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SHORT COMMUNICATION

STEM CUTTINGS AS PROPAGULES FOR VEGETATIVE PROPAGATION OF CASHEW (*Anacardium occidentale* L.)

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Abstract

Due to the existing variability among the populations and the inability of meeting the current demand for planting materials for large-scale cultivation, cashew (*Anacardium occidentale* L.) requires a promising vegetative propagation method. Stem cutting is the best propagule and needs to induce germination which is restricted in the presence of gum. Wiping of gum secreted onto the cut surface of the stem with three solvents, water, acetone, and kerosene just after and after 5, 10, and 15 min of making the cut was tested to remove the gum. The effect of the presence of apical buds and leaves on the stem cuttings and the application of different types of rooting hormones containing IBA onto the cut surface was tested using three commercial varieties, WUCC-13, WUCC-19, and WUCC-21. Data were analyzed using CATMOD and ANOVA procedures. The results denoted a significant effect of gum solvent for maintaining the cutting viability by giving rise to shoots and callus production ($p<0.05$). Acetone was the only effective solvent in developing the callus on the cut surface. The application of Indole-3-butyric acid (IBA) indicated a better growth of shoots ($p<0.001$). Varieties, WUCC-13 and WUCC-21 disclosed better shoot growth. The presence of the apical bud and leaves negatively affected on activation of the axillary buds. Well-developed callus was present on the cut surface indicating the potential of giving rise to adventitious roots.

Keywords: Cashew, Callus formation, Indole Butyric Acid (IBA), Acetone, Rooting hormone, Root induction, Vegetative propagation

INTRODUCTION

Cashew (*Anacardium occidentale* L.; Family Anacardiaceae) a native to Brazil and widely grown in the tropics is considered one of the world's major edible nut crops (Aliyu 2007; Yeboah *et al*. 2020). Cultivation of this crop at a commercial scale has expanded recently in many tropical countries of the world, especially in Eastern and Western Africa, Vietnam, India, and Brazil (Mneney and Mantell 2002). Apart from the nuts, another economically important part is the cashew apple, from which juice, jam, snacks, and alcohol are produced (Aliyu 2005). Kernel secretions and woods also have a certain level of economic value. However, value addition for raw cashew nuts will lead to the development of various innovative products. It will cause to promote market acceptability and give higher income to the producer (Olife *et al*. 2013).

Considering the plant attributes, cashew is considered a drought-resistant tree that performs and thrives well in the tropics. However, the average yield of cashew is considerably low (350 kg/ha) in Sri Lanka (SLCC 2010). One of the main reasons for low productivity is the use of highly variable seedlings with low genetic potential (Jayasinghe and Jayasekara 2002). Cashew is normally propagated from seeds and the majority of the

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cashew plantations were established in the past using seedlings. Being a highly cross-pollinated crop, seed-derived plants are not true -to-type where a wide variation was observed among the trees with respect to the growth, flowering phenology, pest and disease resistance, and even economically important characteristics such as the yield and its quality, due to segregation (Yeboah *et al*. 2020).

Vegetative propagation is the only solution for establishing homogenous plantations with minimum variability, early bearing and uniform yield etc. (Mandal 1997). In order to fullfil the higher demand, an efficient vegetative propagation method is yet to be developed. Lateral grafting and budding are the commonly used vegetative propagation techniques (Thimmappaiah and Sadhana 2002). However, the skilled labour for practical engagement with these techniques is lacking. Propagation by air-layering, cuttings, and micropropagation techniques have also been attempted but with limited success (Martins *et al*. 2019). However, some of these techniques are time-consuming, thus, efficiency is low for large-scale production. Therefore, the supply of planting materials is not sufficient to fulfill the current demand.

Propagation through stem cuttings can be considered as a moderate mass production technique, which would be an option for enhancing the production efficiency. Cuttings would be economical and rapid (Rao *et al*. 1988). However, the reports on rooting induction in the stem cuttings of cashew are lacking, thus it is yet to be optimized. Previous studies reported that the germination of the cashew stem cuttings is very poor and the cuttings got dried up soon after planting them in the nursery (Rao *et al*. 1988). Richardson *et al*., (1979) reported the effect of season of material collection, concentration and the duration of growth hormones. The length of cutting on the rooting medium is vital to the success of germination.

The high content of polyphenolic compounds at the cutting surface together with the gum may inhibit the water and nutrient uptake of the cutting. It can subsequently negatively affect the callus formation at the cut surface preventing the induction of rooting and survival of the stem cuttings. Elimination of those chemical compounds from the cutting surface will be effective to induce callus formation and root development in the stem cuttings. Further, it is required to maintain the viability of the cuttings for a longer period in order to allow for root induction. Therefore, the present study was undertaken to check the effect of using different solvents to remove the secreted chemicals on the cutting surface before planting in the nursery. Furthermore, the preparation of the cuttings also plays an important role in maintaining the viability of the stem cutting. Therefore, the presence of apical bud and the leaves on the cutting were also tested for enhancing the maintenance of the viability of the cuttings. The effect of genotype and type of rooting hormones available at the commercial level was also tested for optimization of the conditions for better callus formation, survival, and root induction.

MATERIALS AND METHODS

Planting materials

Three nodal semi-hardwood cuttings (approx. 7.5-13 cm) from the apical point of the dormant buds were collected from the healthy mother plants. Three varieties, WUCC-13, WUCC-19 and WUCC-21 were used for optimizing the cutting conditions.

Testing the effect of different solvents and application time for removing the secretions from the cutting surface to induce rooting

Slant cuts were made at the lower end of each cutting in which the leaves were trimmed off. A three-factor factorial experiment was conducted to test the effect of three gum solvents, four application times and two root-inducing agent. Water, acetone and kerosene were tested as the solvents for removing the gum on the cutting surfaces of the stems. The surfaces were wiped with cheesecloths that were wetted with each solvent just after making a sharp cut and leaving it for 5, 10, and 15 min. After each treatment, the cut surface was washed thoroughly with soap water followed by washing with tap water. Then the prepared cuttings were planted in the nursery after applying two root-inducing agents separately as treatments, i.e. charcoal powder and 0.3 % Indole-3-butyric acid (IBA). Untreated cuttings were planted in the same media as the control. Potting medium with sand: coir dust (1:1) was used for planting. The planted cuttings were maintained inside a propagator to control micro-environmental conditions and subjected to general management practices. Each treatment combination consisted of 5 cuttings and the experiment was repeated three times.

Optimization of the conditions for maintaining the viability of the cuttings

A three-factor factorial experiment was conducted to test the effect of the presence or absence of apical bud and leaves on the cuttings in three different genotypes, WUCC-13, WUCC-19, and WUCC-21. After wiping the cut surface of each stem cutting with the best solvent selected in the above experiment (acetone) following the same procedure, the cuttings were dipped in rooting hormone. Three commercial rooting hormones containing IBA were used as per the producer's guidelines to check the rooting ability of the cuttings. Cuttings without hormones were used as the control. The cuttings from each treatment were randomly planted in the medium with coir dust and sand (1:1) under the semi-protected environmental condition inside a shade net and lower polytunnel. Seven cuttings were used for each treatment and the experiment was repeated twice. The cuttings were subjected to general management practices.

Both experiments were conducted under propagators with 15 cm spacing. The cuttings were regularly monitored to record the time taken for sprouting (days). One week after planting, the cuttings were observed for the number of sprouts/shoots, shooting position, root development, and callus formation on the cut surface. Further microscopic observations under the light microscope at 10 x magnification were made for testing callus formation using the thin hand sections obtained from un-rooted cutting edges. The growth parameters including, the number of shoots, length of shoots, and the number of fully opened leaves were recorded weekly basis. After one week of planting the cuttings were uprooted weekly for observing the callus formation and root induction (from which an extra set of cuttings were maintained for the purpose) until 5 weeks.

Experimental design and data analysis

All the experiments were conducted in Complete Randomized Design. The recorded data was subjected to the maximum likelihood analysis of variance, which was performed using CATMOD and ANOVA as per requirement, using SAS 9.2 Version. Least Significant Difference (LSD) test was done to separate the means at $\alpha=0.05$ levels where appropriate.

RESULTS AND DISCUSSION

Effect of different solvents and application time for removing the secretions from the cutting surface to induce rooting

The results clearly denoted, that the removal of the secretion from the cutting surface significantly improved the survival of the cuttings, shoot development, and callus formation ($p<0.05$). The cuttings treated with acetone resulted in 45% overall sprouting with 9.2% of callus formation on the cutting surface while water and kerosene oil resulted in 9.1% and 1.7% sprouting respectively with no callus formation. The control treatments neither responded to sprouting nor callus formation (Table 1).

The activation of the axillary buds and shoot development was observed in the cashew stem cuttings between 9-13 days after planting in the nursery bed. The sprouting process denoted variation over a whole lot of cutting where axillary buds at all three nodes or less were activated in some cutting of responded treatments (cuttings treated with acetone). In the control treatment, sprouts were not observed at all. However, the growth of the developed shoots/sprouts varied based on the shoot length and the number of leaves. Some buds developed giving rise to several well-developed leaves (Figure 1a) while others were having weak tiny sprouts with shorter lengths containing a few fully opened leaves. The healthy shoots remained on the cuttings for a longer period (6-8 weeks).

Further, it revealed that kerosene had a toxic effect showing the lowest induction of shoots. It was reported that kerosene toxicity affected seed germination (Kim 2014) and the growth and development of different plant species (Igboama and Ugwu 2016). Water may not be effective in removing the gum efficiently which creates an inhibitory effect on the viability of the cutting and its further development.

Phenolic compounds have various roles in plants. Most importantly, they protect plants from oxidative stress (Jaleel *et al*. 2009). But these phenolic compounds have an added primary effect on the regeneration process by reducing the wound response. A wound response is inherent in the preparation of cuttings. When the wound response is inhibited, it may affect the rooting process (De Klerk *et al*. 2011). Further, it was found that exudation of phenolic compounds at cut surfaces impacts callus formation and results in resistive nature for rooting as well (Sija *et al*. 2015). However, these phenolic compounds are having an impact on the micropropagation of the cashew which causes contamination as reported by Thimmappaiah and Sadhana (2002).

Though there were observations on the shoot development, any root development was not observed in any of the cuttings subjected to different treatments for gum removal. However, as an initial stage of root development, callus formation was observed (Figure 1b) in the cuttings treated with acetone one week after the nursery establishment. Microscopic observations revealed that the callus contained a mass of parenchyma cells (Figures 1c and 1d) which is a primary requirement for the formation of root primordial.

The callus formation is a process that takes place at the site of excision which occurs as a form of a mass of irregular proliferation at the site due to undifferentiated cell divisions (Srikanth *et al*. 2016). The callus contains a mass of parenchyma cells. In most plant species, callus formation is a precursor of adventitious root formation (Owen and Lopez

Table 1: Effect of solvents used for removing the gum from cut surface of cashew stem cuttings

Gum solvent	Sprout formation (%)				Callus formation (%)
	Sprout development of node				
	U	M	L	T	
Water	5.8	2.5	0.8	9.1	0
Acetone	17.5	15	13.3	45.8	9.2
Kerosene	1.7	0	0	1.7	0
Control*	0	0	0	0	0

Note: U=Upper node, M=Middle node, L=Low, T= Total, *without removing the gum

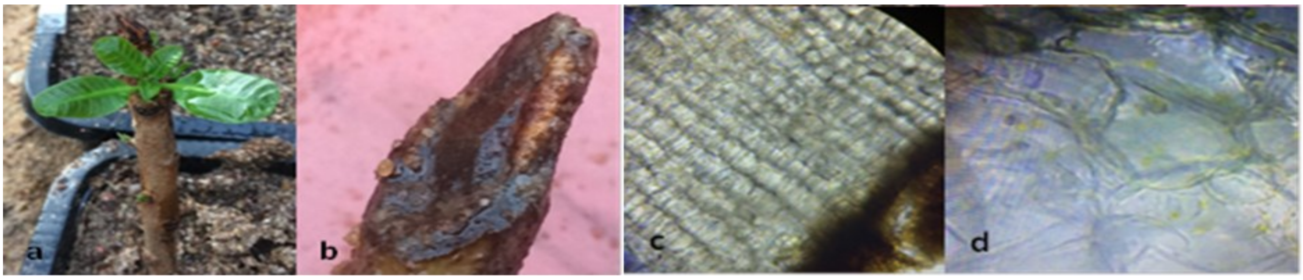

Figure 1: Response of the cashew (*Anacadium occidentale* L) stem cuttings planted in the potting medium *a.* A healthy shoot developed in the stem cutting b. Callus developed on the cut surface c, d. mesophyll cells in the callus

2017). Callus formation is an indicator of the re-differentiation that occurred in the differentiated cells of the cutting surface. Callus plays two important roles in germination. The newly formed cell layer acts as a protected layer for live cutting. It prevents entering the pathogens through the damaged cells of the cutting avoiding rotting. On the other hand, the callus is the primary cell that will give rise to the adventitious root primordial.

The time taken for removing the gum did not have a significant effect on any cutting response (Table 2). However, this phenomenon should be further tested with different time durations. The application of a rooting agent denoted a significant effect on both sprouting and callus formation (Table 2). As per the observations, IBA executed a more positive effect over activated charcoal on sprouting and callus formation with a mean value of 0.17 and 0.25 respectively. Despite the fact that activated charcoal is a good source for adsorbing phenolic compounds, its effect on cutting survival is minimum.

Effect of the presence of apical bud on stem cutting

The presence of apical bud denoted a significant effect on both, the number of shoots per cutting and shoot length per cutting except for the number of leaves (p<00.5; Table 3). The stem cuttings in the absence of an apical bud expressed higher shoot initiation along with the development of axillary buds compared to the cuttings containing the apical buds. In that scenario, it was observed that in some cuttings, all three axillary buds were activated whereas in some cuttings only one or two were developed. However, in the presence of apical buds, any shoot development from axillary buds did not occur while only the activation of the apical bud was observed.

This observation is in agreement with the previous finding that the apical bud prevents the development of axillary buds (Thomas and Hay 2009). All shoots activated at the apical bud got dried out after four weeks of planting, however, the shoots developed at the axillary buds in the cuttings without apical bud remained alive and healthy for more than six weeks. It is important to induce more shoots per cutting in order to supply more energy to the cutting through photosynthesis. Apical dominance is the scenario in which the shoot tip controls axillary bud outgrowth. With the decapitation of the apical bud, branching may occur in the occurrence of a vigorous shoot tip and be controlled by signals arising from the root and stem (Dun *et al*. 2006). Therefore, when preparing the stem cuttings removal of

Table 2: Effect of time of application of the gum solvent after making the sharp cut in the stem cutting and rooting induction agent on sprouting and callus formation of cashew (*Anacadium occidentale* L)

		Mean No. of sprouts[1]	Mean No. of callus[2]
Time of application	0 MAC	0.33	0.22
	5 MAC	0.56	0.33
	10 MAC	0.44	0.33
	15 MAC	0.11	0.11
	Significance	*NS*	*NS*
Type of hormone	Charcoal	0.05^a	0.06^a
	IBA	0.17^b	0.25^b
	X^2	*9.28*	*11.91*
	P value	*0.0023*	*0.0006*

Note: IBA=Indole Butyric Acid; NS: Non-significant, MAC=minutes after cutting the apical bud is important for inducing vigorous growth in the cuttings.

Effect of presence of leaves on the stem cuttings

Results showed that the presence of leaves on the stem cuttings significantly affects the number of shoots, shoot length, and the number of leaves per cutting compares to the cuttings without leaves (P<0.05; Table 3). The mean values for the above-mentioned parameters were higher in the cuttings without leaves. However, Yeboah *et al*. (2020) reported that the presence of leaves on the stem exerts a positive influence via cell division. Leaves are responsible for photosynthesis to provide nutritive materials required for cell division and produce plant growth substances for callus formation. The reduction of photosynthesis and auxin production due to the absence of leaves could affect the success of the propagation process. On contrary, our results denoted a negative relationship with the success of germination of stem cuttings of cashew in the presence of leaves. Increasing the leaf area may cause higher water loss that eventually dries up the cuttings. This makes damage to the cutting over the benefit of photosynthesis during the early days of planting. Therefore, removing or trimming leaves is beneficial when preparing the cashew stem cuttings for nursery planting.

Effect of the variety

The observation denoted a varietal effect on the performances of stem cuttings during the propagation process with a significant effect on all three parameters tested (p<00.5; Table 3). Varieties of WUCC-19 and WUCC-21 executed better for all three parameters over WUCC-13 while WUCC-21 denoted the highest mean values for the number of shoots (0.52), shoots length (5.55) and the number of leaves per cutting (0.58). Results indicated that the potential of vegetative propagation is genetically controlled in cashew therefore the success rate may depend on the variety.

The capability for vegetative propagation in plants varies significantly between species and genotypes. When it comes to propagation by cuttings, the rooting ability also varies in the same scenario. The genetic component of this intra-varietal variability effect may influence the presence of enzymes, inhibitors for the rooting process, endogenous auxins, phenolic, and other rooting co-factors (Leakey 1985; Pissard *et al*. 2008)

Effect of the type of commercial root-inducing hormone

Hormone application had no effect on how the cuttings responded, however, there were differences in the size of the shoots. While some cuttings showed weak and tiny shoots, others had well-developed shoots that produced larger leaves. Auxin is a naturally occurring chemical compound in plants which responsible for inducing cell division and root induction (Yeboah *et al*. 2020). IBA is a plant hormone in the auxin family that is an available ingredient in many commercial plants rooting products (Frick and Strader 2018). Many previous studies related to plant propagation processes reported that IBA affects positively in the success of the germination of stem cutting in a significant manner (Aytekin and Caliskan 2006; Al-Saqri and Alderson 1996; Wiesman, and Lavee 1995).

Adventitious root formation is a key step in the vegetative propagation of cuttings of the woody or horticultural crops (De Klerk *et al*. 2011; Ibironke 2016). The rooting hormone affects the success of root induction in stem cuttings (Topacoglu *et al*. 2016). Auxin is the naturally occurring plant hormone that causes cell division and enhances growth in the form of roots in stem cuttings. However, synthetic forms of auxins are available commercially in the form of IBA, Naphthalene Acetic acid (NAA) and Indole Acetic Acid (IAA) those aid rooting in moderate to difficult species while hastening the root initiation, number of roots, rooting uniformity, and time taken for rooting (Damodaran and Strader 2019).

Aliyu (2007) reported that 74 to 89 days were taken to induce the rooting during the air layering process of cashew. Hence, the time period that maintained the cuttings in the nursery during this study may not be sufficient for root development indicating the importance of waiting for a longer time for recording the

Table 3: Mean response of the stem cuttings of cashew (*Anacadium occidentale* L) for the tested factors of the presence of apical buds and leaves on the cuttings, varieties and application of rooting hormone on the cut surface

Factor	Level	Mean no. of shoots per cutting	Mean shoot length per cutting	Mean no. of Leaves per cutting
Presence of Apical bud	W0	0.60[a]	5.33[a]	0.46[a]
	W1	0.25[b]	2.95[b]	0.35[a]
	P Value	*<.0001*	*0.0005*	*0.22*
	LSD value	*0.11*	*1.32*	*1.32*
Presence of Leaves	L0	0.66[a]	6.39[a]	0.65[a]
	L1	0.19[b]	1.89[b]	0.16[b]
	P Value	*<.0001*	*<.0001*	*<.0001*
	LSD value	*0.11*	*1.32*	*0.18*
Variety	WUCC 19	0.49[a]	4.10[ba]	0.40[ba]
	WUCC 13	0.26[b]	2.77[b]	0.25[b]
	WUCC 21	0.52[a]	5.55[a]	0.58[a]
	P Value	*0.0005*	*0.0036*	*0.01*
	LSD value	*0.13*	*1.62*	*0.22*
Commercial Hormone	H0	0.42[ba]	3.82[ba]	0.42[a]
	H1	0.50[a]	5.50[a]	0.45[a]
	H2	0.46[ba]	3.71[ba]	0.44[a]
	H3	0.32[b]	3.53[b]	0.32[a]
	P Value	*0.15*	*0.13*	*0.77*
	LSD value	*0.16*	*1.87*	*0.26*

Note: Mean value of 14 cuttings is given. W0 – Without apical bud, W1 – With apical bud, L0- Without leaves, L1- With Leaves, H0 – Without hormone, H1 – Hormone 1 , H2 - Hormone 2 , H3 –Hormone 3

P - probability value, values followed by the same letter in a column do not differ significantly, from each at 5% level of probability using General Linear Model (GLM)

LSD value - Least Significant Difference

data on root induction. The vegetative propagation of trees is theoretically similar to the propagation aspects of any other plant. But, yet it is governed by complex interactions of internal and external factors which directly affect the capacity of rooting ability. With the high heterogeneity among the existing plant population of cashew is required suitable and economical vegetative propagation to overcome this issue for commercial large-scale production of true-to-type planting materials. Supposedly, the propagation of cashew by stem cuttings would be a rapid economic means of mass clonal multiplication and crop improvement. Hence the present study was conducted with a focus on finding a suitable method for eliminating the restraining factors on rooting of stem cutting of cashew while identifying the effects of several external and internal factors related to rooting.

However, apart from the tested factors, there might be an influence of some other internal and external factors such as the concentration of endogenous hormones, air temperature, relative humidity etc. on the success of the rooting of cashew stem cuttings. It is a common fact that the success of the rooting of stem cuttings, especially woody plants, depends on the age or physiological stage of the mother plant. Furthermore, environmental conditions, nutrient composition, and the physical structure of rooting media may also impact root growth and sprouting success. Hence, further studies may also require on these aspects for the establishment of a sound protocol for the vegetative propagation of cashew through stem cuttings. The optimized conditions for maintaining the tissue viability are highly important for further development of the root induction process in the aforementioned aspects.

CONCLUSIONS

The study revealed that the elimination of the secretion from the cut surface enhances the response of stem cuttings. The most suitable chemical type for wiping the cut surface is confirmed as acetone by giving rise to an appreciable percentage of cutting viability (46%) and callus formation on the cutting surface (9%). According to the study, the most effective rooting agent was IBA and there is a minimal effect on the type of commercial product. The study further revealed the positive impact of removing the apical buds and the leaves from the cuttings for maintaining viable and active cutting. The varietal effect was significant and WUCC-13 and WUCC-21 have a better potential for propagation through stem cuttings.

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AUTHOR CONTRIBUTIONS

JKWNS-Preparation of the manuscript; PIPP-designing the experiments, analysing the data, editing the manuscript; KVGLMCJ- conducting the experiments; SWSLN-conducting the experiments; HMSPH-managing the field experiment. All authors read and approved the final manuscript.

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