm which took 10 days. Marcot treated with IBA at 600 ppm developed callus in 20 days. The fast development of callus after five days of marcotting might be due to influence of IBA being auxin in promoting rapid cell division brought about by faster conversion of starch to sugars which serve as sources of energy in the processes of cell growth, development, differentiation and dedifferentiation (Thorpe and Murashige, 2010). Further, it could be that cacao shoots contains endogenous auxin which is transported basipetally that initiate callus formation of the marcotted shoots thus, promoting rapid cell elongation in the marcot (Alejar and Sese, 2000).

Earliest callus developed in marcots treated with nanoformulated hormone at 250 ppm within 5 days followed by 200 ppm and 300 ppm within 10 days. Must treatments developed callus at 15 days. Control treatment of IBA took 25 days to develop callus while in the nanoformulated hormone control, it occurred in 20 days (Figure 3-4). The formation of callus as early as 5 days after marcotting might be due to suitable surrounding temperature. In a study on apple (*Malus domestica* Borkh), little, if any callus is formed below 0°C or above 40°C and higher. However, callus production is drastically reduced resulting in increased cell injury and death occurring at higher temperature of 40°C and above. Generally, the ability of marcots to form callus depends on existing active cells in the vicinity of the vascular cambium and phloem to begin to divide and initiate d*e novo* adventitious roots. Nanoformulated hormone particularly at 250 ppm is effective as 900 ppm IBA. Its callus promoting ability could make it a better substitute to IBA.

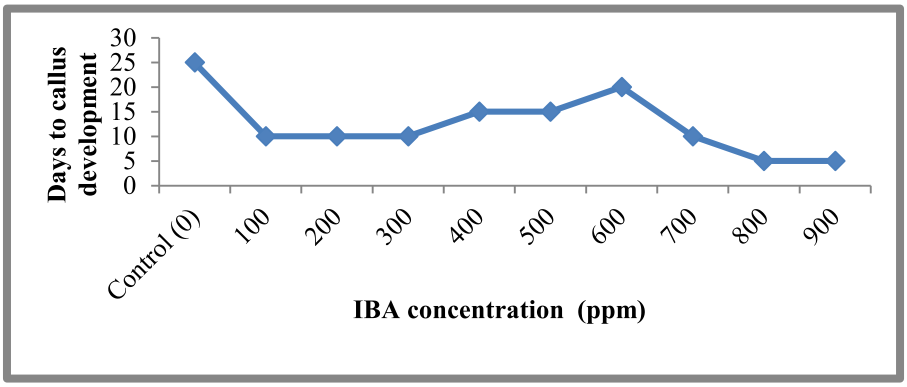

Figure 1: *Days to callus development of Malaysian hybrid cacao as influenced by different IBA concentration*

Figure 2: *Callus formation of Malaysian hybrid marcot 15 days after IBA application*

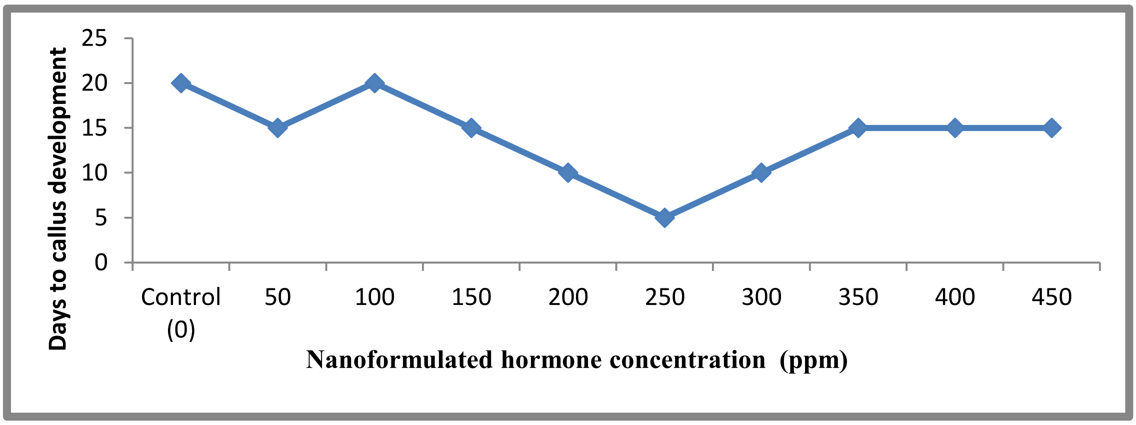

Figure 3: *Days to callus formation of Malaysian hybrid cacao as influenced by different concentration of nanoformulated hormon.*

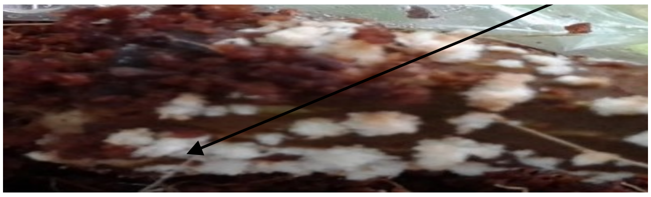

Figure 4: *Callus formed in Malaysian hybrid cacao marcot, 15 days after application of 250 ppm nanoformulated hormone*

Percent Rooting

The effect of IBA and nanoformulated hormone treatment was highly significant on percent rooting (Table 1-2). Aside from accelerating callus formation, higher concentration of IBA also increases percent rooting. Highest percent rooting of 80 % occurred in marcots applied with IBA 900 ppm followed by 800 ppm with 66.67 % rooting (Table 1). Among IBA treatments, IBA 300 ppm and IBA 400 ppm had same percent rooting of 30 %, IBA 500 and IBA 600 ppm were statistically the same (43.33 % and 46.67 %, respectively. IBA 700 ppm produced 60 % rooting. However, control, IBA 100 and IBA 200 ppm treated marcots had the lowest percent rooting of 23, 16. 67 and 20, respectively. These findings were in agreement with the study of Paul and Aditi (2009) who reported that a higher percentage of rooting (62.5 %) was obtained in marcotted water apple [*Syzygium aqueum* (Burm. F.) Altson] treated with 1000 ppm IBA while 500 ppm IBA did not show any significant effect on rooting. Auxins like IBA are known to promote cell elongation. It can trigger competent cells to actively exude protons into the cell wall region by causing a decrease in pH that activates cell wall loosening enzymes. This in turn accelerates breakage of essential cell wall bonds and increases cell extensibility culminating to cell elongation.

Percent rooting was not directly related to concentrations of nanoformulated hormone. Initially, it increased from untreated marcot of 20 % to its peak in 250 ppm nanoformulated hormone at 100 % (Table 2). It decreased thereafter up to 450 ppm where rooting reached 50 %. However, control, 50 and 100 ppm nanoformulated hormone produced statistically similar percent rooting. Likewise, 200 and 400 ppm produced statistically similar percent rooting. 300 ppm produced higher percent rooting of 76.67 % than 400 ppm. Herald *et al.* (2016) reported that nanoformulated hormone has the ability to promote rooting in marcotting of hard–to–root gumamela (*Hibiscus rosa-sinensis* Linn.), bougainvillea (*B. spectabilis* Willd.) and other ornamental plants. It also increased yield of cassava and survival rate of tissue cultured banana over conventional commercial formulations used by farmers and propagators. Nanoformulated hormones increased the phase of cell elongation, cell enlargement, delayed senescence, increased flowering and fruit setting (Bhupinder, 2014).

Number of Roots

The number of roots as influenced by IBA and nanoformulated hormone were significantly different among concentrations (Table 1 and 2). The highest number of roots was produced by IBA 400 (9.00) and 900 ppm (8.33) and lowest at 300 ppm (3.33). Number of roots did not vary significantly among marcots treated with IBA 400, 900 and 600 ppm. All treatments except 200 and 500 ppm are significantly different from control. This agree with the findings of Hore and Sen (2011) who reported that higher concentration of 5000 ppm IBA + 1000 ppm FA (fatty acid) had higher number of roots in cacao marcots than 2000, 3000 and 4000 ppm IBA + 1000 ppm FA. The increase in number of root observed in the marcots with the increased IBA concentration might be due to increased cell wall elasticity which may have accelerated cell division and in turn increased number of roots. This could be triggered by an increase in carbohydrate and metabolic activities. Highest number of roots (15.67) was observed in marcots treated with 250 ppm nanoformulated hormone (Table 2). Marcots applied with nanoformulated hormone lower than 250 ppm did not produce significantly different number of roots. Marcots in the control treatment recorded the lowest number of roots of 3.00. Increased number of roots as an effect of nanoformulated hormone agrees with the findings of Hore and Sen (2011) who reported that application of 5000 ppm IBA + 1000 ppm FA (fatty acid) on cacao marcots induce the production of higher number of roots.

Density of Roots

IBA and nanoformulated hormone concentrations significantly affect root density of marcots (Table 1 and 2). Root density of marcots was highest (4.00) at 900 ppm IBA and lowest (1.00) at 100 ppm IBA. Root density of control was comparable to all except those treated with IBA concentrations 100 ppm and 900 ppm (Table 1). Leakey (2004) reported that marcots treated with auxins root more rapidly and produce more roots. However, different tree species and clones respond differently to various applications and concentrations of auxins even when many other factors are constant. Moreover, increased IBA concentrations accelerate root initiation of root meristems and consequently production of greater number of roots. A highly significant root density (5.00) was observed from marcots treated with 250 ppm nanoformulated hormone (Table 2) and lowest in control (1.67). The rest of nanoformulated hormone treatments were not significantly different from each other. This might be due to factors such as carbohydrate and nitrogen levels of the cacao trees, environmental conditions such as temperature, humidity and light intensity. Leakey (2004) reported that marcots treated with auxins root more rapidly and produce more number of roots.

Number of Lateral Roots

Lateral roots are roots that branch out of the primary roots horizontally and serve to anchor the plant securely into the soil. This branching of roots also contributes to water uptake, and facilitates the extraction of nutrients required for the growth and development of the plant. Highest mean number of lateral roots (15.67) was noted in marcots treated with IBA at 900 ppm and lowest (6.67) at IBA 100 ppm (Table 1). IBA 300, 400 and 800 ppm were significantly different from the control. IBA 900 ppm produced significantly the highest number of lateral roots. Davis *et al.* (2011) observed the highest number of lateral roots (3.20) in marcots treated with 1000 ppm IBA. Nanoformulated hormone effect on number of lateral roots indicates highly significant difference (Table 2). Highest number of lateral roots (16.67) was found in marcots treated with 250 ppm of nanoformulated hormone. However, concentrations of 50, 100, 150 and 200 ppm produce fewer lateral roots that were not significantly different (7.33, 7.67, 10.00 and 10.33, respectively. Marcots in the control and those treated with 450 ppm recorded the lowest number of lateral roots (5.00 and 6.00, respectively) that were not significantly different. Results are in agreement with the study of Stephanie and Ive (2012) who reported that, the factors which influence lateral root branching were either directly via auxin or by having a complementary or antagonistic interaction with auxin. Dubrovsky *et al.* (2008) added that auxins initiate lateral root development by inducing cell divisions and elongation processes necessary for lateral root development.

Roots Diameter

The diameter of the roots did not vary significantly among IBA treatments (Table 1). It ranged from 0.17 to 0.20 mm on marcots treated with control to 300 ppm, 0.27 from 400 ppm to 700 ppm and 0.33 mm on marcots treated with 800 to 900 ppm IBA. Wu *et al*. (2016) asserted that root diameter is related directly to the build up by the plant's biomass in its roots. They further reported that the biomass of a given length of root varies with the square of its diameter and that for a given buildup of carbohydrate, root diameter affects the possible length and number of roots. A decrease in root diameter limits root penetration through the soil, and roots must also develop internal structures dedicated to water and nutrient transport.

Marcots treated with 250 ppm nanoformulated hormone recorded significantly highest root diameter of 0.40 mm. Concentration above 250 ppm produced thinner roots which did not significantly differ from control (Table 2). Control treatment produced lowest root diameter of 0.13 mm. Results suggest that concentrations of nanoformulated hormone higher than 250 ppm did not significantly increase root diameter. The enhancing effect of nanoformulated hormone on root diameter shows that likely, it contains substances with auxin property. This affirms the findings of Herald *et al.* (2016) that nanoformulated hormone significantly affect root diameter of marcots of *Hibiscus rosa-senensis* and other hard to root plants such as gumamela. Further, in many plant species, a positive relationship has been found between the size of the root meristem (and hence apical diameter) and several important developmental characteristics, including: elongation rate, growth duration and gravitropism. The range of variation of root diameter within a root system is also variable and this can be of prime importance for foraging performance.

Root Length

Marcots treated with IBA at 900 ppm had the highest mean root length of 7.77 cm followed by marcots treated with 800 ppm IBA with mean root length of 6.03 cm (Table 1). Root length in all IBA treated marcots significantly differ with control. Results indicate that higher IBA concentration produced longer roots. Findings agree with Caula and Robert (2015) who observed that maximum total root length was recorded with application of 5000 ppm IBA and minimum in the control. They attributed this to the role of IBA in promoting root length growth through the synthesis of enzymes concerned with cell enlargement. The enzymes involved in cell enlargement process are triggered by the auxin at higher concentration. The result observed may be attributed to the primary physiological effect of auxin to promote the elongation of cells in the apical region. Ray *et al.* (2014) reported similar results in litchi where higher total root length was observed in marcots treated with higher IBA concentration of 400 ppm.

These findings were consistent with Zengibal and Ozcan (2006) who concluded that the higher the concentration of auxin, the higher the root performance and that auxins exist naturally in plants and play a key role in root initiation and development by promoting the transport of carbohydrates basipetally in marcotted stems. The effects of nanoformulated hormone on root length shows that marcots treated with 250 ppm nanoformulated hormone had the highest mean root length of 12.17 cm followed by marcots treated with 100 ppm and 200 ppm (Table 2). Mean root length of marcots treated with 50 ppm and those from the control treatment were not significantly different. However, the results indicate that concentrations of nanoformulated hormone above and below the optimum did not produce longer roots.

Specific Root Length

The specific root length is a measure of thinness or thickness of roots as influenced by IBA and nanoformulated hormone concentrations. The higher is the specific root length, the thinner is the root. Results indicated that IBA concentrations significantly increased the specific root length of cacao. IBA at 800 ppm recorded the highest specific root length of 1.70 cm g^{-1} while 300, 500 and 700 ppm IBA gave the lowest mean specific root length of 1.27 cm g^{-1} (Table 1). However, IBA 100, 200, 400, 600 and 900 ppm did not produce significantly different specific root length of 1.30 cm g^{-1} compared with the control. Highest specific root length was obtained in marcots treated with 250 ppm nanoformulated hormone. However, marcots treated with 150 and 200 ppm nanoformulated hormone did not produce significantly different specific root lengths compared to 250 ppm. Lowest mean value of specific root length was recorded on marcots under the control (1.37 cm g^{-1}) (Table 2). Thus, increasing concentration of nanoformulated hormone above the optimum level of 250 ppm produced specific root length of marcots that are almost within the same range.

Table 1: Effects of IBA Concentrations on the different Root Variables of Malaysian Hybrid Cacao Marcots[1’]

Concentration	Percent Rooting (%)	Number of Roots	Root Diameter (mm)	Root Density	Number of Lateral Roots	Dry Matter of Roots (G Plant^{-1})	Root Length (cm)	Specific Root Length (Cm G^{-1})
0 (Control)	23.33e	4.33 c	0.17	2.00 bc	10.67 c	2.23 e	2.90 e	1.30 ab
100 ppm	16.67 e	6.00 b	0.17	1.00 d	6.67 f	2.40 de	3.30 d	1.40 ab
200 ppm	20.00e	4.67 c	0.20	3.67 ab	9.00 e	2.50 de	3.30 d	1.33 ab
300 ppm	30.00de	3.33 cd	0.20	2.00 bc	11.67 c	2.70 cde	3.47 d	1.27 b
400 ppm	30.00de	9.00 a	0.27	2.33 bc	11.33 c	2.47 de	3.30 d	1.37 ab
500 ppm	43.33cd	4.67 c	0.27	1.33 bc	7.67 e	3.73 abc	4.77 c	1.27 b
600 ppm	46.67cd	7.67 ab	0.27	3.33 abc	14.33 ab	3.67 bc	4.73 c	1.33 ab
700 ppm	60.00bc	6.00 b	0.27	2.00 bc	14.67 ab	3.97 ab	5.17 bc	1.30 b
800 ppm	66.67ab	6.67 b	0.33	2.67 bc	13.33 c	3.50 bcd	6.03 b	1.70 a
900 ppm	80.00a	8.33 a	0.33	4.00 a	15.67 a	4.80 a	7.77 a	1.60 ab
Average	41.66	6.07	0.25	2.43	11.50	3.20	4.47	1.39
HSD	17.54**	1.60*	0.07^{ns}	0.79**	2.76*	0.36**	0.39**	0.13*
CV (%)	14.39	28.12	32.36	34.20	25.55	12.00	9.19	9.98

Note: [1’]Means within column with the same letter(s) are not significantly different at 5% HSD; ** = highly significant.

In addition, Each and every root parameters are interrelated and cannot be discussed separately from one another. Specific root length (SRL) is the ratio of root length to dry mass of primary lateral roots. It determines the capacity of the root to acquire resources base on the ability of the root to reach nutrients (resources acquisition) and it is directly related to density of the root. Cacao varieties with high SRL stand the chance to build more root length for a given dry-mass output and are generally considered to have higher rates of nutrient and water uptake (per dry mass), shorter survival rate and higher relative growth rates compare to varieties with low SRL. High SRL is related to low root diameter or low root density, the nature of their relationship determines the structure of the root. Cacao marcots with thin roots may have the tendency of less penetrative force into the soil and this may affect the absorption and transport of water, nutrients and other dissolved substances. On the other hand, varieties with low root density may have lower penetration deep into the soil but higher rates of water, nutrient and other substances uptake more especially under conditions of high field capacity.

Root Dry Matter Yield

Marcots treated with IBA at 900 ppm produced the highest root dry matter of 4.80 g compared to the control with the lowest dry weight of 2.23 g (Table 1). Results indicate that the higher IBA concentration significantly produced higher root dry weight of marcots. These findings were consistent with that of Zengibal and Ozcan (2006) who concluded that the higher the concentration of auxin, in this case IBA, the higher the root performance. Also, Fett-Neto *et al.* (2001) reported that marcotting of guava treated with 4000 ppm IBA significantly produced the highest dry weight (0.43 g); cashewnut marcots treated with 5000 ppm IBA gave the highest root dry-weight per stem. In a similar trend, the effect of different concentrations of nanoformulated hormone on root dry matter showed highly significant difference (Table 2). Apparently, highest number of roots obtained from marcots treated with 250 ppm nanoformulated hormone also produced the highest root dry matter (3.67 g plant^{-1}) followed by 300 ppm (2.90 g plant^{-1}). Mean root dry weights from marcots treated with 150 ppm, 200 ppm and 350 ppm did not vary significantly. The control treatment produced the lowest root dry (1.43 g plant^{-1}).

Carbohydrate and Total Nitrogen Concentration of Marcots before and after IBA and Nanoformulated Hormone Application

The average of carbohydrate and total nitrogen levels of the cacao trees before and after IBA treatment application were CHO: 8.20 and 17.39; N: 1.36 and 1.07, respectively 40 days after the experiments. Even though, it is not a factor to consider in rooting. Carbohydrate can promote rooting while nitrogen can promote shoot growth (Hartmann *et al*., 2002). The ratio of carbohydrate to nitrogen before and after IBA and nanoformulated hormone application were 6.02 and 16.25; 6.02 and 16.01, respectively (Table 3). However, the significant effect of IBA and nanoformulated hormone concentrations on marcotted cacao roots may be attributed to the influence of the applied IBA and nanoformulated hormone concentrations that facilitated high absorption, transportation and accumulation of high level of carbohydrate and other root growth and development promoting substances which in turn induces higher callus formation leading to high root proliferation, growth and development.

Table 2: Effects of Nanoformulated Hormone Concentrations on the different Root Variables of Malaysian Hybrid Cacao Marcots$^{1'}$

Concentration	Percent Rooting (%)	Number of Roots	Root Diameter (cm)	Root Density	Number of Lateral Roots	Dry Matter of Roots (G Plant^{-1})	Root Length (cm)	Specific Root Length (cm g^{-1})
0 (Control)	20.00e	3.00 e	0.13 b	1.67 c	5.00 c	1.43 e	2.00 e	1.37 d
50 ppm	27.76e	5.33cd	0.23 ab	3.00 b	7.33 bc	2.10 cd	2.47 e	1.17 d
100 ppm	26.67e	9.00b	0.30 ab	3.33 b	7.67 bc	1.60 de	4.00 d	2.50 ab
150 ppm	50.00d	8.67bc	0.30 ab	3.00 b	10.00 b	2.47 bc	5.13 bc	2.13 bc
200 ppm	60.00cd	10.33b	0.30 ab	2.67 b	10.33 b	2.53 bc	6.63 b	2.33 bc
250 ppm	100.00a	15.67a	0.40 a	5.00 a	16.67 a	3.67 a	12.17 a	3.40 a
300 ppm	76.67b	8.67bc	0.17 b	3.00 b	7.67 bc	2.90 ab	6.60 b	2.27 bc
350 ppm	66.67 bc	7.67bcd	0.17 b	2.67 b	8.67 c	2.43 bc	5.70 bc	2.33 bc
400ppm	56.67cd	5.33cd	0.17 b	2.67 b	7.00 bc	2.27 bcd	6.00 b	2.67 ab
450ppm	50.00d	6.19de	0.18 b	2.33 b	6.00 c	1.90 cde	5.25 bc	2.57 ab
Average	53.44	7.87	0.24	2.93	8.63	11.60	5.60	2.27
HSD	11.68**	1.10**	0.24**	0.54**	1.24**	0.25**	0.59**	0.33**
CV (%)	7.48	14.86	32.26	19.57	15.23	11.60	11.12	15.28

Note: $^{1'}$Means within column with the same letter(s) are not significantly different at 5% HSD; ** = highly significant.

This scenario seemed to be evident in the averages of carbohydrate and nitrogen analysis where the average level of carbohydrate after termination of the experiment was higher than the levels before the treatment application. This could mean that there occurs a higher amount of carbohydrate accumulation in the marcotted cacao shoots. Yeboah *et al.* (2009a) reported that the physiological factors influencing rooting of marcots occur within the plant and are very essential in the promotion of rooting of the marcots. Auxins and other essential plant rooting substances move from the apex to the base where they exert their effect and the girdling of the plant stems (disruption) of the movement of these important substances from the apex to the base of the stems results in inducement of rooting in the marcots. Girdling or otherwise constricting the cacao stem, blocks the downward translocation of carbohydrates, hormones and other substances responsible for rooting and the upper part of the girdled stem accumulates high amounts of these vital substances thereby promoting rooting of the marcotted cacao stems (Hartmann *et al*., 2002).

Result of nitrogen analysis in same experiment was dissimilar with that of carbohydrate. N level after termination of the experiment were lower than the before IBA and nanoformulated hormone application. This could be due to low mobilization of N brought about by low effect of IBA and nanoformulated hormone on N and perhaps due to less influence on callus development which may lead to higher root growth and development since it is needed during shoot development as reported by Hartmann *et al*. (2002) that carbohydrate can promote rooting while nitrogen can promote shoot growth. Root diameter was not significantly affected by IBA concentrations, this might be due to some of the limitations encounter such as removal of high cambial layer causing excessive damaged to the shoots, cutting into wood which damaged the wood and increased breakage of roots, drying of medium, drought stress of plants among others.

Table 3: Average Carbohydrate and total Nitrogen Concentration of Marcotted Malaysian Hybrid of Cacao before and after IBA and Nanoformulated Hormone Application

Hormone	**Carbohydrate (%)**		**Total Nitrogen (%)**	
	Before	After	Before	After
IBA Nanoformulated	8.20	17.39	1.36	1.07

CONCLUSION AND RECOMMENDATIONS

The results of the study indicate that vegetative propagation by marcotting in combination with nanoformulated rooting hormone and IBA can be a suitable method for the production of planting materials of cacao (*Theobroma cacao* **L.**) within a short period of time. Further, the study revealed that IBA at 900 ppm and nanoformulated hormone at 250 ppm is the most effective rooting hormone concentrations used for Malaysian hybrid cacao. For IBA treated marcots, dissimilarities were recorded in days to callus development, root length, highest percentage rooting, root dry weight, root density, number of roots and number of lateral roots. For nanoformulated hormone treated marcots, similar root characters were observed to have differences in days to callus initiation, root length, percent rooting, root diameter, number of roots, number of lateral roots, and specific root length. Increasing concentrations of IBA tends to increase rooting responses of marcots. For nanoformulated hormone, marcots tend to decrease in rooting characteristics at every increasing level of the treatment. In a similar manner, proximate analysis reveals changes in nitrogen and carbohydrate concentrations before and after treatment application.

Results of the present study on the different root parameters as affected by different concentrations of nanoformulated hormone demonstrate the potential of the hormone in vegetative propagation of cacao. The application of nanoformulated hormone in vegetative propagation could pave the way for studying auxin actions on plants as it will allow the documentation of how plant roots develop and acclimatize to their environment particularly for the improvement of soil retention of water or liquid by nanomaterials. Further, results of this study showed that with the use of nanoformulated hormone an increase in rooting is possible. This is supported with the findings of the initial test in crops like gumamela, bougainvillea, cassava and cultured banana. Nanoformulated hormone was found to increase the phase of cell elongation, cell enlargement, delayed senescence, increased flowering and fruit setting (Herald *et al.*, 2016). It was also found that nanoformulated hormone significantly enhanced the growth of rice (Fernando *et al*., 2010).Nanoformulated hormone can be an alternative to commercial IAA or NAA rooting hormones. It therefore offers a sustainable option to nurserymen and growers. However, of all the two hormones used, nanoformulated hormone seemed to be the best.

ACKNOWLEDGEMENTS

Sincere thanks to Professor. Domingo E. Angeles for sponsoring the entire proximate Analysis for carbohydrate, nitrogen and coir dust, his encouragement and support throughout the study. Sincere gratitude is extended to the members of the Advisory Committee and the entire staff of Interdisciplinary Studies Center on Food and Nutrition Security University of the Philippines Los Baños, Laguna, Philippines.

REFERENCES

Alejar, A. A. and Sese, D. M. (2000). *Fundamentals of Plant Physiology.* Plant Physiology Society of the Philippines. Pasig City, Metro Manila.

AOAC. (2016). *Official methods of Analysis of AOAC International.* 20th Ed., AOAC International, Gaithersburg, MD, USA. www.eoma.aoac.org.

Asare, R. (2013). *A review on cocoa agro-Forestry as a means for biodiversity conservation.* World Cocoa Foundation Partnership Conference, Brussels, 17.

Bareja, B. G. (2013). *Learn marcotting, it's quite easy.* Crop Agriculture Review. Available: www.cropsreview.com/marcotting.html. (Accessed 10 April, 2017).

Bhupinder, S. K. (2014). Nanotechnology in agri – food production: an overview. *Dove Press Journal: Nanotechnology, Science and Application,* 1: 31 – 53.

Robert, N. T. and Caula, A. (2015). *Plant propagation concepts and laboratory exercises*. Suite 300 Boca Brown. USA

Davis, A. S., Aghai, M. M., Pinto, J. R. and Apostol, K. G. (2011). Growth, gas exchange, foliar nitrogen content, and water use of sub-irrigated and overhead irrigated *Populus tremuloides* Michx. *Seedlings Hort Science*, **46**(9): 1249-1253.

Dubrovsky, J. G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M. G., Friml, J., Fernando, L. M., Menca, F. E. and Paterno, E. S. (2010). Isolated and partial structure, elucidation of gibberellin produced by plant growth promoting bacteria (PGPB) and its effect on the growth of hybrid rice (*Oryza sativa* L.). *Philip. J. of Crop Sci*., **35**: (2) 12-22.

Fett-neto, A. G., Fett, J., Goulart, L. W. V., Pasquali, G., Termignoni, R. R. and Ferreira, A. G. (2001). Distinct effects of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globulus*. *Tree Physiol*., **21**(7): 457-464.

Hartmann, H. T., Kester, D. C., Davies, F. T. and Geneve, R. L. (2002). *Plant propagation and practices* (6th edition). Prentice Hall International Edition. Englewood Cliffs, N. J., Prentice Hall, Inc. 205 – 374Pp.

Herald, N. F. B., Lilia, M. F., Juan, M. K. P., Erlinda, M. S. P., Pablito, M. M., Florinia, Q. L., Marites, Q. L. and Jayrald, C. K. (2016). *Nano-formulated plant growth regulators derived from bacteria for the production of high value crops.* 4th Philippine Society of Materials Science and Nanotechnology Scientific Conference. De La Salle University, Taft Avenue, Manila, August 25-27.

ICCO. (2015). *Quarterly Bulletin of Cocoa Statistics:* Vol. XIII. No. 1, Cocoa year 2015/ 2016. UK.

Leakey, R. R. B. 2004. *Physiology of vegetative reproduction*. **In:** Burley, J., Evans, J, Youngquist, J. A. (eds.) **Encyclopaedia of Forest Sciences**. *Academic Press. London*, 1655-1668Pp.

Paul, R. and Aditi, C. H. (2009). IBA and NAA of 1000 ppm induce more improved rooting characters in air-layers of waterapple (*Syzygium javanica* **L.**). *Bulgarian J. Agric. Sci.,* **15**(2):123-128.

Ray, P. S., Martin, J. L., Otani, E. A., Dillmann, W. H. and Reynolds, D. K. (2014). *Preliminary studies in Western Samoa using various parts of the coconut palm (Cocos nucifera L.) as a growing media. Acta Hort*. **37**:1983.

Sen, S. K. and Hore, J. K. (2011). Effect of growth regulators and synergists on air layering of waterapple (*Syzygium jambos (*Linn.) *Alston. Orissa J. Agric. Res.*, **5**(1-2): 20-29.

Stephanie, S. and Ive, D. S. (2012). Root system architecture: insights from Arabidopsis and cereal crops. *Philos Trans R Soc. Lond B Biol. Sci*., **367**(1595): 1441-1452.

Thorpe, T. A. and Murashige, T. (2010). Some histo–chemical changes underlying shoot initiation in tobacco callus cultures. *Canad. J. Bot.,* **4**: 227 285.

Wu, Q., Pages, L. and Wu, J. (2016). Relationships between root diameter, root length and root branching along lateral roots in adult, field grown maize. *Ann. Bot.,* **117**: 379-390.

Zengibal, H. and Pzcan, M. (2006). The effect of IBA treatments on rooting of cuttings in kiwi fruits (*Actinidia delicioba*, A. chev.). *J. Fac. Agr. OMu.* **21**(1): 40-43.