SHOOT MULTIPLICATION AND PLANT REGENERATION FROM *IN VITRO* CULTURES OF DRUMSTICK TREE (*Moringa oleifera* Lam.)

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SUMMARY

To obtain plantlets of drumstick tree (*Moringa oleifera* Lam.) with identical genetic make up for propagation or field production, *in vitro* cultures of drumstick tree were produced. For multiple of shoot induction, the stem explants of drumstick tree plantlets were cultured on Murashige and Skoog (MS) agar medium supplemented with different concentrations of 6-benzyl adenine purine (BAP) and maintained at 25 ± 2°C for 5 weeks. The results showed that sterilization with 0.1% $HgCl_2$ solution for 8 minutes gave a clear explant rate of 53.3% and a germination rate of 51.7%. Multiple of shoot induction was present in the stem explants that were cultured on the MS medium supplemented with BAP at the concentration range of 0.5 - 3.0 mg/l or the combination of BAP at 1.5 mg/l, TDZ at the range of 0.2 - 1.0 mg/l and NAA at the range of 0.2 - 1.0 mg/l. The MS medium containing BAP at 1.5 mg/l was found to produce 95.3% shoot formation with the highest average number of 8.4 shoots per explant. For plant regeneration, the shoot explants were cultured on ½ MS + 7 g/l agar + 15 g/l sucrose supplemented with IBA and IAA at difference concentration root induction. The results showed that ½ MS + 7 g/l agar + 15 g/l sucrose supplemented with IBA at 0.3 mg/l and IAA at 0.2 mg/l produce 100% root formation with the highest average number of 4.2 roots per explant and 3.5 cm in length. Tissue culture protocol reported in this study is an alternative means of micropropagation of drumstick plantlets with uniform genotypes for breeding selection and field production.

Keywords: Drumstick tree, *Moringa oleifera* Lam, plant regeneration, shoot multiplication.

1. INTRODUCTION

Drumstick tree (*Moringa oleifera* Lam.) nowadays is currently commercially planted and popularly utilized in more than 80 countries for medical, cosmetic, beverage technology/production, and nutrient and functional foods. Traditional propagation of drumstick tree is by direct seedling. This method takes between 3 - 14 days and results in one plant per seed.

Drumstick trees obtained from seeds vary in genotypes and hence in their phenotypes leading to variation in fruit (pod) production and nutritional values. Selected characteristics of plants may be maintained via *in vitro* propagation. Steintz *et al*. (2009) successfully propagated *Moringa oleifera* Lam., *Moringa stenopetala* (Baker f.) Cufod. and *Moringa peregrina* Forssk. by using basal Murashige and Skoog (MS) medium in the absence of plant growth regulators. The *in vitro* propagation was implemented by 3 following steps: 1) multiple shoot regeneration from cotyledonary node of decapitated seedlings, 2) axillary shoot growth from single node shoot segments and 3) rooting of excised shoots. This article reported the multiple shoots induction and regeneration of *in vitro Moringa oleifera* Lam. plantlets from shoot cultures. Stem explants of drumstick tree plantlets were used as explants to shoot multiplication study in MS medium in the presence of different concentrations of 6-Benzyl adenin purine (BAP) or BAP concentrations at 1.5 mg/l with TDZ and NAA at different concentrations. Shoots explants obtained from study of shoot multiplication were continuously used for multiple induction on MS or ½ MS medium supplemented with 0.5 mg IBA/l and sucrose at different concentrations. Regeneration of drumstick plantlets was then explored by using IBA and IAA at various concentrations. The procedure reported in this article is useful for regeneration of drumstick plantlets from shoots which is a mean of propagation of plants with the identical genetic traits for field studies.

2. MATERIALS AND METHODS

2.1. Plant materials

Seeds and young shoots of drumstick tree were collected at Ninh Thuan province, Vietnam.

2.2. Research methods

2.2.1. General method

The experiments were arranged in a Completely Randomized Design (CRD) with three replicates. All culture mediums were adjusted to pH 5.8 before autoclaving at 121^0C for 15 minutes. The cultures were maintained at room temperature (at 25 ± 2^0C), the light intensity of 35 µE/m^2/s and lighting time of 16 hours/day for 5 weeks.

2.2.2. Preparation of Explant and Establishment of Sterile Cultures

Seeds are cleaned with diluted soapy water (10%), rinsed with sterile distilled water. The seeds were sterilized by NaClO solution (20%, 30%) and solution of $HgCl_2$ (0.1%) with different sterilizing time, then wash disinfectant with sterile distilled water, soak seeds in sterile distilled water with a time of 20 minutes. The sterilized seeds were cultured on MS medium supplemented with agar 7g/l and sucrose 30g/l. The cultures were maintained in the same condition at 25 ± 2°C for 5 weeks. Percentage of clean explants and clean germinated explants were recorded.

2.2.3. Multiple shoot induction

Stem explants, from *in vitro* drumstick tree plantlet, were cut into sections of size of 1.0 - 1.5 cm and transplanted into multiple shoot regenerative medium: (1) (MS + 30 g/l sucrose + 7 g/l agar) supplemented with different BAP concentrations at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. (2) (MS + 30 g/l sucrose + 7 g/l agar) supplemented with BAP concentrations at 1.5 mg/l with TDZ concentrations at from 0.2 to 1.0 mg/l and NAA concentrations at from 0.2 to 1.0 mg/l. (3) MS or ½ MS + 7 g agar/l + 0.5 mg IBA/l and sucrose supplementation in different concentrations. Percentage of shoot formation, average shoots per explant and the characteristics of explants growth were recorded.

2.2.4. Plant regeneration

Shoot explants, from 1.5 at appropriate concentration of BAP, were cut into the small pieces and placed on the medium (1/2MS + 7 g/l agar + 15 g/l sucrose) supplemented with IBA and IAA at different concentrations. The concentration of IBA ranged from 0.2 mg/l to 0.8 mg/l, and IAA concentrations ranged from 0.2 mg/l to 1.0 mg/l medium. Percentages of shoot and root formation, average shoots and roots per explant and the characteristics of explants growth were recorded.

3. RESULTS AND DISCUSSION

3.1. Preparation of Explant and Establishment of Sterile Cultures

3.1.1. Influence of defection concentration and time with NaClO to clean explant formation ability from seeds

Table 1. Effect of concentration and sterilization time by NaClO on the ability to create clean explants after 2 weeks of culture

Category	NaClO concentration (%)	Time (minute)		Average B
		5	10	
Clean explant rate (%)	20	70.8	91.7	81.2 b
	30	80.0	95.8	87.9 a
Average A		75.4 b	93.7 a	
CV% = 3.0; F_A^{**}; F_B^{*}; F_{A*B}^{ns}				
Clean germinated explant rate (%)	20	65.5 c	87.5 a	76.5 a
	30	70.0 b	54.2 d	62.1 b
Average A		67.7	70.5	
CV% = 3.0; F_A^{ns}; F_B^{**}; F_{A*B}^{**}				

*In the same average group, the values with the same accompanying characters do not have statistical significance P < 0.05; *: statistically significant difference at P < 0.05; **: P < 0.01; ns: is a difference not statistically significant; A: time factor; B: NaClO concentration factor.*

The study results showed that the concentration of NaClO and different sterilization time had a statistically significant ($P < 0.05$) effect on the rate of clean explants of *drumstick tree*. In the same concentration of NaClO, the more sterilization time increases, the better rate of clean explants gets. At the same time of sterilization, increase of the concentration of NaClO also improved the rate of clean explants. There was no interaction between NaClO concentration and sterilization time to clean the explant rate ($P > 0.05$). Using 30% NaClO, sterilizing for 10 minutes gave the highest rate of clean explants (95.8%). In contrast, using a concentration of 20% NaClO, sterilizing for 5 minutes produced the lowest rate of clean explants (70.8%).

Table 1 also showed that the concentration of NaClO significantly affected ($P < 0.05$) to the rate of clean regenerated explants. During 5 minutes of sterilization, the rate of clean regenerated explants ranged from 65.5 to 70.0% by increasing the concentration of NaClO from 20 - 30%; on the other hand, during the 10-minute sterilization period, increase of NaClO concentration reduced from 87.5% to 54.2% of the rate of clean regenerated explants. There was an interaction between NaClO concentration and sterilization time to clean regenerated explants ($P < 0.05$). In which the combination of 20% NaClO concentration and 10 minutes sterilization time gave the highest rate of clean regenerated explants (87.5%); But using 30% NaClO concentration sterilized in 10 minutes, the lowest rate of clean regeneration explants was produced (54.2%).

The reason for sterilization with 20% NaClO in 10 minutes gives better results than 30% NaClO could be that NaClO in high concentration may be a strong disinfectant with toxicity to the cell, so when increasing the concentration, the ratio of clean explants increases but the rate of clean explants regenerated decreases.

3.1.2. Effect of concentration and sterlization time with $HgCl_2$ on the ability to form clean explants from shoots

In this study, in addition, *drumstick trees* embryos were used as a source of research materials to develop propagation processes. The experiment was also carried out on the *drumstick tree* shoots collected from well-grown mother plants to build *in vitro drumstick tree* propagation process.

The results of the sterilization of clean explants from the shoots presented in Table 2 showed that when the sterilization time increased from 5 to 12 minutes, the rate of clean explants increased from 18.3% - 75.0%, respectively. However, prolonged sterilization time (10, 12 minutes) gave a high rate of clean explants but the rate of re-germinated clean explants decreased (37.5% - 20%). This can be explained that if the time of sterilization by chemical $HgCl_2$ increase, destruction of pathogens (bacteria, fungi) will be better but it also increases vulnerability of plant tissue. In formula of 5-minute sterilization time, number of clean explants achieved was 22/120 explants, accounting for 18.3%, which is the lowest in this formula. But 22 clean explants were capable of regenerating shoot. From 4 experimental treatments on sterilization time in this study, the treatment of $HgCl_2$ 0.1% sterilized for 8 minutes gave 53.3% of clean explants and the highest germinated clean explant rate of 51.7% on the number of explants tested. This means that it is a suitable formula to sterilize clean explants from the shoots of *drumstick tree.*

Table 2. Effect of sterilization time by $HgCl_2$ on the ability to form clean explants from *Drumstick tree* shoots after 2 weeks of culture

Chemical	Time (minute)	Clean explant		Regenerated clean explant	
		No. of clean explants (explant)	Clean explant rate (%)	No. of regenerate clean explants	Regenerated clean explant rate (%)
$HgCl_2$ 0.1%	5	22 c	18.3 c	22 c	18.3 c
	8	64 b	53.3 b	62 a	51.7 a
	10	87 a	72.5 a	45 b	37.5 b
	12	90 a	75.0 a	24 c	20.0 c
CV%		3.5	3.5	5.8	5.8
P		<0.01	<0.01	<0.01	<0.01

Our results obtained were similar to those published by various authors on other species. Study of *in vitro* cultivation of the shoots *of Calamus tetradactylus* Hance, Bui Van Thang et al. (2013) used 0.1% $HgCl_2$ to disinfect shoots twice (1st time from 5 - 7 minutes, 2nd time from 3 to 5 minutes) in shoot sterilizations. Their results showed that the sterilization time (1st time: 7 minutes; 2nd time: 3 minutes) gave the best results and produced a clean explant rate of 85.5% and a clean regenerated explant rate of 76.3%. Similarly, Vu Quang Nam et al. (2013) reported that sterilization with 0.1% $HgCl_2$ for 7 minutes produced the best results and the rate of clean explants reached 72.0%, the rate of regenerated clean explants reached 87.6% in *in vitro* propagation of *Celastrus hindsii*.

3.2. Multiple of shoot induction

3.2.1. Effect of BAP content on shoot formation

In plant tissue culture, BAP plays a very important role in strongly stimulating shoots formation, determining multiplier and shoots quality. According to reports of Joarder et al. (1993), BAP of cytokinin group is suitable for development of shoots of Neem tree, induction of shoot formation in woody plants (Zamam et al., 1991, Thakur et al., 1997). Using BAP in the shoot formation induction of d*rumstick tree* was also reported by Eufrocinio (2010), Thidarat et al. (2011) and Saini et al. (2012). These authors reported that using the same concentration of BAP growth regulator for different varieties of *drumstick tree* produced different shooting rate and the number of regenerated shoots. In our study, effect of concentration of BAP growth regulator (ranged from 0.5 - 3.0 mg/l) on the regeneration of shoots for a variety of *drumstick tree* originating from Ninh Thuan province was investigated.

The shoots *of drumstick tree* were regenerated after 2 weeks of culture on the starting medium. The stems were cut into small pieces of size 1.0 - 1.5 cm, transplanted into the induction medium to form the shoots. In this experiment, MS medium was used and supplemented with BAP with different concentrations, such as 0.5 mg/l; 1.0 mg/l; 1.5 mg/l; 2.0 mg/l; 2.5 mg/l; and 3.0 mg/l. Experimental results were collected after 3 weeks and presented in table 3.

Table 3. Effect of BAP content on the ability to form multiple shoots

Formula	BAP (mg/l)	Rate of regenerated shoots (%)	No. of shoots /explant	Shoot length (cm)
MS0	0.0	65.0	1.2	2.5
MS1	0.5	74.3	2.3	2.7
MS2	1.0	90.3	5.8	4.5
MS3	1.5	95.3	8.4	4.2
MS4	2.0	90.0	4.5	2.1
MS5	2.5	70.3	3.3	2.2
MS6	3.0	72.7	2.5	1.7
CV%		2.78	5.21	4.37
P		< 0.01	< 0.01	< 0.01

Table 3 showed that there were statistical differences ($p < 0.01$) between the experiment treatments. In the medium without BAP supplementing (MS0), the explants still regenerated shoots at the rate of 65% and the average number of shoots/explant was 1.2 shoots. The medium supplemented with BAP concentration of 0.5 - 3.0 mg/l gave the rate of regeneration of shoots ≥ 70.5% and the average number of shoots/explant ≥ 2.3, higher than the non-BAP-supplementary medium. BAP growth regulator had a significant effect on the rate of shoot regeneration from *drumstick tree* when *in vitro* cultured.

Figure 1. *Drumstick tree* shoots on MS medium supplemented with 1.5 mg/l BAP

The nutrient medium formula of MS supplemented with BAP concentration from 1.0 to 2.0 mg/l gave a high rate of shoot regeneration (> 90%), the average number of shoots/explant ranged from 4.5 to 8.4 shoots. Among 7 experimental formulas, the medium formula supplemented with 1.5 mg/l BAP produced the highest rate of shoot regeneration

(95.3%), average number of shoots/explant (8.4 shoots) and the average length of shoots (reached 4.2 cm). Using lower BAP (0.5 mg/l) or higher BAP (2.5 - 3.0 mg/l) concentrations produced lower the rate of shoot regeneration (70 - 74%), average number of shoots/explant (2.3 to 3.3 shoots) and shoot length than the best medium formula. Our study results obtained here are similar to those published by various authors. Thidarat et al (2011) studied *in vitro* propagation of *drumstick tree* originated from Chiang Rai province, Thailand and reported that using a concentration of 1 - 2 mg/l BA produced the shoot regeneration rate of 100%, average number of shoots/explant ranged from 5.2 - 10.8 shoots, higher than those when using BA at other concentrations. When using BAP concentration of 1.0 - 2.0 mg/l in Micropropagation of *drumstick tree* (Variety-PKM1) originated from the Indian University of Agricultural Sciences also produced the highest rate of regenerating shoots from 90 - 94% and the average number of shoots/explant was 8.3 - 9.0 shoots (Saini et al., 2012). Therefore, using BAP or BA at a concentration of 1.0 - 2.0 mg/l for the period of *drumstick tree* shoot regeneration can be suitable. Previous results of various authors also show that the difference of *drumstick tree* origins significantly affected the regeneration rate and the number of shoot/explant. In our micropropagation of *drumstick tree* from Ninh Thuan province in shoot regeneration period, explants were cultured on a nutrient medium of MS + 30 g/l sucrose + 7 g/l agar supplemented with 1.5 mg/l BAP, which is the best medium formula among experimental medium formulas.

3.2.2. Effect of BAP, TDZ, NAA combination on shoot formation

Besides supplementing BAP growth regulators of 1.5 mg/l, in this experiment TDZ and NAA at different concentrations (TDZ: 0.2 mg/l; 0.5 mg/l; 1.0 mg/l; và NAA: 0.2 mg/l; 0.5 mg/l; 1.0 mg/l) were supplemented to determine the combination of growth regulators with suitable concentration for shoot formation of *drumstick tree*, in culture medium.

The results showed that when using 1.5 mg/l BAP growth regulator in combination of 0.2 - 1.0 mg/l TDZ and NAA growth regulators produced the rate of shoot formation ranged from 60.5 to 85.7% and the number of shoots/explant ranged from 2.1 to 6.2 shoots (Table 4), lower than the shoot formation culture medium by supplementing with only a single BAP of 1.5 mg/l (rate of shoot formation of 95.5%, the number of shoots/explant was 8.4 shoots). Reports of various authors also show that using single BAP produced the best result in *in vitro* propagation of *drumstick tree* at the multiple shoots formation. When using other substances (like TDZ, NAA, IBA), the ratio of shoots formation and the number of helpful shoots/explant were low (Eufrocinio, 2010; Thidarat, 2011; Mylene and Evalour, 2011).

Table 4. Effect of BAP, TDZ, NAA combination on shoot formation

Medium formula	BAP (mg/l)	TDZ (mg/l)	NAA (mg/l)	Shoot regenration rate (%)	No. of shoots /explant	Shoot length (cm)
MS7	1.5	0.2	0.0	85.7	6.2	3.8
MS8		0.5	0.0	72.5	5.3	4.0
MS9		1.0	0.0	60.5	5.0	2.7
MS10		0.0	0.2	75.0	3.4	2.5
MS11		0.0	0.5	68.8	2.2	2.0
MS12		0.0	1.0	63.0	2.1	1.5
CV%				3.73	1.47	4.92
P				< 0.01	< 0.01	< 0.01

Study on the combination of growth regulators (treatment MS7 – MS12) showed that the formula of medium (MS7) supplemented with BAP 1.5 mg/l and TDZ 0.2 mg/l produced the rate of regenerated shoots of 85.7% and 6.2 shoots per explant, the highest formula of medium in our experiment. Medium formula supplemented with 1.5 mg/l BAP and 1.0 mg/l NAA produced the lowest rate of multiple shoots formation, number of shoots/explant and shoot length.

Figue 2. *Drumstick tree* shoots on MS medium supplemented with 1.5 mg/l BAP and 0.2 mg/l TDZ

3.3. Plant regeneration

3.3.1. Effect of nutrient medium and sucrose content on root formation

In *in vitro* propagation, the nutrient medium greatly affects on growth and development of the plant. It do not only influence on the shoot formation, but also affect significantly on root formation. For each plant species, nutritional needs at the stage of rooting are significantly different. The period of complete plant formation often reduces mineral nutrients content and especially the sugar content for sturdy plantlets, better adaptation when plantlets planted in natural environment. After 2 weeks of culture on root formation medium, the results were presented in table 5. There was an interaction between the nutrient medium and sucrose content effected on the rooting rate ($P < 0.01$); in which the highest rooting rate from shoot (90%) obtained from the interaction of 15 g/l sucrose content with both MS and ½ MS mediums, the lowest rate was 60.6% from the interaction of 10 g/l sucrose content with MS medium.

The interaction between nutrient medium and sucrose content ($P < 0.01$) also affected on *the* root length of *drumstick tree*. The interaction between sucrose content of 15 g/l + ½ MS produced the highest root length of 3.6 cm, while the interaction between sucrose content of 10 g/l + MS produced the lowest (2.0 cm).

There was no interaction between nutrient medium and sucrose content ($P > 0.05$) affected on the number of roots/shoots. However, the independent effect of each factor was statistically significant ($P < 0.01$), in which the combination of sucrose content of 15 g/l + ½ MS produced the highest number of roots per shoot (4.8 roots), and sucrose 10 g/l + MS produced the lowest roots (3.2 roots).

Table 5. Effect of nutrient medium and sugar content on root formation of drumstick tree after 2 weeks of culture

Category	Sucrose (g/l)	MS	1/2MS	Average B
Rooting rate (%)	10	60.6 c	65.2 c	62.9 d
	15	90.0 a	90.0 a	90.0 a
	20	82.6 ab	90.0 a	86.4 b
	30	70.2 b	72.3 b	71.2 c
Average A		75.9 b	79.3 a	
CV% = 1.9; F_A^{**}; F_B^{**}; F_{A*B}^{**}				
No. of roots/shoot	10	3.2	3.5	3.4 d
	15	4.4	4.8	4.6 a
	20	4.1	4.5	4.3 b
	30	4.0	4.2	4.1 c
Average A		3.9 b	4.3 a	
CV% = 2.7; F_A^{**}; F_B^{**}; F_{A*B}^{ns}				
Root length (cm)	10	2.0 f	2.5 e	2.3 c
	15	2.5 e	3.6 a	3.1 a
	20	2.7 de	3.4 b	3.1 a
	30	2.8 d	3.0 c	2.9 b
Average A		2.5 b	3.1 a	
CV% = 1.5; F_A^{**}; F_B^{**}; F_{A*B}^{**}				
Plant quality	10	+	++	
	15	+	++	
	20	+	+	
	30	+	+	

Plant quality: + bad quality trees (trees have full leaves and roots, but the roots appear large scar tissue); ++ good quality plants (trees have full leaf stems, roots, root parts appear small scar tissue); +++ plants are of good quality (trees have full leaf stems, roots, root parts do not appear scar tissue).

Monitoring process showed that all of the *drumstick tree* plantlets appeared callus at the root base, which affecting plant quality in plant transfer. This result is similar to some previous publications (Eufrocinio, 2010; Mylene and Evalour, 2011; Thidarat, 2011; Lalida, 2013). The medium of MS + 7 g agar/l + 15 g sucrose/l + 0.5 mg IBA/l supplemented with 15 g sucrose/l and the medium of ½ MS + 7 g agar/l + 15 g sucrose/l + 0.5 mg IBA/l supplemented with 15 – 20 g sucrose/l produced the highest rate of rooting (90%). The medium of ½ MS + 7 g agar/l + 15 g sucrose/l + 0.5 mg IBA/l supplemented with 15 g sucrose/l produced the highest number of roots forming per shoot (4.8 roots), root length of 3.6 cm and the root base appears only small callus, as well.

In summary, the basic nutrient medium to culture *drumstick tree* shoots in the period of forming complete plantlet is ½ MS and 15 g sucrose/l.

3.3.2. Effect of concentration of IBA and IAA on root formation

After determining the basic nutrient medium and appropriate sugar content, our experiments continued conducting tests of suitable type and content of growth regulators for studying effect of these growth regulators on the rooting ability of the shoot in period of rooting and form complete plantlets.

Shoot explants were cut and transplanted to the root formation induction medium. Using rooting medium formula 1/2MS + 7 g/l agar + 15 g/l sucrose supplemented with different concentrations of IBA and IAA. After 2 weeks of culture under lighting conditions, the obtained results were shown in table 6.

Table 6. Influence of IBA and IAA on root formation of drumstick tree

Formula	IBA (mg/l)	IAA (mg/l)	% Rooting	No. of roots/shoot	Root length (cm)	Plant quality
R9	0.0	0.0	0.0			
R10	0.2	0.0	82.5	4.0 ± 0.30	2.6 ± 0.25	++
R11	0.4	0.0	100	4.6 ± 0.53	2.8 ± 0.20	++
R12	0.8	0.0	75.0	4.2 ± 0.43	1.8 ± 0.34	+
R13	0.0	0.2	90.2	4.0 ± 0.62	2.5 ± 0.44	++
R14	0.0	0.5	100	3.8 ± 0.57	2.2 ± 0.63	++
R15	0.0	1.0	100	3.5 ± 0.36	1.5 ± 0.60	+
R16	0.2	0.2	92.8	3.8 ± 0.65	3.0 ± 0.40	+++
R17	0.3	0.2	100	4.2 ± 0.40	3.5 ± 0.33	+++
CV%			1.36	2.91	5.41	
P			< 0.01	< 0.01	< 0.01	

The results obtained in Table 6 showed that after 2 weeks of culture on basic nutrient medium without growth regulators (R9), 100% of cultured shoots did not have the roots. In the medium formulas supplemented with IBA or IAA with different contents, *drumstick tree* shoots rooted. However the rates vary significantly (ranged from 82.5 - 100%). The average number of roots/shoot and root length (ranged from 3.5 to 4.6 roots and 1.5 to 3.5 cm, respectively. Combined medium formula (R16 and R17) supplemented with IBA or IAA at small content (0.2 - 0.3 mg/l IBA and 0.2 mg/l IAA) produced rooting efficiency of 92.8 – 100%, the average number of roots of 3.8 – 4.2 roots/shoot and root length of 3.0 – 3.5cm, better plant quality than others (The root part did not appear callus). Saini et al. (2012) reported similarly that the period of rooting from shoots of *drumstick trees* of Indian origin on nutrient medium supplemented with 2.85 µM IAA and 4.92 µM IBA produced the rooting rate of 100%, 15 roots/shoot and average root length of 8 cm. When culture medium supplemented with IBA and NAA at high concentrations (0.8 - 1.0 mg/l), the rooting rate of *drumstick tree* shoots was relatively high, however, shoots appeared large scar tissue at their base, affecting the plantlet quality.

According to the study conducted by Hongfeng and Qiang (2008), the best rooting stimulating medium for *drumstick trees* shoots was ½ MS + 0.4 mg/l IBA + 0.2 mg/l NAA + 7 g/l Karagum + 20g/l sucrose. Eufrocinio (2010) reported MS medium supplemented with 30 g/l sucrose + 5.0 g/l agar + 0.25 µM NAA produced the highest rooting rate, with 6.8 roots per shoot after one week of culture.

Figure 3. Complete *in vitro Moringa oleifera* roots

From our study results, the medium formula of ½ MS + 7 g/l agar + 15 g/l sucrose supplemented with IBA at 0.3 mg/l and IAA at 0.2 mg/l produced 100% rooted plants, complete plantlets which ensure eligibility for taking outside at nursery.

4. CONCLUSION

The disinfection formula for *in vitro* culture from *Moringa oleifera* shoot was 0.1% $HgCl_2$ for 8 minutes.

The most suitable nutrient medium formula to regenerate and form multiple shoots of *in vitro drumstick tree* was: MS + 30 g/l sucrose + 7 g/l agar + 1.5 mg/l BAP. It produced the rate of regenerated shoots of 95.5%, the average number of shoots/explant of 8.4 shoots and the average shoot length of 4.2 cm, good quality of shoots.

The best rooting medium formula was: ½ MS + 7 g/l agar + 15 g/l sucrose + 0.3 mg/l IBA + 0.2 mg/l IAA produces 100% rooting rate, the average root number of 4.2 roots/shoot, the average root length of 3.5 cm, plants grow well.

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XÂY DỰNG KỸ THUẬT NHÂN GIỐNG *IN VITRO* CÂY CHÙM NGÂY (*Moringa oleifera* Lam.)

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TÓM TẮT

Hiện nay ở Việt Nam phương pháp nhân giống cây Chùm ngây chủ yếu bằng hạt và giâm cành, tuy nhiên các phương pháp này có những nhược điểm. Để khắc phục và tiến tới cung cấp với số lượng lớn giống Chùm ngây cho sản xuất trên quy mô lớn, có những đặc tính ưu việt và đồng nhất. Nghiên cứu này trình bày kết quả nhân giống Chùm ngây hoàn chỉnh trong điều kiện *in vitro*. Kết quả nghiên cứu cho thấy khử trùng bằng dung dịch $HgCl_2$ 0,1% trong thời gian 8 phút cho tỷ lệ mẫu sạch 53,3% và tỷ lệ mẫu nảy mầm 51,7%. Công thức môi trường cảm ứng tạo đa chồi sử dụng môi trường MS bổ sung BAP ở nồng độ khác nhau: 0,5 - 3,0 mg/l hoặc tổ hợp BAP 1,5 mg/l, TDZ: 0,2 - 1,0 mg/l và NAA: 0,2 - 1,0 mg/l. Môi trường MS bổ sung 1,5 mg/l BAP cho tỷ lệ mẫu tái sinh chồi cao nhất (95,3%), số chồi trung bình/mẫu cũng cao nhất 8,4 chồi và chiều dài trung bình của chồi đạt 4,2 cm. Đối với cảm ứng tạo rễ và tái sinh cây hoàn chỉnh, các chồi Chùm ngây *in vitro* có lá đạt tiêu chuẩn kích thước (đạt từ 3 - 5 cm) được cắt và cấy chuyển sang môi trường cảm ứng tạo rễ (1/2MS + 7 g/l agar + 15 g/l sucrose) bổ sung IBA và IAA với các nồng độ khác nhau. Kết quả cho thấy môi trường dinh dưỡng cảm ứng tạo rễ bổ sung 0,3 mg/l IBA và 0,2 mg/l IAA cho tỷ lệ chồi ra rễ 100%, số rễ/chồi đạt cao nhất 4,2 rễ và chiều dài rễ đạt 3,5 cm.

Từ khóa: Cảm ứng tạo chồi, cảm ứng tạo rễ, cây Chùm ngây, tái sinh cây.

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