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Micropropagation and Production of *Rondeletia odorata* Jacq from *In vitro* Technique

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to investigate the most suitable treatments for micropropagation of *Rondeletia odorata* Jacq. *Rondeletia* are of slow growth, and not many cultivator of plants care to give time and space to raise them.

The explants were sterilized by immersion in a Clorox solution at 5, 10, 15, 20 or 25% at 20 min plus 3 drops of Tween 20 and Hg₂Cl at 0.1, 0.3, 0.5 and 0.7 mg/l at 1 min. Shoot tips and auxiliary buds survived after surface sterilization were cultured on MS-strength medium (full, half and quarter salt strength), supplemented with NAA at 0.0, 0.1, 0.5 and 1.0 mg/l and their combination. For multiplication stage, 2iP at 0.0, 0.5, 1.0, 2.0 3.0 or 4.0 mg/l and IBA at 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l. The second experiment BA at 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l and Kin at 0.0, 0.5, 1.0 and 1.5 mg/l were used. Shoot was cultured on MS-strength medium at full, half or quarter salt strength with activated charcoal at 0.0 or 3.0 g/l and different concentrations of NAA at 0.0, 0.1, 0.5 or 1.0 mg/l. In the same time, IBA and IAA at 0.0, 0.1, 0.5, or 2.5 mg/l was added alone for rooting. All rooted shoots were acclimatized on peat moss and sand with 1:1, 1:2 or 1:3 (v/v) under plastic tunnel under plastic house conditions.

Study results, 15 % Clorox plus 0.5mg/l Hg₂Cl gave good results for surface sterilization. For the explants were collected gradually from the mother plants at establishment stage, the shoots were tallest at August and June. MS at half strength and 0.5 mg/l NAA induced the highest shoot length and number of leaves in establishment stage. In the

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multiplication stage at the first experiments, the highest shoot length and number of leaves was recorded on MS medium supplemented with 2.0mg/l IBA. At the second experiments, the highest number of shoots was found at 1.5mg/l BA. The highest number of roots; root length and callus formation was formed at quarter strength of MS medium and 0.5mg/l NAA. A high percentage of plant survival (22.22%) was achieved at pots containing only peat moss.

This study was achieved to the most suitable protocol for micropropagation of *Rondeletia odorata* Jacq. This Acclimatization, however, need further work to increase establishment at greenhouse.

Keywords: *Micropropagation; tissue culture; explants; in vitro; callus; Rondeletia.*

1. INTRODUCTION

Rondeletia odorata Jacq is a genus from the family Rubiaceae, Originally from Panama and Cuba. Bailey & Bailey [1] showed that *Rondeletia* are of slow growth, and not many cultivator of plants care to give time and space to raise them, nevertheless some of the species, notably *Rondeletia odorata* Var. major, deserve to be more widely grown.

El-shamy et al. [2] found that sodium hypochlorite (NaOCl) at 2 % and 0.5mg/l mercuric chloride (Hg₂Cl) were sterilization significant with the highest value of *Zantedeschia aethiopica* survived explants. Siva et al. [3] reported that emerging explants of *Oldenlandia umbellata* were grew on 2.5mg/l Naphthalene acetic acid (NAA) was suitable for callus induction; Shoots where formed when embryogenic calli cultured on Murashige & Skoog (MS) medium containing 1.5 mg/l Benzyladenine (BA), 0.3mg/l NAA and 1% coconut milk (CM). Alahakoon et al. [4] suggested that shoot tips of *Ixora odorata* were cultured on half MS with 2mg/l 6-benzylamino purin (BAP) and 500 mg/l cefotaxime. Axillary shoots were transferred on half MS supplemented with 200 mg/l hygromycin. Roja [5] found that plantlets of *Ophiorrhiza rugosa* were regenerated from shoot initiated from axillary meristems on medium containing 4.0mg/l BA and 0.05mg/l NAA. Radha et al. [6] reported that the highest number of shoots was obtained on medium supplemented with 1 mg/l BA and 0.02mg/l IAA. Shoot regeneration of *Rubia cordifolia* was subcultured on 0.5 mg/l BA. Xiong et al. [7] reported that shoot developed roots with 100% on half MS medium. Adding 0.5% activated carbon (AC) and 1.0mg/l Indole butyric acid (IBA) induced rooting of *Emmenopterys henryi*. Radha et al. [6] noted that the best root of *Rubia cordifolia* (98%) was achieved on 1 mg/l IBA followed by 1mg/l Indole acetic acid (IAA). Senthilkumar et al. [8] reported that 82 % of hardened plantlets of *Ophiorrhiza mungos* survived well in the hardening medium containing red soil, coir waste and vermicompost in the ratio of 1:1:1 by volume.

The objectives of the experiment were to investigate the most suitable protocol for micropropagation of *Rondeletia odorata* Jacq. There isn't any work on *in vitro* propagation of *Rondeletia*. Our initial experiment proved that this species is difficult to propagate as the shoot tips displayed strong apical dominance and produced large number of shoots. In addition, many shoots developed *in vitro* were browned.

2. MATERIALS AND METHODS

The experiments of this study were consummated in Plant Tissue Culture Laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, during 2008 – 2011 year.

The mother plants were grown naturally at the open field condition at Zohria Botanical garden. The parts used as explants were shoot tips and axillary buds. Explants were incubated at growth room under light intensity of 3000 lux, 16 h photoperiod provided by white fluorescent lamp at average temperature of $24\pm 2^{\circ}\text{C}$.

2.1 Surface Sterilization of Explants

The explants were washed with drops of a commercial liquid detergent and Clorox. They were washed for two hours under a running tap water. The explants were sterilized by immersion in a Clorox solution (commercial bleach, 5.25% sodium hypochlorite) at 5, 10, 15, 20 or 25% at 20 min plus 3 drops of Tween 20 and Hg_2Cl at 0.1, 0.3, 0.5 and 0.7 mg/l at 1min.

2.2 Explants Date on Establishment Stage

For the date, the explants were collected gradually from the mother plants every month to formulate 12 treatments (12 months) to know the most propel month for micropropagation.

2.3 Establishment Stage

Explants survived were cultured on MS-strength medium (full, half and quarter salt strength), supplemented with NAA at 0.0, 0.1, 0.5 and 1.0mg/l and their combination. The PH media was adjusted to 5.7 and solidified containing 200mg/l Polyvinyl pyrrolidone (PVP).

2.4 Multiplication Stage

To determine the most suitable medium for multiplication stage, twenty treatments were initiated to study the effect of 2-isopentenyl aminopurine (2iP) at 0.0, 0.5, 1.0, 2.0 3.0 or 4.0mg/l and IBA at 0.0, 0.5, 1.0, 1.5 and 2.0mg/l giving thirty treatments. The second experiment BA at 0.0, 0.5, 1.0, 1.5 and 2.0mg/l and Kinetin (Kin) at 0.0, 0.5, 1.0 and 1.5mg/l were used.

2.5 Rooting Stage

Each shoot of *Rodeletia odorata* (3 cm in length with 5 leaves) was cultured on MS-strength medium at full, half or quarter salt strength with activated charcoal (AC) at 0.0 or 3.0g/l and different concentrations of NAA at 0.0, 0.1, 0.5 or 1.0 mg/l were initiated from the use of NAA. In the same time, IBA and IAA at 0.0, 0.1, 0.5, or 2.5 mg/l was added alone. AC at 3.0g/l was added to all media to improve root formation.

2.6 Acclimatization Stage

All rooted shoots were acclimatized on peatmoss and sand with 1:1, 1:2 or 1:3 (v/v) under plastic tunnel under plastic house conditions. The survival percentage (%) of plantlets was

calculated after one month. After acclimatization stage, no abnormalities physical appearance and growth habits were observed on the transplanted plants.

2.7 Experimental Design and Statistical Analysis

Snedecor and Cochran, [9] a complete randomized design was employed in all experiments. Analysis of variance was used to show statistical differences between treatments using the L.S.D. at probability level (5%). Each treatment consisted of three jars containing three shoots in each jar.

3. RESULTS AND DISCUSSION

3.1 Effect of Clorox and Mercuric Chloride on Surface Sterilization of *Rondeletia* Explants

In respect to Clorox effect on surface sterilization of *Rondeletia odorata*, data in Table 1 reveal that Clorox affected significantly on surface sterilization of *Rondeletia* buds. Number of uncontaminated explants increased with the increase of Clorox concentration, giving the highest mean value (21.25%) at the concentration of 15%, whereas lower or higher rates decreased the uncontamination percentage. Concerning the effect of Hg₂Cl, the data cleared that 0.5 mg/l resulted the highest uncontamination percentage (16.0%), whereas higher or lower rates of mercuric chloride decreased this value. The interaction between Clorox and Hg₂Cl affected significantly giving the highest value of uncontaminated explants (50%) at 15 % Clorox plus 0.5 mg/l Hg₂Cl. Present results on sterilization of explants are in line with those of Saifullah et al. [10] on *Ixora coccinea* and Huang et al. [11] on *Morinda officinalis*.

Table 1. Effect of mercuric chloride concentration and Clorox concentration (%) on survival percentage at surface sterilization of *Rondeletia odorata* explants

Clorox (%)	Hg ₂ Cl (mg/l)				Mean (A)
	0.1	0.3	0.5	0.7	
5	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	5.00	1.25
15	0.00	20.00	50.00	15.00	21.25
20	0.00	5.00	30.00	15.00	12.50
25	0.00	0.00	0.00	0.00	0.00
Mean (B)	0.00	5.00	16.00	7.00	
LSD_{0.05} for					
Clorox, %(A)	1.60				
Hg ₂ Cl, mg/l (B)	1.43				
(AxB)	3.20				

3.2 Effect of Monthly Sampling Dates on Establishment Stage of *Rondeletia odorata*

Concerning the sampling date, results demonstrated in Table 2 showed that the highest value for the number of shoots (50) had been obtained for August sampling. The longest shoots value (3.5cm) had been obtained on shoots of explants collected in June and August.

Table 2. Effect of monthly sampling dates on shoots of *Rondeletia odorata* explants

Sampling dates (monthly)	Explants	
	Number of shoots	Shoot length (cm)
January	10.00	2.00
February	10.00	2.00
March	15.00	2.00
April	20.00	2.50
May	30.00	3.00
June	35.00	3.50
July	40.00	3.00
August	50.00	3.50
September	35.00	2.00
October	30.00	2.50
November	25.00	2.50
December	15.00	2.00
LSD _{0.05}	8.24	0.72

3.3 Effect of MS Medium Strength and NAA on Establishment Stage of *Rondeletia odorata*

3.3.1 Shoot length

The data presented in Table 3 showed that the highest mean value for shoot length had been measured for that cultured on MS medium at half strength. The different concentrations of NAA, the highest mean value for shoot length was found when medium contains 0.5 mg/l NAA. The interaction between MS strength and the different concentrations of NAA, the data showed that MS at half strength supplemented with 0.5 mg/l NAA induced the highest shoot length, while higher concentration decreased this value at the same strength and same level of NAA. This means that the moderate strength of MS (half strength) supplemented with moderate concentration of NAA (0.5) were superior for shoot elongation than the other salt strength and NAA concentration.

3.3.2 Number of leaves

The data in Table 3 showed that MS medium strength and NAA concentration affected significantly on number of leaves. MS medium at half strength resulted in the highest mean value for number of leaves. This cleared that MS medium at half strength was more effective than full or quarter strength. The highest mean value had been obtained at 0.5 mg/l NAA. This means that low or high concentration of NAA were not favorable for leaf formation. The best combination was on MS at half strength and 0.5 mg/l NAA that gave the highest number of leaves (Plate 1a). These results are in line with that obtained on *Coffea Arabica*, Pasqual and Barros [12]; Kahia [8] and Andrade et al. [13].

Table 3. Effect of MS medium strength supplemented with different concentration of NAA on shoot length (cm) and number of leaves at establishment stage of *Rondeletia odorata*

MS (strength)	NAA (mg/l)				Mean (A)					Mean (A)
	0.00	0.10	0.50	1.00		0.00	0.10	0.50	1.00	
	Shoot length (cm)					Number of leaves				
Full	3.67	3.00	3.67	3.67	3.50	1.00	1.17	1.67	1.50	1.33
½	3.33	4.67	5.67	3.67	4.33	1.50	1.67	2.33	1.67	1.79
¼	2.67	4.33	3.67	3.33	3.50	1.67	1.67	1.83	1.67	1.71
Mean (B)	3.22	4.00	4.33	3.56		1.39	1.50	1.94	1.61	
LSD _{0.05} for MS (A)			0.47					0.21		
NAA(B)			0.54					0.24		
(AxB)			0.93					0.42		



Plate 1a. Shoot formation of *Rondeletia odorata* on (0.0, 0.1, 0.5 or 1.0 mg/l) NAA and 1/2 MS during establishment stage

3.4 Effect of 2ip and IBA on *Rondeletia odorata* in Multiplication Stage

3.4.1 Shoot length

The data exhibited in Table 4 indicated that steady decreases in shoot length had been obtained as the increase in 2ip concentrations from 0.0 to 4.0 mg/l. The mean value for shoot length was increased by increasing the concentration level of IBA as compared with the control in each case. The highest mean value for shoot length was 2.61 cm at 2.0 mg/l IBA. Regarding the interaction between 2ip and IBA, the shoots cultured on MS medium supplemented with 2 mg/l IBA produced the highest mean value for shoot length.

3.4.2 Number of leaves

The highest mean value for number of leaves was recorded at 0.0 mg/l 2ip, however the values decreased gradually, while the shoots were cultured on MS medium supplemented with 4.0 mg/l 2ip. The highest number of leaves was obtained at 2.0 mg/l IBA as compared with control. The highest value for number of leaves was recorded on MS medium supplemented with 2.0 mg/l IBA and 0.0 mg/l 2ip. The effect of IBA was more than the effect of 2ip. The results agree with that obtained on *Coffea arabica*. Meanwhile, Zeatin and 2ip failed to promote proliferation at any used level with Ebrahim et al. [14]. Whereas it disagreed with that obtained by Pontikis [15] on *Gardenia jasminoides* was achieved on a modified Anderson medium supplemented with 100 mg/l myo-inositol, 4 mg/l thiamine HCl and 0.1 mg/l IBA, 0.1 mg/l Gibberellic acid (GA3) or BA.

Table 4. Effect of MS medium supplemented with different concentrations of 2ip and IBA on shoot length and number of leaves at multiplication stage of *Rondeletia odorata*

2ip (mg/l)	IBA (mg/l)											
	0.0	0.5	1.0	1.5	2.0	Mean (A)	0.0	0.5	1.0	1.5	2.0	Mean (A)
	Shoot length (cm)						Number of leaves					
0.0	2.33	2.17	2.83	3.17	4.17	2.93	3.33	4.00	4.33	5.33	6.67	4.73
0.5	1.83	2.33	2.67	3.33	3.50	2.73	3.33	3.67	3.67	4.33	5.00	4.00
1.0	1.67	1.83	2.00	2.33	2.67	2.10	3.00	3.33	3.33	3.67	4.33	3.53
2.0	1.50	1.83	1.83	2.17	2.17	1.90	2.67	3.00	3.33	3.33	3.67	3.20
3.0	1.33	1.67	1.67	2.00	1.83	1.70	2.33	3.00	3.00	2.67	3.33	2.87
4.0	1.17	1.33	1.33	1.67	1.33	1.37	2.00	2.67	2.33	2.33	3.00	2.47
Mean (B)	1.64	1.86	2.06	2.44	2.61		2.78	3.28	3.33	3.61	4.33	
LSD_{0.05} for												
2ip (A)						0.1975						0.3647
IBA(B)						0.1803						0.3330
(AxB)						0.4416						0.8156

3.5 Effect of BA and Kin on Multiplication Stage of *Rondeletia odorata*

3.5.1 Shoot length

Data revealed that the highest mean value had been recorded at the concentration of 1.5 mg/l BA, followed by 2.0mg/l BA, whereas the lowest value was obtained for that of control treatment (0.0 mg/l NAA). There was a decrease in shoot length with increasing of Kin concentrations. The highest mean value for shoot length was obtained for control treatments as compared to the other test concentrations of Kin. The interaction between BA and Kin concentrations, the results showed that the tallest shoot was obtained when BA added at 2.0mg/l at 0.0 mg/l Kin (Plate1b).

3.5.2 Number of shoots

Data in Table 5 demonstrated that BA and Kin in addition to their combination affected significantly on this character. Increasing the concentration of BA to 1.5 mg/l resulted in the highest mean value followed by 1.0 at 2.0mg/l BA as compared with the control. Application of Kin resulted in decreasing mean value from the highest value for 0.0mg/l to reach its minimum value at the highest concentration of Kin (1.5mg/l). Data also showed that BA and Kin at all tested concentrations caused an increase in number of *Rondeletia* shoots as

compared with the control. The highest number of shoots was found as a result of the combined 1.5mg/l BA at 0.0mg/l Kin.



Plate 1b. Shoot formation of *Rondeletia odorata* at (0.0, 0.5, 1.0, 1.5 or 2.0 mg/l) BA during multiplication stage

3.5.3 Number of leaves

The highest mean value for number of leaves had been obtained for control then decreased to 7.13, 6.60 and 6.06 leaves by increasing the concentration of Kin to 0.5, 1.0 and 1.5 mg/l respectively. The highest value for number of leaves was recorded on MS medium supplemented with 1.5 or 2.0 mg/l BA without Kin. These results showed that great shoot development was obtained on MS medium supplemented with BA. BA was more effective on production of higher shoots and leaves and this agreed with that reported by Pontikis [15] and Mishra and Sreenath [16]. On the other hand, it disagreed with that confirmed by Dumanois et al. [17] on *Gardenia jasminoides* showing that the morphogenetic activity decreased for the *in vitro* propagation.

3.6. Effect of Auxins on Rooting Stage of *Rondeletia odorata*

3.6.1 Effect of Indole butyric acid

In general, the data in Table 6 the addition of IBA to the rooting media affected significantly on increasing the shoot length and number of leaves by increasing the IBA concentration. Concerning the effect of IBA concentrations, indicated that rooting of *Rondeletia* shoots was not affected by the different concentrations of IBA. The results here were in agreement that with reported on rooting for *Ixora singaporensis* on MS medium supplemented with IBA, Kartha et al. [18] and Malathy and Pai [19].

Table 5. Effect of MS medium supplemented with different concentrations of BA and Kin on shoot length, number of shoots and number of leaves at multiplication stage of *Rondeletia odorata*

BA (mg/l)	Kin (mg/l)					Mean (A)	Kin (mg/l)					Mean(A)	Kin (mg/l)					Mean(A)
	0.0	0.5	1.0	1.5	Mean (A)		0.0	0.5	1.0	1.5	Mean(A)		0.0	0.5	1.0	1.5	Mean(A)	
Shoot length (cm)						Number of shoots					Number of leaves							
0.0	1.00	2.00	3.33	3.67	2.50	0.00	0.00	0.00	0.00	0.00	3.67	3.67	4.67	6.33	4.58			
0.5	3.00	3.33	3.67	4.33	3.58	0.00	1.00	0.00	0.00	0.25	5.67	6.33	7.33	6.33	6.42			
1.0	3.67	4.00	3.67	2.67	3.50	0.67	0.67	0.00	0.00	0.33	6.33	6.67	7.00	6.33	6.58			
1.5	7.67	5.33	4.67	3.33	5.25	3.67	2.00	1.00	0.00	1.67	13.33	9.67	7.33	6.00	9.08			
2.0	8.00	4.67	3.67	3.33	4.92	2.67	1.33	0.00	0.00	1.00	13.33	9.33	6.67	5.33	8.67			
Mean (B)	4.67	3.87	3.80	3.47		1.40	1.00	0.20	0.00		8.47	7.13	6.60	6.06				
LSD_{0.05} for																		
BA (A)					0.41	Kin (B)					0.24	(AxB)					0.49	
Kin (B)					0.37	(AxB)					0.21	BA (A)					0.44	
(AxB)					0.82	Kin (B)					0.48	(AxB)					0.98	

Table 6. Effect of IBA concentration on rooting stage of *Rondeletia odorata*

IBA (mg/l)	Shoot length (cm)	No. of leaves	No. of roots
0.00	3.44	5.56	0.00
0.10	3.78	6.44	0.00
0.50	5.22	8.78	0.00
2.50	4.33	7.33	0.00
LSD _{0.05}	0.24	0.46	0.00

3.6.2 Effect of IAA

The data in Table (7) proved that no root formation occurred at all concentrations of IAA on *Rondeletia* shoots.

Table 7. Effect of IAA concentration on rooting stage of *Rondeletia odorata*

IAA (mg/l)	Shoot length (cm)	No. of leaves	No. of roots
0.00	3.44	5.56	0.00
0.10	3.78	7.11	0.00
0.50	4.56	9.33	0.00
2.50	4.00	7.78	0.00
LSD _{0.05}	0.60	0.49	0.00

3.7 Effect of NAA

3.7.1 Number of roots

Data recorded in Table 8 demonstrated that number of *Rondeletia odorata* roots was significantly affected with cultivation on MS medium at different strength and NAA either with AC. The highest mean value for the effect of MS medium strength had been obtained at half strength without AC. The highest mean value was recorded for that treated with 0.5 mg/l NAA. Regarding the interaction between MS medium and NAA concentrations (Plate1c), it was found that MS medium at quarter strength combined with 0.5 mg/l NAA increased the number of roots to reach.

Table 8. Effect of MS salt strength medium, NAA concentration and their interaction on number of roots during rooting stage of *Rondeletia odorata*

MS medium	NAA (mg/l)				Mean(A)
	0.00	0.10	0.50	1.00	
Full	0.00	0.00	0.00	0.00	0.00
½	0.56	1.44	2.22	1.33	1.39
¼	0.22	0.44	2.56	0.56	0.94
Full + AC	0.00	0.00	0.00	0.00	0.00
½+ AC	0.00	1.56	1.67	0.22	0.86
¼+ AC	0.00	0.22	0.89	0.22	0.33
Mean (B)	0.13	0.61	1.22	0.39	
LSD_{0.05} for MS (A)		0.13			
NAA (B)		0.10			
(AxB)		0.25			



Plate 1c. Effect of activated charcoal at quarter strength of MS medium supplemented with 0.5 mg/l NAA during rooting stage of *Rondeletia odorata*

3.7.2 Root length

Similar trend had been obtained as shown in Table 9, the highest mean value was recorded for that cultured on MS medium at half strength. NAA at 0.5 mg/l was gave the highest value for root length. Regarding the interaction between culture media (MS) and NAA, the longest roots had been obtained on MS at quarter strength without AC and presence of 0.5mg/l NAA.

Table 9. Effect of MS salt strength medium and NAA concentration on root length (cm) during rooting stage of *Rondeletia odorata*

MS medium	NAA (mg/l)				Mean (A)
	0.00	0.10	0.50	1.00	
Full	0.00	0.00	0.00	0.00	0.00
½	0.56	1.44	1.67	1.44	1.28
¼	0.22	0.44	2.56	0.44	0.92
Full + AC	0.00	0.00	0.00	0.00	0.00
½+ AC	0.00	0.44	1.78	0.22	0.61
¼+ AC	0.00	0.22	0.56	0.22	0.25
Mean (B)	0.13	0.42	1.09	0.39	
LSD_{0.05} for MS (A)		0.12			
NAA (B)		0.09			
(AxB)		0.23			

3.7.3 Shoot length

Data in Table 10 revealed that the highest mean value of shoot length was formed at quarter strength of MS medium supplemented with 3 g/l AC as compared with full or quarter MS without AC. Shoot length was increased by increasing the concentrations. The highest shoot length was obtained at the highest concentration of NAA. The longest shoots were produced on MS medium at quarter strength with 3 g/l AC plus 1.0 mg/l NAA.

Table 10. Effect of MS salt strength medium and NAA concentration on shoot length (cm) during rooting stage of *Rondeletia odorata*

MS medium	NAA (mg/l)				Mean (A)
	0.00	0.10	0.50	1.00	
Full	3.22	3.67	4.33	4.33	3.89
½	3.56	4.22	4.56	4.78	4.28
¼	4.56	5.22	5.56	5.78	5.28
Full + AC	3.44	4.44	5.22	6.22	4.83
½+ AC	4.33	5.22	5.67	6.56	5.45
¼+ AC	5.78	5.67	5.78	7.33	6.14
Mean (B)	4.15	4.74	5.19	5.83	
LSD_{0.05} for MS (A)					0.16
NAA (B)					0.13
(AxB)					0.33

3.7.4 Number of leaves

The data in Table 11 indicated that both of culture media at different strength and NAA at different concentration affected significantly on the number of leaves. MS at quarter strength medium with 3 g/l AC resulted in the highest mean value for number of leaves. NAA steadily increase in number of leaves due to the increase in NAA concentration value for 1.0 mg/l NAA giving the highest mean. Quarter MS supplemented with 3 g/l AC and supplemented with 1.0 mg/l NAA resulted in the highest value for number of leaves. These results were agreed *in vitro* rooting. Shoots were treated with NAA in the culture medium by Kahia [20].

Table 11. Effect of MS salt strength medium, NAA concentration and Activated charcoal on number of leaves during rooting stage of *Rondeletia odorata*

MS medium	NAA (mg/l)				Mean (A)
	0.00	0.10	0.50	1.00	
Full	5.33	5.67	6.33	7.67	6.25
½	5.78	6.33	7.44	8.56	7.03
¼	6.67	7.78	9.22	10.67	8.59
Full + AC	5.56	6.22	7.44	8.67	6.97
½+ AC	6.56	7.44	7.89	10.00	7.97
¼+ AC	7.78	8.78	9.67	11.44	9.42
Mean (B)	6.28	7.04	8.00	9.50	
LSD_{0.05} for MS (A)					0.18
NAA (B)					0.15
(AxB)					0.37

3.7.5 Callus formation

The data in Table 12 showed that there was highest significant effect on callus formation as result of cultivation of shoots on MS medium at different strength and NAA concentration at MS quarter strength medium without AC resulted in the highest mean value. These results clearly demonstrated that MS medium at quarter strength and 0.5 mg/l NAA gave the highest value for callus formation. These results are in line with these reported by Lakshmanan et al, [21] on *Coffea arabica*. That NAA was highly effective in inducing rooting, producing the maximum amount of basal callus. The presence of basal callus, did not affect transplantation or the establishment of plantlets in soil as the callus was removed easily without damaging the root system.

Table 12. Effect of MS salt strength medium and NAA concentration on callus formation during rooting stage of *Rondeletia odorata*

MS medium	NAA (mg/l)				Mean (A)
	0.00	0.10	0.50	1.00	
Full	2.00	2.00	2.33	2.00	2.08
½	2.00	2.33	3.00	2.00	2.33
¼	2.00	2.33	4.00	2.33	2.67
Full + AC	1.00	1.00	1.00	1.00	1.00
½+ AC	1.00	2.00	2.33	2.33	1.92
¼+ AC	2.00	2.00	3.33	2.00	2.33
Mean (B)	1.67	1.94	2.67	1.94	
LSD_{0.05} for	MS (A)	0.3042			
NAA (B)		0.2483			
(AxB)		0.6083			

Footnote: Results for callus formation/explant were calculated visually as scores (according to Pottino, 1981[22]): Negative (-)=1, Below average (+)=2, Average (++)=3
Good (+++)= 4

3.7.6 Effect of peatmoss+sand mixtures on acclimatization stage of *Rondeletia odorata*

The results in Table 13 indicated that a high percentage of plant survival (22.22%) was achieved. By using peatmoss as planting substrate, i.e. plants succeeded to be transplanted to greenhouse conditions in pots containing peatmoss (Plate1 d). The other mixtures didn't give any survival for plantlets. These results disagreed with that concluded by (Huang, et al. [11], the rooting seedlings of *Morinda officinalis* were transplanted in sand or burnt soil with plant ash, and the highest survival rate was 90%.

Table 13. Effect of peatmoss and sand on survival (%) during acclimatization stage of *Rondeletia odorata*

Peatmoss	Sand	Survival %
1	0	22.22
1	1	0.00
1	2	0.00
1	3	0.00
LSD _{0.05}		11.10

4. CONCLUSION

The longest shoots had been obtained on shoots of explants collected in June and August of *Rondeletia odorata* Jacq. The highest value of uncontaminated explants (50%) was found at 15 % Clorox plus 0.5 mg/l Hg₂Cl for surface sterilization. MS at half strength supplemented with 0.5mg/l NAA were superior for shoot elongation and Number of leaves at establishment stage. The highest value for multiplication stage was recorded on MS medium supplemented with 1.5 or 2.0 mg/l BA without Kin. MS medium at quarter strength combined with 0.5 mg/l NAA was the most suitable for rooting stage. The highest percentage of plant survival was achieved in pots containing peatmoss at greenhouse during acclimatization stage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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