

Callus Induction and *In vitro* Regeneration from Leaf and Cotyledon Explants of Noni (*Morinda citrifolia* L.)

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Key words: Micropropagation, Noni, Regeneration, Callus, Cotyledons, Leaf segments

Abstract

Noni (*Morinda citrifolia* L.) is considered as an important medicinal plant due to its medicinal properties. In this study, young leaves and cotyledons of Noni were used as explants for callus induction on MS medium supplemented with different concentrations of 2,4-D (1.0-5.0 mg/l). The results demonstrated that both leaf and cotyledon explants exhibited the maximum callus induction on MS medium supplemented with 3.0 mg/l 2,4-D. Here cotyledon explants showed better performance on callus induction than that of leaf explants. For the enhancement of plantlet regeneration, induced calli were transferred to MS₀, ½MS₀, and ¼MS₀ medium where different concentrations and combinations of BAP (1.0-5.0 mg/l), Kn (0.5-2.5 mg/l) and NAA (0.2-1.0 mg/l) were used either single or in combination. Results showed that ½MS₀ medium produced the best performance on regeneration (79.76%). Calli derived from cotyledons showed better regeneration ability than that of the leaves. Consequently, proliferation of shoots (9.1 ± 0.50) from cotyledon derived callus were formed to be higher than that of leaf segment derived callus (5.6 ± 0.34). MS medium with 4.0 mg/l BAP and 2.0 mg/l Kn produced the best results in increasing shoot length (4.02 ± 0.22 cm). Here ½MS₀ medium supplemented with 2.0 mg/l IBA showed the best performance on root induction (93.80 ± 1.78) and increasing the length of roots (1.76 ± 0.03 cm).

Introduction

Morinda citrifolia L. referred to as Noni or Indian mulberry is a traditional indigenous medicinal plant that grows in tropical and subtropical climates worldwide (Jayaprakash et al. 2017). Throughout the year, this plant produces flowers and fruits. Noni plants grow incredibly quickly and begin to produce fruits within the first year after sowing (Basar et al. 2010). This plant can be recognized by its enormous, glossy, dark green, elliptical leaves, white tubular flowers with ovoid to globes heads, and white corolla. A fruit with a yellowish-white, ovoid, lumpy body that made up of many fused, ripening ovaries that are separately distinct from the white flower. The fruit has a pungent, rotting

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scent similar to butyric acid, whereas the unripe fruit is dark green in appearance. When the fruit is torn, the pulp is gelatinous, light-dull yellowish white, and bitter; there are several firm, triangular, reddish-brown pits (Ali et al. 2016). In traditional oriental medical systems, every parts of *M. citrifolia* L. such as the leaves, fruits, barks, and roots was utilized as a febrifuge, cathartic and for the treatment of various ailments including sapaemia, dysentery, and spongy gums (Sreeranjini and Siril 2014).

Multiple medicinally active principles with therapeutic benefits, including antioxidant, antibacterial, antiviral, and anticancer activity is found in *M. citrifolia* L., according to present pharmacological analysis (Sreeranjini and Siril 2014). Sterols, minerals, putative proxeronine and vitamins are also found in Noni (Gajakosh et al. 2010). Traditional uses of this plant for the remedy of different diseases of human being are really praiseworthy. Biochemical, phytotherapeutic and toxicological characteristics as well as the most present advances in the production and standardization of goods made from Noni fruits (Almeida et al. 2019). Noni fruit juice has also the liver protective functions (Wang et al. 2008). Noni seeds are rich in linoleic, oleic, and palmitic acids, along with beta-sitosterol, campesterol, and stigmasterol (West et al. 2011).

As an annual shrub, *M. citrifolia* can produce fruits at various stages of ripeness simultaneously on the same plant (Chan-Blanco et al. 2006). This is an exceptional characteristics of this plant. Fruits of this plant have the high medicinal values for the remedy of different diseases of human being. That is why the cultivation of this plant is the demand of time. Therefore, seedlings are necessary for the cultivation of this plant. But seed germination rate both in *ex vitro* and *in vitro* conditions is very poor and it takes approximately six months. Under these circumstances, the techniques of *in vitro* micropropagation can be applied for the production of adequate seedlings of Noni. During this study *in vitro* regeneration was carried out through callus induction using the explants of leaf segments and cotyledons of seeds of Noni. Moreover, experiments were also conducted to develop roots from the *in vitro* raised shoots to get complete plantlets of Noni.

Materials and Methods

This study was conducted to enhance the micropropagation process of Noni, which is cultivated widely all over the country. Young leaves and immature fruits of Noni were collected from the research field of the Medicinal Plants Germplasm, Institute of Biological Sciences, University of Rajshahi, Bangladesh for conducting this experiment. Cotyledons were collected from immature seeds and very juvenile expanding leaves (1-2 months old) were used as explants for *in vitro* callus induction. The leaves which were collected to use as explants were washed with running tap water for 20 min and then treated with 35 to 40% sodium hypochlorite (NaOCl), Tween-20 and liquid detergent for 20 min. Then the explants were taken inside the laminar air flow chamber under sterile condition and washed with the sterile distilled water up to removing the foam which was formed in conical flask at the time of sterilizing. After that the explants were excised

and divided into segments of 0.5 to 1.0 cm. Cotyledons of seeds were collected from fruits directly inside the laminar airflow chamber under sterile condition. Then sterilized leaf segments and cotyledons were used as the explants for callus induction and regeneration of plantlets. For these purposes MS medium (with 30 g/l sucrose and 7.0 g/l agar) supplemented with different concentrations and combinations of 2,4-D (1.0-5.0 mg/l), BAP (1.0-5.0 mg/l), Kn (0.5-5.0 mg/l), IBA (0.5-2.0 mg/l), IAA (0.5-2.0 mg/l) and NAA (0.5-2.0 mg/l) were used. After adjusting the pH (5.80), the medium was sterilized in an autoclave at 121°C for 15 min.

Five explants were inoculated in every culture vessel in sterile condition and kept them at $25 \pm 2^\circ\text{C}$ for 4 to 6 weeks in dark condition. After 6 weeks the induced calli were transferred from the dark condition to the another fresh callus inducing medium. After 8 to 10 weeks, suitable calli were chosen and inoculated on MS0, $\frac{1}{2}$ MS0, $\frac{1}{4}$ MS0 and full MS medium with different concentrations of BAP (1.0-5.0 mg/l) either single or in combination with Kn (0.5-2.5 mg/l) and NAA (0.2-1.0 mg/l).

Then regenerated plantlets were sub-cultured on MS medium with different concentrations of phytohormones like BAP and Kn individually and in combinations for shoot induction. Well developed shoots were transferred on the full strength and half strength MS medium with different concentrations of IBA (0.5-2.0 mg/l), IAA (0.5-2.0 mg/l) and NAA (0.5-2.0 mg/l) individually for development of roots. The well rooted plantlets were transferred to pots after acclimatization.

Data were recorded on the basis of callus induction, proliferation shoots and numbers of induced roots as well as the lengths of roots. Mean values were recorded from 10 culture vessels for every treatment and all experiments were repeated in thrice. Five explants were taken in each vessel for this study. Data were subjected to analysis of variance in order to estimate and evaluate the primary effects of media, PGRs, and their actions and interactions on callus formation and *in vitro* regeneration of Noni plantlets. These results were expressed as Mean \pm SE, and the Duncan's Multiple Range Test (DMRT) was used to estimate the variation among the mean values at 5% level of significance.

Results and Discussion

Under this studies leaf segments and cotyledons were used for the callus induction. The highest percentage of callusing from cotyledons (94%) and leaf segments (85%) were found on full strength of MS medium supplemented with 3.0 mg/l 2,4-D (Fig. 1). So, it was found that MS medium supplemented with 3.0 mg/l 2,4-D was appeared to be better medium for callus induction from leaf segments and cotyledons. As explant cotyledons showed better performance than leaf segments. It was noticed that 2,4-D was the effective PGR for *in vitro* callus induction in Noni by other investigators (Irvani et al. 2010, Soorni and Kahrizi 2015, Zaman et al. 2021, Mahood et al. 2022). On the contrary, Rahman and Bari (2012) reported that the combination of 2.0 mg/l BAP + 0.5 mg/l NAA showed the

best performance for callus induction in *Ricinus communis* L. Hassan et al. (2008) reported that the MS basal medium supplemented with 1.5 mg/l BAP + 0.2 mg/l NAA demonstrated the best performance for callus induction in a perennial medicinal herb of *Scoparia dulcis*.

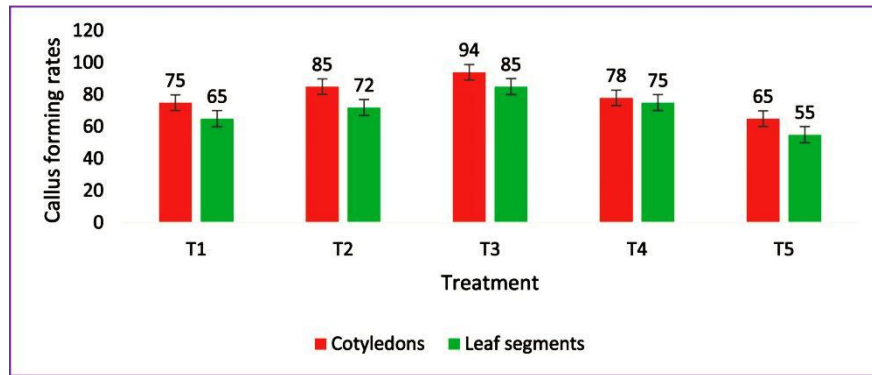


Fig. 1. Callus derived from cotyledons and leaf segments of Noni using different concentration of 2,4-D. T₁ = 1.0 mg/l, T₂ = 2.0 mg/l, T₃ = 3.0 mg/l, T₄ = 4.0 mg/l, T₅ = 5.0 mg/l. Callus developed stages showed by red coloured bar from cotyledons, and green coloured bar from leaf segments.

Calli derived from both explants were sub-cultured and regenerated plantlets were found on full MS (60.71%), ½MS (79.76%) and ¼MS (54.76) (Fig. 2). On the other hand, MS medium in addition with different concentrations of BAP singly and in combination with Kn and NAA did not responded equally. To the best of our knowledge, this is the first report of regeneration of Noni plantlets on ½MS medium derived from leaf and cotyledon producing calli (79.76%).

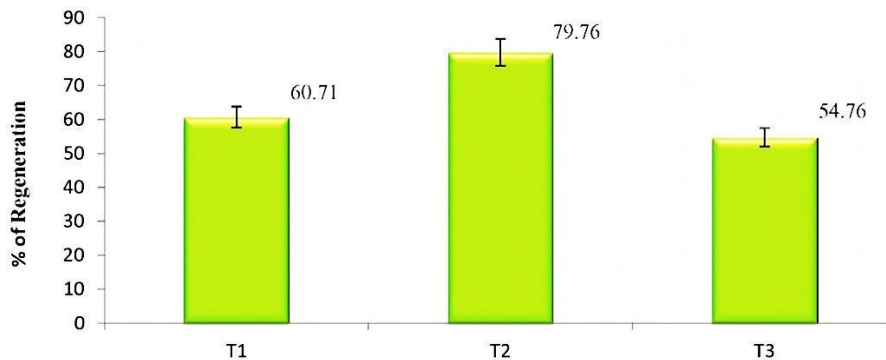


Fig. 2. Plantlets regenerated from cotyledons and leaves producing calli of Noni. T₁ = Full strength of MS, T₂ = ½MS, and T₃ = ¼MS.

MS medium supplemented with different concentrations and combinations of PGRs was used for the proliferation of shoots following sub-culturing of the regenerated plantlets obtained from cotyledon derived calli (Fig. 3a-c). The MS medium

supplemented with 4.0 mg/l BAP (7.9 ± 0.57) and 3.0 mg/l Kn (7.2 ± 0.66) produced the highest number of proliferated shoots individually (3d-f). However, MS medium supplemented with 4.0 mg/l BAP and 2.0 mg/l Kn together were found to be more effective (9.1 ± 0.50) than MS medium supplemented with 4.0 mg/l BAP (7.9 ± 0.57) and 3.0 mg/l Kn (7.2 ± 0.66) individually (Table 1).

Table 1. Effects of different concentrations and combinations of PGRs on shoots induction from cotyledons after 15, 30 and 45 days of culture initiation in Noni.

PGRs (mg/l)		Number of shoots (Mean \pm SE)		
BAP	Kn	After 15 days	After 30 days	After 45 days
1.0	-	1.2 ± 0.13^b	5.4 ± 0.56^b	6.6 ± 0.54^b
2.0	-	1.3 ± 0.15^b	5.7 ± 0.62^b	7.0 ± 0.63^b
3.0	-	1.4 ± 0.16^{ab}	6.0 ± 0.65^{ab}	7.4 ± 0.69^b
4.0	-	1.7 ± 0.18^{ab}	6.4 ± 0.52^{ab}	7.9 ± 0.57^{ab}
5.0	-	1.3 ± 0.15^b	5.7 ± 0.62^b	7.0 ± 0.63^b
-	1.0	1.3 ± 0.15^b	4.9 ± 0.41^b	6.2 ± 0.44^b
-	2.0	1.2 ± 0.13^b	5.1 ± 0.35^b	6.3 ± 0.40^b
-	3.0	1.5 ± 0.18^{ab}	5.9 ± 0.53^{ab}	7.2 ± 0.66^b
-	4.0	1.2 ± 0.13^b	5.2 ± 0.44^b	6.4 ± 0.43^b
-	5.0	1.2 ± 0.13^b	5.1 ± 0.41^b	6.3 ± 0.40^b
1.0	0.5	1.2 ± 0.13^b	5.5 ± 0.58^b	6.7 ± 0.62^b
2.0	1.0	1.3 ± 0.15^b	5.8 ± 0.55^b	7.1 ± 0.60^b
3.0	1.5	1.2 ± 0.13^b	6.1 ± 0.57^{ab}	7.3 ± 0.67^b
4.0	2.0	1.8 ± 0.17^a	7.5 ± 0.43^a	9.1 ± 0.50^a
5.0	2.5	1.2 ± 0.13^b	5.4 ± 0.34^b	6.6 ± 0.40^b
MS0 (Control)		1.2 ± 0.13	1.7 ± 0.21	2.9 ± 0.23

PGRs = Plant growth regulators. Values were expressed Mean \pm SE (Standard Error). Every treatment was repeated in thrice with ten culture vessels. Means in a column with the different letter (indicated in superscript) are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT in SPSS 25)

On the other hand, MS medium supplemented with different concentrations and combinations of PGRs was used for the proliferation of shoots following sub-culturing of the regenerated plantlets obtained from leaf derived callus (Fig. 4b). MS medium supplemented with 4.0 mg/l BAP (5.3 ± 0.30) and 3.0 mg/l Kn (4.9 ± 0.23) showed the highest proliferation of shoots (Fig. 4a-g). When the PGRs used together in MS medium (4.0 mg/l BAP and 2.0 mg/l Kn) showed more effective (5.6 ± 0.34) results than singly using 4.0 mg/l BAP (5.3 ± 0.30) and 3.0 mg/l Kn (4.9 ± 0.23) (Table 2). The present results showed that quite similarity of findings with Gajakosh et al. (2010) and Siddique and Islam (2015) where they used MS medium supplemented with different concentrations of BAP and Kn for inducing multiple shoots.

Table 2. Effects of different concentrations and combinations of PGRs on shoots number regenerated from leaf producing calli after 15, 30 and 45 days of culture initiation of Noni.

PGRs (mg/l)		Number of shoots (Mean \pm SE)		
BAP	Kn	After 15 days	After 30 days	After 45 days
1.0	-	1.1 \pm 0.13 ^c	3.5 \pm 0.22 ^{bcd}	4.7 \pm 0.15 ^{bcd}
2.0	-	1.3 \pm 0.14 ^{bc}	3.7 \pm 0.21 ^{abcd}	5.0 \pm 0.15 ^{abcd}
3.0	-	1.2 \pm 0.13 ^c	3.9 \pm 0.23 ^{abcd}	5.1 \pm 0.18 ^{abcd}
4.0	-	1.6 \pm 0.18 ^{ab}	4.1 \pm 0.28 ^{ab}	5.3 \pm 0.30 ^{ab}
5.0	-	1.4 \pm 0.16 ^{bc}	3.6 \pm 0.34 ^{abcd}	5.0 \pm 0.21 ^{abcd}
-	1.0	1.2 \pm 0.13 ^c	3.2 \pm 0.25 ^d	4.4 \pm 0.22 ^d
-	2.0	1.3 \pm 0.15 ^{bc}	3.3 \pm 0.15 ^{cd}	4.6 \pm 0.22 ^{bcd}
-	3.0	1.5 \pm 0.19 ^{abc}	3.7 \pm 0.30 ^{abcd}	4.9 \pm 0.23 ^{abcd}
-	4.0	1.3 \pm 0.13 ^c	3.5 \pm 0.22 ^{bcd}	4.7 \pm 0.15 ^{bcd}
-	5.0	1.1 \pm 0.10 ^c	3.4 \pm 0.16 ^{bcd}	4.5 \pm 0.17 ^{cd}
1.0	0.5	1.3 \pm 0.15 ^{bc}	3.6 \pm 0.22 ^{abcd}	4.9 \pm 0.18 ^{abcd}
2.0	1.0	1.2 \pm 0.13 ^c	3.8 \pm 0.13 ^{abcd}	5.0 \pm 0.15 ^{abcd}
3.0	1.5	1.2 \pm 0.13 ^c	4.0 \pm 0.21 ^{abc}	5.2 \pm 0.33 ^{abc}
4.0	2.0	1.8 \pm 0.17 ^a	4.3 \pm 0.21 ^a	5.6 \pm 0.34 ^a
5.0	2.5	1.3 \pm 0.15 ^{bc}	4.2 \pm 0.13 ^{ab}	5.5 \pm 0.17 ^a
MS0 (Control)		1.1 \pm 0.18	1.3 \pm 0.15	2.4 \pm 0.27

PGRs = Plant growth regulators. Values were expressed Mean \pm SE (Standard Error). Each treatment was repeated in thrice with ten culture vessels. Means in a column with the different letter (indicated in superscript) are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT, by SPSS 25).

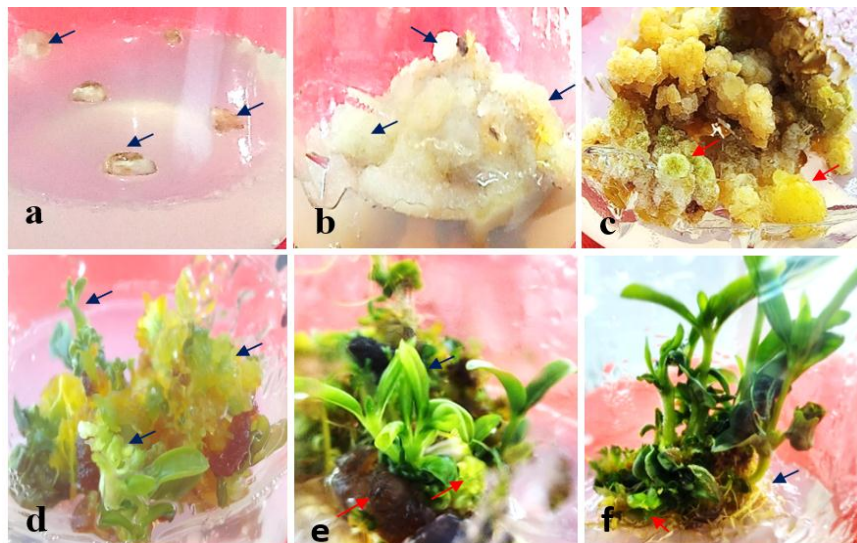


Fig. 3(a-f). Callus induction and regeneration of plantlets from Noni cotyledons: (a) initiation of cotyledons on culture medium, (b) formation, changing color and proliferation of callus after three weeks of culture initiation, (c) start to regeneration of plantlets after six weeks, (d) proliferation of regenerated plantlets after eight weeks of culture initiation, (e) increasing shoots and development, and (f) completely plantlets with multiple shoots and well developed roots after twelve weeks.

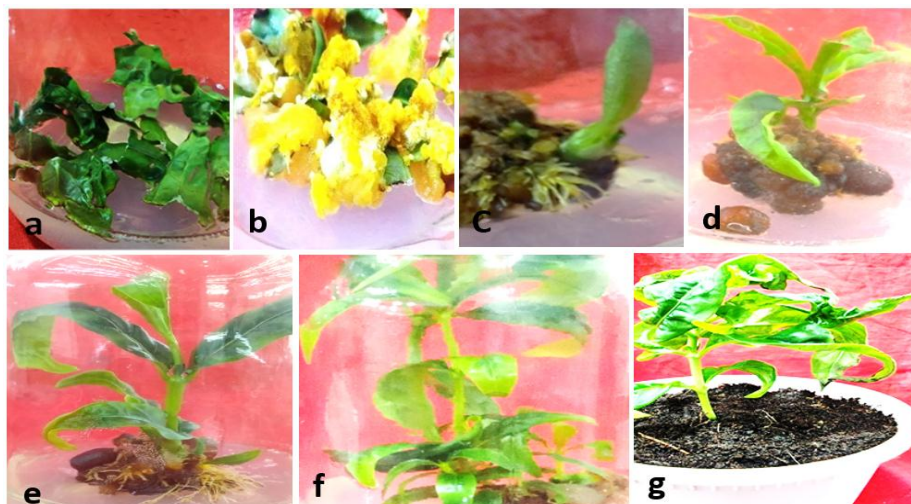


Fig. 4(a-g). Callus induction and regeneration of plantlets from Noni leaves: (a) leaf segments on medium after one week of culture initiation, (b) callus formation after four weeks of culture initiation, (c) regeneration of seedling from callus after 8 weeks, (d) growth and development of shoot after 9 weeks, (e) root formation and development of shoot after ten weeks, (f) well developed plantlets with root and shoots after twelve weeks, and (g) mature and well developed plantlet transferred into a plastic pot.

Table 3. Effects of different concentrations and combinations of PGRs on increasing sub-cultured shoot length, regenerated from callus after 15, 30 and 45 days of culture initiation in Noni.

PGRs (mg/l)		Plant height in cm (Mean \pm SE)		
BAP	Kn	Length of shoots after 15 days	Length of shoots after 30 days	Length of shoots after 45 days
1.0	-	1.59 \pm 0.10 ^{ab}	1.96 \pm 0.14 ^{abc}	3.55 \pm 0.15 ^{bc}
2.0	-	1.58 \pm 0.10 ^{ab}	1.99 \pm 0.12 ^{abc}	3.57 \pm 0.12 ^{bc}
3.0	-	1.58 \pm 0.10 ^{ab}	2.01 \pm 0.12 ^{abc}	3.59 \pm 0.12 ^{bc}
4.0	-	1.73 \pm 0.03 ^{ab}	2.14 \pm 0.12 ^{ab}	3.84 \pm 0.19 ^{ab}
5.0	-	1.56 \pm 0.07 ^{ab}	1.99 \pm 0.10 ^{abc}	3.55 \pm 0.07 ^{bc}
-	1.0	1.53 \pm 0.06 ^{ab}	1.74 \pm 0.12 ^c	3.27 \pm 0.13 ^c
-	2.0	1.53 \pm 0.06 ^{ab}	1.78 \pm 0.12 ^{bc}	3.31 \pm 0.08 ^c
-	3.0	1.64 \pm 0.08 ^{ab}	2.04 \pm 0.15 ^{abc}	3.55 \pm 0.07 ^{abc}
-	4.0	1.53 \pm 0.06 ^{ab}	1.78 \pm 0.11 ^{bc}	3.31 \pm 0.11 ^c
-	5.0	1.51 \pm 0.06 ^b	1.77 \pm 0.11 ^{bc}	3.28 \pm 0.11 ^c
1.0	0.5	1.52 \pm 0.06 ^{ab}	1.97 \pm 0.11 ^{abc}	3.49 \pm 0.13 ^{bc}
2.0	1.0	1.52 \pm 0.06 ^{ab}	1.99 \pm 0.10 ^{abc}	3.51 \pm 0.09 ^{bc}
3.0	1.5	1.51 \pm 0.06 ^b	2.02 \pm 0.10 ^{abc}	3.53 \pm 0.10 ^{bc}
4.0	2.0	1.76 \pm 0.05 ^a	2.21 \pm 0.12 ^a	4.02 \pm 0.22 ^a
5.0	2.5	1.52 \pm 0.06 ^{ab}	2.01 \pm 0.10 ^{abc}	3.53 \pm 0.10 ^{bc}
MS0 (Control)		1.52 \pm 0.06	1.54 \pm 0.09	3.06 \pm 0.11

PGRs = Plant growth regulators. Values were expressed Mean \pm SE (Standard Error). Every treatment was repeated in thrice with ten culture vessels. Means in a column with the different letter (indicated in superscript) are significantly different ($p < 0.05$) according to according to Duncan's Multiple Range Test (DMRT, by SPSS 25).

Over all cotyledons derived calli showed more effective results on regeneration (9.1 ± 0.50) than leaf segments derived calli (5.6 ± 0.34) in obtaining more number of shoots in a specific time period (Table 1 and 2; Fig. 1 and 2). The shoots without roots were transferred on MS medium supplemented with different concentrations and combinations of PGRs (BAP, Kn). It was observed that MS medium supplemented with 4.0 mg/l BAP and 2.0 mg/l Kn together was the most effective for the growth and development of shoots (4.02 ± 0.22), (Table 3). Then full strength of MS and $\frac{1}{2}$ MS medium supplemented with different concentrations of PGRs like IBA (0.5-2.0 mg/l), IAA (0.5-2.0 mg/l) and NAA (0.5-2.0 mg/l) were used separately to determine the formation of roots and the growth of roots of regenerated plantlets (Table 4).

Table 4. Effects of MS medium with different concentrations of PGRs (IBA, IAA, NAA) on forming roots and increasing length of roots after 45 days of culture initiation in Noni.

Media	PGRs			Number of roots (Mean \pm SE)	Length of roots in cm (Mean \pm SE)
	IBA	IAA	NAA		
$\frac{1}{2}$ MS	0.5	-	-	79.80 \pm 0.84 ^b	1.58 \pm 0.04 ^{bcd}
	1.0	-	-	80.80 \pm 1.55 ^b	1.63 \pm 0.03 ^{abc}
	1.5	-	-	84.50 \pm 2.66 ^b	1.75 \pm 0.05 ^a
	2.0	-	-	93.80 \pm 1.78 ^a	1.76 \pm 0.03 ^a
Full MS	0.5	-	-	80.30 \pm 0.71 ^b	1.69 \pm 0.04 ^{ab}
	1.0	-	-	84.10 \pm 0.50 ^b	1.57 \pm 0.03 ^{bcd}
	1.5	-	-	86.10 \pm 2.11 ^b	1.58 \pm 0.03 ^{bcd}
	2.0	-	-	85.70 \pm 2.22 ^b	1.59 \pm 0.03 ^{bcd}
$\frac{1}{2}$ MS	-	0.5	-	57.20 \pm 2.67 ^{fg}	1.52 \pm 0.04 ^{cde}
	-	1.0	-	61.80 \pm 3.36 ^{def}	1.55 \pm 0.02 ^{bcd}
	-	1.5	-	65.30 \pm 2.49 ^{bcd}	1.55 \pm 0.03 ^{bcd}
	-	2.0	-	71.20 \pm 1.83 ^c	1.62 \pm 0.03 ^{abcd}
Full MS	-	0.5	-	63.90 \pm 0.97 ^{cdef}	1.42 \pm 0.06 ^{efg}
	-	1.0	-	68.90 \pm 1.53 ^{bc}	1.46 \pm 0.05 ^{defg}
	-	1.5	-	62.00 \pm 2.69 ^{cdef}	1.47 \pm 0.05 ^{cdef}
	-	2.0	-	58.20 \pm 2.54 ^{fg}	1.49 \pm 0.04 ^{cdef}
$\frac{1}{2}$ MS	-	-	0.5	59.20 \pm 2.14 ^{efg}	1.31 \pm 0.05 ^{gh}
	-	-	1.0	62.60 \pm 2.88 ^{cdef}	1.42 \pm 0.06 ^{efg}
	-	-	1.5	62.40 \pm 1.72 ^{cdef}	1.34 \pm 0.06 ^{fgh}
	-	-	2.0	66.90 \pm 2.01 ^{bcd}	1.36 \pm 0.06 ^{fgh}
Full MS	-	-	0.5	45.300 \pm 2.71 ^j	1.33 \pm 0.07 ^{fgh}
	-	-	1.0	50.70 \pm 2.02 ^{ij}	1.36 \pm 0.08 ^{fgh}
	-	-	1.5	45.60 \pm 1.92 ⁱ	1.35 \pm 0.05 ^{fgh}
	-	-	2.0	55.10 \pm 1.15 ^{gh}	1.24 \pm 0.03 ^h
MS0 (Control)			38.5 \pm 0.55	1.1 \pm 0.03	

PGRs = Plant growth regulators. Values were expressed Mean \pm SE (Standard Error). Every treatment was repeated in thrice with ten culture vessels. Means in a column with the different letter (indicated in superscript) are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT by SPSS 25).

From this experiment it was noticed that ½MS medium supplemented with 2.0 mg/l IBA was the most effective for the regeneration and development of roots and the effectiveness of IBA were also proved in some medicinal and tree plants for rooting (Mamun et al. 2002, Baul et al. 2010, Amri 2012, Sen et al. 2014). On the contrary Rahe et al. (2020) noticed that ½MS medium supplemented with 0.25 mg/l IBA was found to be the best medium for root formation in *Bacopa monnieri* L. Biswas et al. (2007) showed that ½MS medium supplemented with 0.50 mg/l IBA was also found to be the best for root formation in *Aristolochia tagala* Champ. Akter et al. (2020) also noticed that ½MS with 0.5 mg/l NAA is the best medium in *Ocimum americanum* for root formation. Here it was observed that we can produce more plantlets in a short time through this method for the sustainable development of this plant.

The present study successfully demonstrated that both leaf segments (85%) and cotyledons (94%) of *Morinda citrifolia* are responsive explants for *in vitro* callus induction and plant regeneration (79.76%). Among the two, cotyledon explants exhibited a higher frequency of callus formation and subsequent shoot regeneration, indicating their higher morphogenic potential. The choice of plant growth regulators, especially the combinations and concentrations of auxins and cytokinins, played a crucial role in optimizing callus induction, regeneration of plantlets, shoot proliferation (9.1 ± 0.50) and shoot development (4.02 ± 0.22 cm). These findings provide a basic protocol for an efficient micro-propagation studies in Noni, contributing to its conservation and large-scale propagation.

Acknowledgements

The authors would like to express their appreciation to the Ministry of Education, Government of the People's Republic of Bangladesh, for approving study leave to MWH. Special thanks to the UGC and IBS, RU for granting fellowship for this study. The authors are grateful to the Plant Biotechnology and Genetic Engineering Laboratory, Institute of Biological Sciences, University of Rajshahi for providing research and field facilities.

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(Manuscript received on 12 October, 2025; revised on 20 November, 2025)