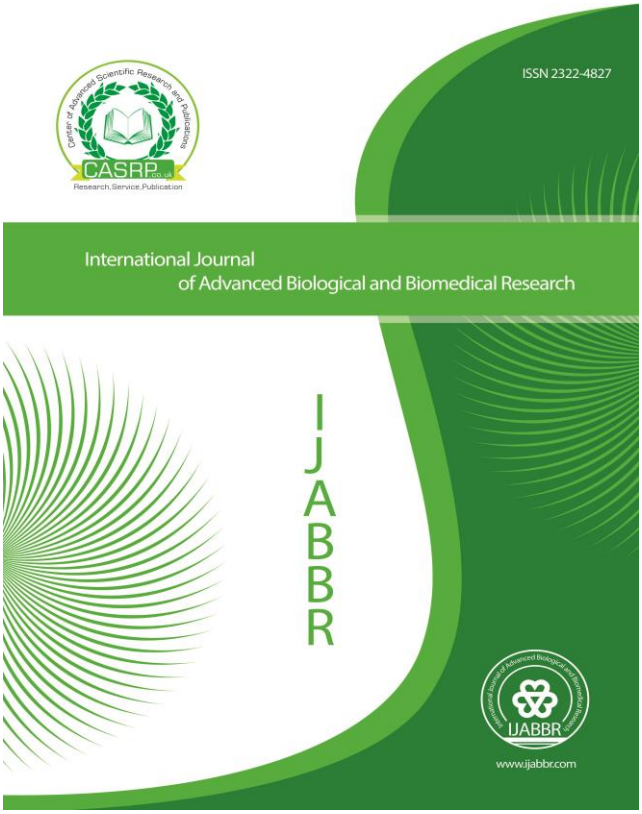

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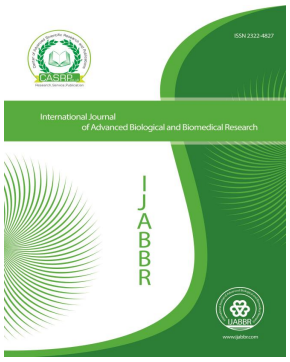

Original Article Open Access

Vegetative propagation of Rambutan (*Nepheliumlappaceum*) by marcotting: effect of indole-3-butyric acid concentration

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Abstract

Rambutan tree (*Nepheliumlappaceum* L.) is an important but a lesser known fruit tree in Ghana and has several nutritional and medicinal uses. Efforts to establish plantation of Rambutan in Ghana to ensure its sustainable use is challenged with unavailable planting materials because the seeds are recalcitrant in nature, loses viability easily when exposed to dryness. Seeds are therefore sown directly after extraction from fruit, even with this, most of the seeds do not germinate. A vegetative propagation technique by marcotting was devised to produce planting materials within 3 months. Four plant species of the same physiological age and spaced 4m apart were tested in complete random design fashion. Marcots were treated with four Indole-3-Butyric Acid **(**IBA) concentrations (0 Mg/L, 2000 Mg/L, 4000 Mg/L and 6000 Mg/L). Data collected were analysed on number of calluses formed in marcots, survival, shoot and root formation and root length. Comparable but highest survival of marcots was recorded in the 2000 Mg/L of IBA (14.67 $\pm$0.33) representing 97.78%. Marcots with 2000 Mg/L IBA concentration recorded highest mean number of roots (8.67 $\pm$0.33) formed. Root length ranged from 33.60 $\pm$0.52 in the 6000 Mg/L to 19.77 $\pm$1.26 in the control (0 Mg/L). Marcots with no IBA recorded 9.00 $\pm$0.58 as mean number of callused marcots. It is concluded that vegetative propagation technique by marcotting can be a suitable technique for Rambutan planting materials.

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1. Introduction

Rambutan tree (*Nepheliumlappaceum* L.) is an evergreen tropical fruit tree of the family Sapindaceae. It is a relative of the lychee and longan and grows to a height between 12-20m (Morton, 1987). Origin of Rambutan is linked to West Malaysia and the island of Sumatra (Indonesia) where it is known as "Rambut" meaning hair in Malay (Watson, 1984). The tree now has wide distribution including Central America, Northern Australia, India, New Guinea, Sri Lanka, Hawaii and Africa. The tree grows well in humid tropical regions with well distributed annual rainfall (2500-3000 mm). Rambutan tree has several nutritional and medicinal uses. The fruits and kernel are eaten and the roots used for treating fever. Leaves and skin of fruit are used as dye and also some tongue diseases can be treated with the bark (Abbey, 2000). The wood is hardwood and used for constructional purposes. Rambutan plays significant role in improving income of small households and also the local economy. In Ghana there are no commercial plantations of Rambutan to take advantage of its economic potentials. However, according to (Tindall, 1994; Nakasome and Paul, 1998), commercial production of Rambutan trees increased foreign exchange earning in tropical Australia, central and southern parts of Africa, and South America. In spite of the economic importance of Rambutan efforts to establish its plantations in Ghana is limited primarily because of unavailable planting materials/stock. The known Rambutan plantations are those on the field gene bank of the Plant Genetic Resources Research Institute, Council for Scientific and Industrial Research (CSIR-PGRRI), Bunso and the University of Ghana's Agricultural Research station, Kade which are for conservation and research purposes. Rambutan propagation is either by seed or vegetative method. The seeds lose viability easily due to its recalcitrant nature. Seeds are therefore sown directly after extraction from fruit (Bryan, 2001). This limits distribution of seeds and consequently availability of planting material for plantation establishment. Factors such as imbalance sex ratio, long gestation period and root-shy also limit seed propagation (Abbey, 2000). Tindall, (1994) reported that Rambutan is difficult to root using stem cuttings although the few studies conducted indicated partial survival. Large scale production of Rambutan would depend on reliable and efficient planting material production system. Rambutan can be a choice fruit tree for alleviation of rural poverty as the tree fruits profusely and has high market demand.

Studies have shown important products of the underutilised Rambutan fruits, such as jams, juices and candied fruits in income generation and poverty reduction of small scale entrepreneurs in developing countries (Azam-Ali, 2003). Planting materials are basic necessity in breeding programmes, plant genetic conservation and commercial plantations. Vegetative propagation by marcotting has been used to produce true to type planting materials for many tree species (Hartmann et al., 2002). The physical attachment of the stem to the plant during rooting permits continuing translocation of water, minerals, carbohydrates and hormones through the vascular tissues which makes it far more successful than methods like seed propagation and stem cuttings (Pauku, 2005). The method is deemed appropriate to complement seed propagation which has some challenges. Seed propagated Rambutan has longer gestation period than vegetative propagated ones (Verheiji, 1991). There is germination range and fruit bearing period of 7 to 20 days and after 5 to 6 years respectively for seed propagated Rambutan while marcotted Rambutan bear fruits between 1 to 4 years (Verheiji, 1991). Marcotting could be used to duplicate plants of superior genotype as vegetative propagation bars against genetic variations. Tindall (1994) also explained that, by applying marcotting technique, resulted in 80% rooting in immature branches of cultivar without using growth hormone.

IBA was found to be best and most effective rooting hormone because of its non-toxic characteristics to plants over wide range of concentration levels (Opuni-Frimpong et al., 2008). Dessalegn and Reddy (2003); Debnath (2008) recorded IBA concentrations ranging from 2000- 6000 Mg/L to be successful. The aim of the study was to determine the suitability of vegetative propagation technique by Marcotting for the production of Rambutan planting materials and there attempted to address the question: at what concentration of indole-3-butyric acid would give the optimum rooting, germination and shoot formation with marcotting. Specifically, the

objective of this study was to identify indole-3-butyric acid (IBA) concentration that would best stimulate survival, rooting, calluse and shoot formation of marcotted Rambutan

2. Materials and methods

2.1. Study area description

The study was undertaken at the Plant Genetic Resources Research Institute of the Council for Scientific and Industrial Research (PGRRI-CSIR), at Bunso in the Eastern Region of Ghana with an area of 19,323 square kilometers. The Eastern Region occupies 8.1 per cent of the total land area of Ghana, making it the sixth largest region in Ghana. It has an estimated population of 2,106,696 representing 11.1 per cent of Ghana's population. Bunso lies on latitude 6°28'N, longitude 0.4°56'W with temperatures in the region varying from 24°C to about 28°C and an average rainfall of 1750 mm. Farming is the major occupation of the inhabitants with some of the other occupations being carpentry and fishing.

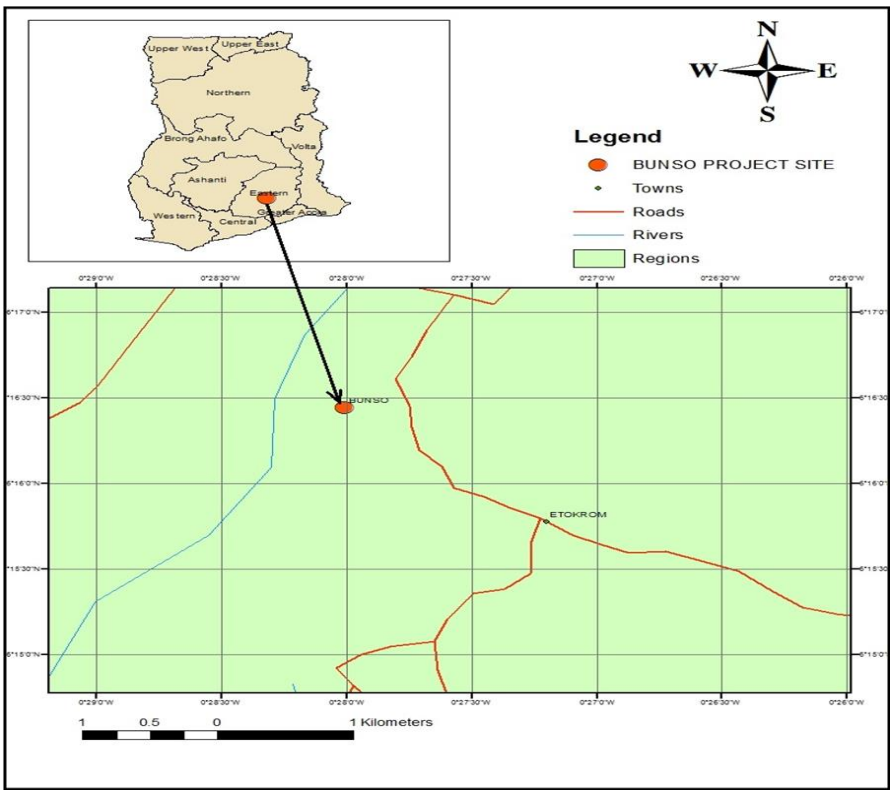

Fig. 1. Study area map (Bunso).

2.2. Experimental design

The experiment was set up in a complete randomized design with three Rambutan trees of the same age and spaced 4m apart. A vegetative propagation by marcotting was used and four treatments with different levels of concentrations of Indole-3-butyric acid (0 mg/l, 2000mg/l, 4000mg/l and 6000mg/l) were applied to initiate root and shoot growth. Each treatment contained forty-five marcots (45) making 180 marcots as the total experimental units. In trying to avoid bias, simple random technique was applied in selecting potential branches for marcotting.

2.3. Preparation of rooting media

River sand and palm fibre were used as the rooting media. The media were sterilized separately by barrel sterilization at 105°C for four (4) hours. The river sand and palm fibre media were mixed in proportions of 1: 2 (v/v). The soils were mixed thoroughly on thick sheet of polythene with a shovel. Stones and other foreign

materials were removed. Indole-3-butyric acid (IBA) concentrations of 0 Mg/L, 2000 Mg/L, 4000 Mg/L and 6000Mg/L were used as rooting hormone for the treatments. IBA concentration of 2000 mg/l was prepared by weighing 200 mg of powdered Stock IBA.The weighed IBA powder was then poured into a volumetric flask containing 100 ml of 50% ethanol and stirred continuously till completely dissolved. The process was repeated in the right stock IBA and 50% ethanol proportions to prepare the other concentrations.

2.4. Treatment selection

Leafy shoots measured 30cm in length from the apex and approximately 0.9 cm in diameter were selected from healthy branches of a matured *Nepheliumlappaceum* tree at the field gene bank of CSIR-PGRRI, Bunso (Davies et al., 2011). The barks of selected shoots were girdled by removing 2 cm wide strip of the bark. The debarked surfaces were scraped of all phloem and cambium tissues to retard healing of the exposed surfaces. The IBA solution was applied on the girdled surfaces of sampled shoots. Half hand-full of moistened rooting media was placed in $20cm^2$ transparent polyethylene film and wrapped around the girdled surface to serve as root substrate. It was tied at both ends by a thread. Monitoring of the setup was done weekly for 12 weeks and this is in accordance with work done by Pauku (2005). The marcots were excised from the plant with pair of secateurs after 12 weeks for examination. The transparent polyethylene film and the rooting medium were removed and the marcots were examined for callus, roots and sprouts or shoot formation.

2.5. Data collection and analysis

Data collection was done after twelve weeks after setting up the experiment. Parameters considered for data collection were; Survived marcots, number of marcots that rooted, total number of roots per marcot, root mean length, number of marcots that formed shoots and marcots that callused. Data were analyzed in one-way analysis of variance at 5% level of significance using Genstat Statistical Software version 10.3. Fisher's Pairwise Comparison test was used to separate treatment means where there was significant differences at $\alpha = 0.05$.

3. Results and discussion

3.1. Effect of IBA Concentrations on marcots survival of Rambutan

One-way analysis of variance tests on the survival of Rambutan marcots showed comparable results ($p>0.05$) with varying IBA concentrations at 5% level of significance (Table 1). Results indicated higher survival of marcots with IBA concentration than the control. Highest survival of marcots was recorded in the 2000 Mg/L of IBA (14.67 $\pm$0.33) representing 97.78%. Also, the 6000 Mg/L and 4000 Mg/L recorded mean survival of marcots 14.00$\pm$0.58 and 13.33 $\pm$0.88 representing 93.33% and 88.89% respectively. Marcots with no IBA (0 Mg/L) recorded the lowest mean survival of 12.33 $\pm$1.45 and expressed percentage (%) of 82.22. The results indicate explicitly how the rooting hormone, IBA induces marcot branches for survival. Rooting hormone increases the rate of cell division in the meristem thereby speeding up the rate at which the branches heal or the growth rate of the new organ (Jemaa et al., 2011), and this in turn increases the survival rate. This result is supported by the study of Rahman et al. (2000) which recorded survival percentages of 44.4%, 77.77%, 72.22, 66.66%, 61.10%, 49.97% and 33.33% induced by 3000mg/l, 2500mg/l, 2000mg/l, 1500mg/l, 1000mg/l, 500mg/l concentrations of IBA and 0mg/l (no auxin) depicting how IBA induces branch healing or rooting. This increases the survival rate of marcots. Among IBA treatments, survival rate decreases slightly as concentration increases with the exception of 4000 Mg/L that had some branches breaking due to windy weather condition.

3.2. Effect of IBA concentration on rooted marcots

Comparable ($p>0.05$) number of rooted marcots were observed for Rambutan under different concentrations of IBA at 5% level of probability as indicated in Table 2. Higher number of roots were recorded for marcots with IBA concentration than the control. Marcots with 2000 Mg/L IBA concentration recorded highest mean number of roots (8.67 $\pm$0.33), 4000 Mg/L (8.00 $\pm$1.15) and 6000 Mg/L (7.60 $\pm$0.33). The control recorded the lowest mean number of roots (5.67 $\pm$1.20) by Rambutan marcots. Although there were no significant differences among the treatments, marcots with IBA gave higher rooting percentages than the control. This might be due to the rooting hormone stimulating cell division at the girdled area. This confirmed work done by Tchoundjeu et al. (2002) on *Prunusafricana* where stem cuttings treated with IBA had a higher rooting percentage (85%) compared to

untreated cuttings (40%) at the end of an experiment (10 weeks). Again work done by Mishra et al. (1994) indicated that rooting of *Terminaliaarjuna* was best induced by 2000ppm IBA (60%) as compared with the control (3.3%). According to Davies and Hassig (1990), the production in plant through cell division, multiplication and specialization is controlled by plant growth substances especially auxins. This implies that treating marcots with auxins (IBA) can increase or decrease the percentage rooting, root initiation and number of roots per marcots. This probably explains the reason why low IBA (2000 Mg/L) recorded the highest percentage rooting (57.78) and high IBA (6000 Mg/L) recorded (51.11) These findings are also consistent with the findings of Debnath, (2008) who conducted a research on the effect of IBA on root proliferation of stevia and found out that increasing IBA concentrations increases sprouting, percentage rooting, root length and call us intensity but to a certain limit.

Table 1
Variation of IBA concentration on the number of survived marcots.

Treatment/ IBA concentration (Mg/L)	Mean ± S.E survival of Marcots	Range		Percentage survival of Marcots (%)
		Maximum	Minimum	
0	12.33 ±1.45	15.00	10.00	82.22
2000	14.67 ±0.33	15.00	14.00	97.78
4000	13.33 ±0.88	15.00	12.00	88.89
6000	14.00 ±0.58	15.00	13.00	93.33
P-value	0.374			
% Cv	11.6			

S.E= Standard error; Cv= Coefficient of variation. Mean survival of marcots under different IBA concentrations are not significantly different at $p > 0.05$.

Table 2
Variation of IBA concentration on the number of rooted marcots.

Treatment/ IBA concentration (Mg/L)	Mean ± S.E number of rooted Marcots	Range		Percentage number of rooted Marcots (%)
		Maximum	Minimum	
0	5.67 ±1.20	8.00	4.00	37.70
2000	8.67 ±0.33	9.00	8.00	57.78
4000	8.00 ±1.15	10.00	6.00	53.33
6000	7.60 ±0.33	8.00	7.00	51.11
P-value	0.163			
% Cv	20.6			

S.E= Standard error; Cv= Coefficient of variation. Mean number of rooted marcots under different IBA concentrations are not significantly different at $p > 0.05$.

3.3. Effect of IBA concentration on total number of roots formed

Test results at 5% level of probability revealed significant differences ($p<0.05$) among marcots of Rambutan with varying IBA concentrations (Figure 2). Highest mean number of roots formed ranged from 118.00 ±4.45 in the 4000 Mg/L to 88.00 ±7.33 in the control (0 Mg/L). Marcots with IBA concentrations of 2000 Mg/L and 6000 Mg/L also recorded 100.00±4.73 and 93.00 ±6.45 mean number of roots formed, respectively. Results show that rooting hormone was effective on total number of root formed and this is in conformity with the findings by Stem *et al.*, (1978) who observed that IBA treatment stimulates the production of profuse rooting. This result contradicts the findings by Dessalegn and Reddy, (2003) who reported 6000mg/l concentration of IBA to be the best for rooting and number of roots with *Simmondsiachinensis.*

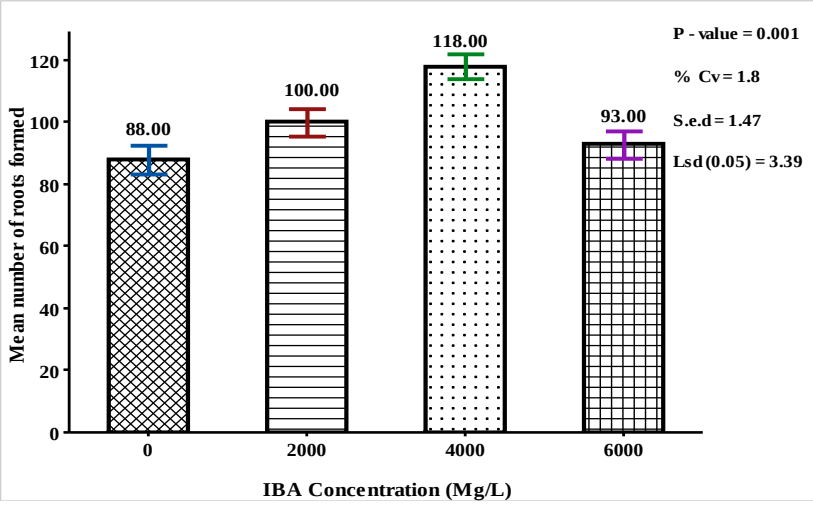

Fig. 2. Variations in number of roots formed by Rambutan marcots in different IBA concentrations.

3.4. Effect of IBA concentration on mean root length

Test results from one-way analysis of variance at a probability level of 5% on effect of IBA concentrations on Rambutan marcots revealed significant differences ($p<0.05$) in the lengths of roots formed. Mean root length ranged from 33.60 ±0.52 in the 6000 Mg/L to 19.77 ±1.26 in the control (0 Mg/L). Root length was 30.13 ±0.35 in marcots with 2000 Mg/L of IBA. Also, 26.48 ±1.33 was recorded as the mean root length of Rambutan marcots in IBA of 4000 Mg/L concentration (Figure 3). Results were consistent with the findings of Zengibal and Ozcan (2006), which concluded that the higher the concentration of auxin, the higher the root performance (root activity).

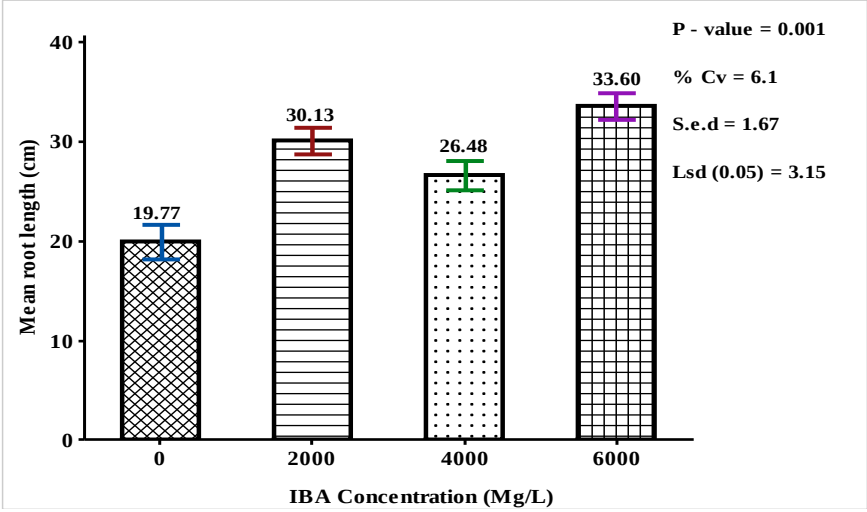

Fig. 3. Length of roots formed by Rambutan marcots in different IBA concentrations.

3.5. Effect of IBA concentration on marcots that callused

Effects of IBA concentrations on callused marcots revealed significantly comparable ($p>0.05$) results at 5% level of probability (Figure 4). Marcots with no IBA recorded 9.00 ±0.58 as mean number of callused marcots. Also, marcots with 6000 Mg/L of IBA recorded a mean number of marcots that callused as 6.67 ±0.88. However, marcots with 2000 and 4000 Mg/L recorded mean number of callused marcots of 6.33 ±0.88 and 6.33 ±0.67 respectively. This result is in conformity with work done by Canli and Bozkuri (2009), where the callus formation was highest under the control treatment (0mg/l) than the IBA treatments (500ppm, 1000ppm, 1500ppm and 2000ppm) used. They concluded that, root formation was not necessarily depended on callus formation, because roots were able

to form in most of the marcots without any intermediate callus phase. Results also contradicted Krishnan et al. (2003) where IBA 3000ppm promoted 100% more callusing success percentage than the control (no auxin) in *Simaroubaglauca.* This results shows that IBA was not effective in callusing and it is suggested that for callusing formation on *Nepheliumlappaceum*, 0 Mg/L (no auxin) should be considered appropriate.

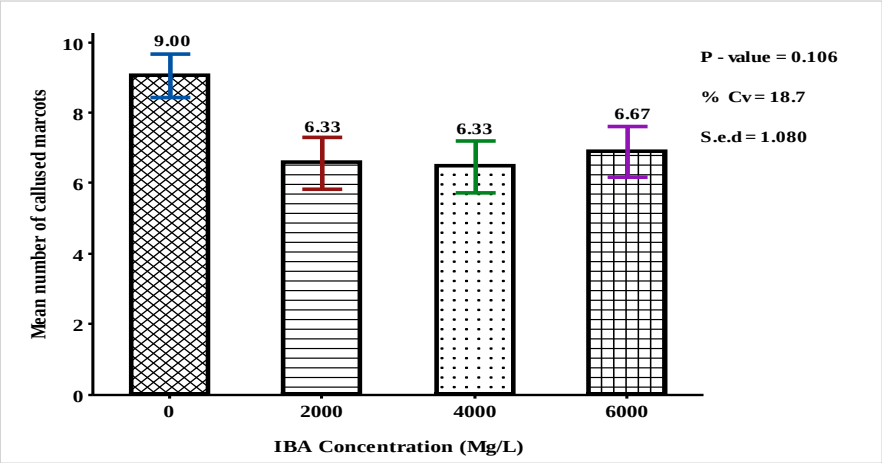

Fig. 4. Variation in number of callused formed by Rambutan marcots in different IBA concentrations.

3.6. Effects of IBA concentration on shoot formation

Analysis of variance test at a probability level of 5% on effect of IBA concentrations on Rambutan marcots showed comparable results ($p>0.05$) in shoot formation. Mean number of shoots formed ranged from 13.67 $\pm$0.88 in the 2000 Mg/L to 10.67 $\pm$2.33 in the control (0 Mg/L). Shoot formed was 12.67 $\pm$0.88 in marcots with 4000 Mg/L of IBA. Also, 11.00$\pm$2.00 was recorded as the mean shoot formed by Rambutan marcots in IBA of 6000 Mg/L concentration (Figure 5). This result indicated that Indole-3-Butyric Acid (IBA) was more effective on propagation of the species (Kesari et al., 2009; Patricio et al., 2006) compared to the control (0 Mg/L).

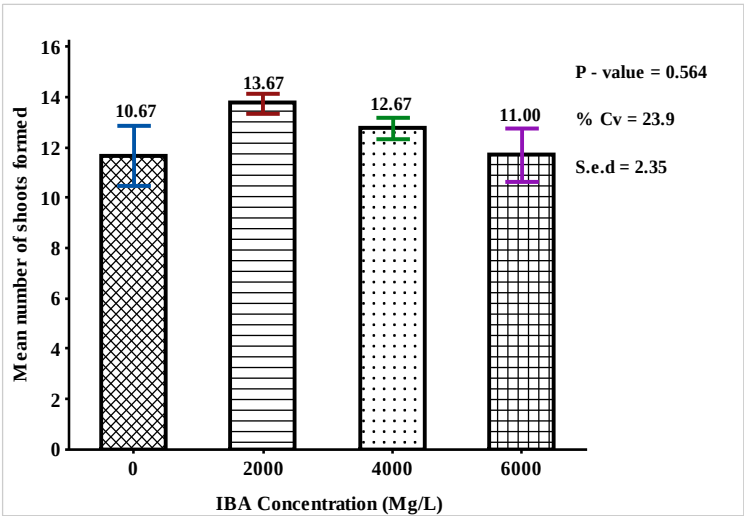

Fig. 5. Variation in number of shoots formed by Rambutan marcots in different IBA concentrations.

4. Conclusion

The study has provided baseline information of the marcotting technique, the rooting hormone (IBA) and the effect of IBA on survival, rooting, root length, callusing and shoot formation of Rambutan marcots. In developing

planting materials for the production of *Nepheliumlappaceum* (Rambutan), highest survival of marcots was recorded in the 2000 Mg/L of IBA (14.67 ±0.33) representing 97.78%. Also, the 6000 Mg/L and 4000 Mg/L recorded mean survival of marcots 14.00 ±0.58 and 13.33 ±0.88 representing 93.33% and 88.89% respectively. Marcots with no IBA (0 Mg/L) recorded the lowest mean survival of 12.33 ±1.45 and expressed percentage (%) of 82.22. Marcots with 2000 Mg/L IBA concentration recorded highest mean number of roots (8.67 ±0.33), 4000 Mg/L (8.00 ±1.15) and 6000 Mg/L (7.60 ±0.33). The control recorded the lowest mean number of roots (5.67 ±1.20). Highest mean number of roots formed ranged from 118.00 ±4.45 in the 4000 Mg/L to 88.00 ±7.33 in the control (0 Mg/L). Marcots with IBA concentrations of 2000 Mg/L and 6000 Mg/L also recorded 100.00 ±4.73 and 93.00 ±6.45 mean number of roots formed, respectively. Mean root length ranged from 33.60 ±0.52 in the 6000 Mg/L to 19.77 ±1.26 in the control (0 Mg/L). Root length was 30.13 ±0.35 in marcots with 2000 Mg/L of IBA. Marcots with no IBA recorded 9.00 ±0.58 as mean number of callused marcots. Also, marcots with 6000 Mg/L of IBA recorded a mean number of marcots that callused as 6.67 ±0.88. However, marcots with 2000 and 4000 Mg/L recorded mean number of callused marcots of 6.33 ±0.88 and 6.33 ±0.67 respectively.

This result showed that vegetative propagation by marcotting can be a suitable propagation method for the development of planting materials of *Nepheliumlappaceum*. The study further revealed IBA 2000mg/l as the most effective rooting hormone for propagating *Nepheliumlappaceum* recording the highest percentage rooting (57.78%), percentage survival (97.78%) and percentage shoot formation (91.11%) though statistically there was no significant different among treatments.

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