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RESEARCH ARTICLE

In Vitro Micropropagation of Noni (*Morinda citrifolia* L.) through Nodal Segments and Shoot Tips Culture

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Abstract

The present study was conducted to develop an efficient *in vitro* micropropagation protocol for Noni, an important medicinal plant, using nodal segments and shoot tips. In this study the Murashige and Skoog medium was considered with various concentrations and combinations of BAP and Kinetin. For the evaluation of shoot and root induction, their proliferation and the increased number of shoots and roots, various auxins (IBA, IAA and NAA) were used. The highest length of shoots (5.2±0.15 cm) and the highest number of shoots (3.2±0.13) were found on MS medium supplemented with BAP (4.0 mg/l) and Kinetin (2.0 mg/l) from nodal segments. In this case a data were recorded after 12 weeks of culture initiation. After 42 days, the highest number of roots per shoot (32.5±0.40), the highest length of roots (1.9± 0.07 cm) and percent of shoots (86.0%) induced roots were recorded on half strength (½) MS medium containing IBA (2.0 mg/l). The aftermaths of this study showed that nodal segments were better explants than shoot tips for the *in vitro* micropropagation of Noni.

Keywords: *In vitro* micropropagation, *Morinda citrifolia*, Nodal segments, Noni, PGRs, Shoot tips.

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Introduction

Morinda citrifolia L. is a fruit-bearing, tropical, evergreen shrub with therapeutic properties. The species, sometimes known as Indian mulberry or Noni in Bangladesh, is a member of the Rubiaceae family (Afolabi et al. 2020). This evergreen tree is native to Polynesia and grows throughout the world's tropical and subtropical zones (Elakkuvan and Manivannan 2015). It can withstand brackish water stagnation and high soil salinity (Sunder et al. 2016). Tropical regions of eastern Asia and Australia have long used the tropical plant *M. citrifolia* for therapeutic purposes. The treatment of excruciating inflammatory diseases such as arthritis is one of its most popular applications (Basar et al. 2010). It is a small plant or tall shrub that grows in both wet and dry environments, in acidic and alkaline conditions, as well as in barren soil (Shekhawat et al. 2015). Many chemical compounds that are necessary for human life can be found in this medicinal plant.

It is in fact advantageous to use methods of tissue culture for *in vitro* proliferation and extraction of significant chemical substances (Hossain et al. 2009, Gajakosh et al. 2010, Paul et al. 2013). The plant has received the recognition that it deserves recently as an important source of anthraquinones and neuro-pharmaceuticals. Numerous biochemical compounds with curative properties, including antioxidant, antibacterial, antiviral, and anticancer activities are found in *M. citrifolia*, according to modern pharmaceutical analysis. It has been shown that approximately 160 important phytochemicals include anthraquinones, organic acids, and alkaloids being the three main types of compounds remain in this valuable plant (Sreeranjini and Siril 2014). Traditional propagation by seeds is challenging, and the resulting offspring are not true to form. Therefore, superior genotypes are rapidly multiplied through in vitro cultivation (Mamun et al. 2002, Elakkuvan and Manivannan 2015). Noni has growing market demand due to its health benefits and unique flavors. On the other hand, scalable plant production helps

meet commercial demand for fruits, extracts, or nutraceutical products, supporting local farmers and agri-businesses. The fruits of Noni contain bioactive compounds with anti-cancer, anti-microbial, and anti-diabetic properties that can support the development of natural health supplements, creating alternatives to synthetic drugs. Noni juice marketed for commercial use and encapsulated noni powder have gained popularity in Asia, North America, and Europe (Srinivasahan and Durairaj 2014). Noni fruit juice has hepatoprotective activity (Nayak et al. 2011). Noni plants (*M. citrifolia*) are rich in medicinal and nutritional properties but are not widely cultivated in Bangladesh.

Micropropagation allows year-round, large-scale plant production to meet both local and export demands. It also leads to higher yield and disease resistance, boosting farmer income, encourages biotechnology research in Bangladesh, and enhances capabilities in plant tissue culture, phytochemistry, and functional food development. *In vitro* micropropagation helps rapid mass production of high-quality, disease-free planting materials, conservation of rare or underutilized genetic resources and ensuring uniformity and availability of this elite plant variety.

Materials and Methods

This research was conducted to establish the *in vitro* micropropagation methods for Noni, aiming to facilitate extensive cultivation of this plant throughout the country. Mature and fresh Noni stems with shoot tips were collected from the research field of the Medicinal Plants Germplasm, Institute of Biological Sciences, University of Rajshahi to conduct this experiment. Then the nodal segments and shoot tips (1-1.5 cm) were cut down from the collected stems with a surgical blade. The nodal segments and shoot tips were washed with running tap water for 30 minutes and then treated with 35 to 40% sodium hypochlorite (NaOCl), Tween-20 and liquid detergent for 20 minutes. Then the explants were taken inside the laminar air flow chamber under sterile conditions and washed with the double-distilled water to remove the foam that had formed in the conical flask at the time of sterilizing the explants. After that, the explants were treated with 100% ethanol for 30 seconds and 70% ethanol for 1 minute and then washed the explant with double distilled water to remove the traces of ethanol for 3 to 4 times. Before that the MS medium was made with different concentrations and combinations of different PGRs. The medium was adjusted to pH 5.7±0.1 before autoclaving at 121°C for 20 minutes.

The sterilized nodal segments and shoot tips (1 to 1.5 cm) were inoculated in culture vessels containing MS medium with different concentrations of BAP (1.0, 2.0, 3.0, 4.0, 5.0 mg/l) and Kinetin (1.0, 2.0, 3.0, 4.0, 5.0 mg/l) in single and different concentrations BAP (1.0, 2.0, 3.0, 4.0, 5.0 mg/l) and Kinetin (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) in combined and kept them in the growth chamber at 25± 2°C. After 2 weeks, shoots were induced from the inoculated explants. After that the shoots were grown on MS medium with above mentioned BAP and kinetin in single and combined and the shoot height and number were measured after 4, 8, and 12 weeks. After that well developed shoots without roots were inoculated on the full strength and half strength MS media with different concentrations of IBA, IAA and NAA (0.5- 2.5 mg/l). Rooting percentage, number of roots per shoot, and root length were recorded after 21 and 42 days. Plantlets with strong roots were then transferred to plastic pots with sand and sterilized garden soil and kept in the shade house of the IBSc research field, University of Rajshahi, for acclimatization.

Data about the length of shoots and roots, number of shoots and roots in different culture media in the above mentioned duration were recorded. Every experiment was conducted three times with ten culture medium vessels and the mean values of thirty culture vessels were obtained for each treatment. To evaluate the main impact of media, PGRs, and their action and interaction on the development of shoots and roots of Noni plantlets, data were subjected to analysis of variance. The Duncan's Multiple Range Test (DMRT) was utilized to separate the means at a 5% level of significance when the data required to be presented as means± SE.

Results and Discussion

In this experiment nodal segments and shoot tips were used for *in vitro* micropropagation. Explants were inoculated on MS medium with above mentioned different concentrations of BAP and Kinetin in single and combined to observe the growth and development of shoots and also their proliferations after 4, 8 and 12 weeks respectively.

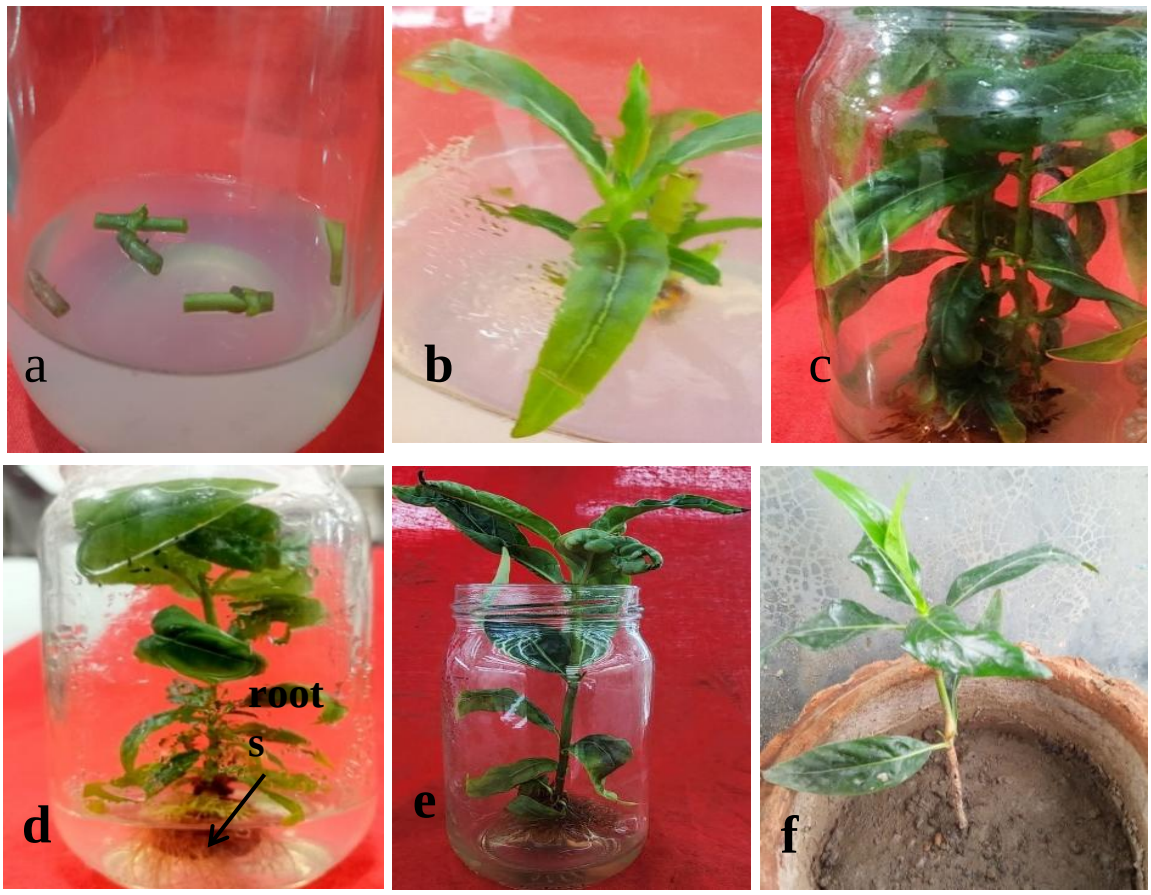

Fig. 1(a-f): *In vitro* micropropagation of Noni from nodal segments. a) Initiation of nodal segments, b) induction and development of shoots, c) formation of multiple shoots, d) well rooted plant, e) acclimatization of plant, f) plants grown in pot.

In this study the highest length of shoots (5.2±0.15 cm) and the highest number of shoots (3.2±0.13, Table 1) for nodal segment explants was found on MS medium supplemented with BAP (4.0 mg/l) and Kinetin (2.0 mg/l) in combination. On the other hand, the highest length of shoots (4.7±0.15 cm) and the highest number of shoots (2.3±0.15, Table 1) for shoot tip explants was found on MS medium supplemented with BAP (4.0 mg/l) and Kinetin (2.0 mg/l) in combination.

In comparisons between the above mentioned explants on the same medium nodal segments were better for *in vitro* micropropagation of Noni. According to the findings of other researchers only the BAP 2.0 mg/l was given the best results in this case in the other plants (Pawar et al. 2015, Ugandhar et al. 2012).

Table 1: Effects of MS medium with different concentrations and combinations of PGRs on shoots development after 12 weeks of culture initiation in Noni.

PGRs (mg/l)	Length (cm) of shoots (Mean±SE)						Increased shoot numbers after 12 w	
	4 w		8 w		12 w			
MS + BAP	NS	ST	NS	ST	NS	ST	NS	ST
1.0	1.4±0.16^{a}	1.3±0.15^{a}	2.5±0.17^{b}	2.2±0.13^{b}	3.8±0.13^{bc}	3.5±0.22^{bc}	1.3±0.15^{b}	1.1± 0.10^{b}
2.0	1.5±0.17^{a}	1.4±0.16^{a}	2.7±0.15^{ab}	2.4±0.16^{ab}	3.9±0.10^{bc}	3.7±0.15^{ab}	1.4±0.16^{ab}	1.2±0.13^{ab}
3.0	1.6±0.16^{a}	1.5±0.17^{a}	2.9±0.10^{a}	2.5±0.17^{ab}	4.2±0.13^{ab}	3.9±0.10^{ab}	1.6±0.16^{ab}	1.4±0.16^{ab}
4.0	1.8±0.13^{a}	1.7±0.15^{a}	3.1±0.10^{a}	2.8±0.13^{a}	4.5±0.17^{a}	4.1±0.18^{a}	1.8±0.13^{a}	1.6±0.15^{a}
5.0	1.4±0.16^{a}	1.3±0.15^{a}	2.4±0.16^{b}	2.3±0.15^{b}	3.6±0.22^{c}	3.2±0.13^{c}	1.5±0.17^{ab}	1.3±0.15^{ab}
MS + Kin								
1.0	1.2±0.13^{b}	1.1±0.10^{a}	2.1±0.18^{b}	1.9±0.18^{b}	3.2±0.13^{a}	2.9±0.10^{c}	1.2±0.13^{b}	1.1±0.10^{a}
2.0	1.4±0.16^{ab}	1.3±0.15^{a}	2.2±0.20^{b}	2.0±0.15^{b}	3.7±0.15^{abc}	3.5±0.22^{ab}	1.4±0.16^{a}	1.2±0.13^{a}
3.0	1.7±0.15^{a}	1.5±0.17^{a}	2.8±0.13^{a}	2.6±0.16^{a}	4.2±0.20^{a}	3.9±0.10^{a}	1.5±0.17^{a}	1.3±0.15^{a}
4.0	1.4±0.16 ^{ab}	1.3±0.15^{a}	2.5±0.17^{ab}	2.5±0.17^{a}	3.8±0.20^{ab}	3.6±0.22^{ab}	1.1±0.10^{b}	1.2±0.13^{a}
5.0	1.4±0.16^{ab}	1.2±0.13^{a}	2.3±0.15^{ab}	2.2±0.13^{ab}	3.5±0.22^{bc}	3.2±0.13^{bc}	1.2±0.13^{b}	1.2±0.13^{a}
MS + BAP + Kin								
1.0+0.5	1.6±0.16^{a}	1.4±0.16^{a}	2.5±017^{c}	2.3±0.15^{c}	4.1±0.18^{b}	3.9±0.10^{b}	2.3±0.15^{c}	1.5±0.17^{b}
2.0+1.0	1.7±0.15^{a}	1.5±0.17^{a}	2.8±0.13^{bc}	2.6±0.16^{bc}	4.3±0.15^{b}	4.1±0.18^{b}	2.5±0.17^{bc}	1.9±0.18^{ab}
3.0+1.5	1.6±0.16^{a}	1.6±0.16^{a}	3.1±0.10^{b}	2.8±0.13^{b}	4.5±0.17^{b}	4.2±0.13b	2.6±0.16^{bc}	2.0±0.15^{a}
4.0+2.0	1.9±0.07^{a}	1.8±0.11^{a}	3.5±0.22^{a}	3.2±0.13^{a}	5.2±0.15^{a}	4.7±0.15^{a}	3.2±0.13^{a}	2.3±0.15^{a}
5.0+2.5	1.7±0.15^{a}	1.5±0.17^{a}	2.7±0.26^{bc}	2.4±0.16^{bc}	4.2±0.25^{b}	3.9±0.10^{b}	2.8±0.13^{b}	2.1±0.18^{a}
MS0 (Control)	0.90±0.04	0.87±0.04	1.4±0.16	1.3±0.15	2.3±0.21	2.1±0.18	0.94±0.03	0.92±0.04

PGRs = Plant growth regulators; W= Weeks after culture initiation; NS = Nodal segments; ST = Shoot tips; Values were expressed in Mean±SE (Standard Error). Every treatment was repeated in three times with ten culture vessels. Means in a column with the different letter (superscript) are significantly different according to least significant difference (LSD) at $p<0.05$ level.

Then properly developed shoots without roots were inoculated on MS0 and ½ MS0 media with above mentioned concentrations of IBA, IAA and NAA in single for *in vitro* root formation and measured the percent of shoots induced roots. Number of roots per shoot and the length of the roots of every plantlet were also measured after 21 days and 42 days respectively.

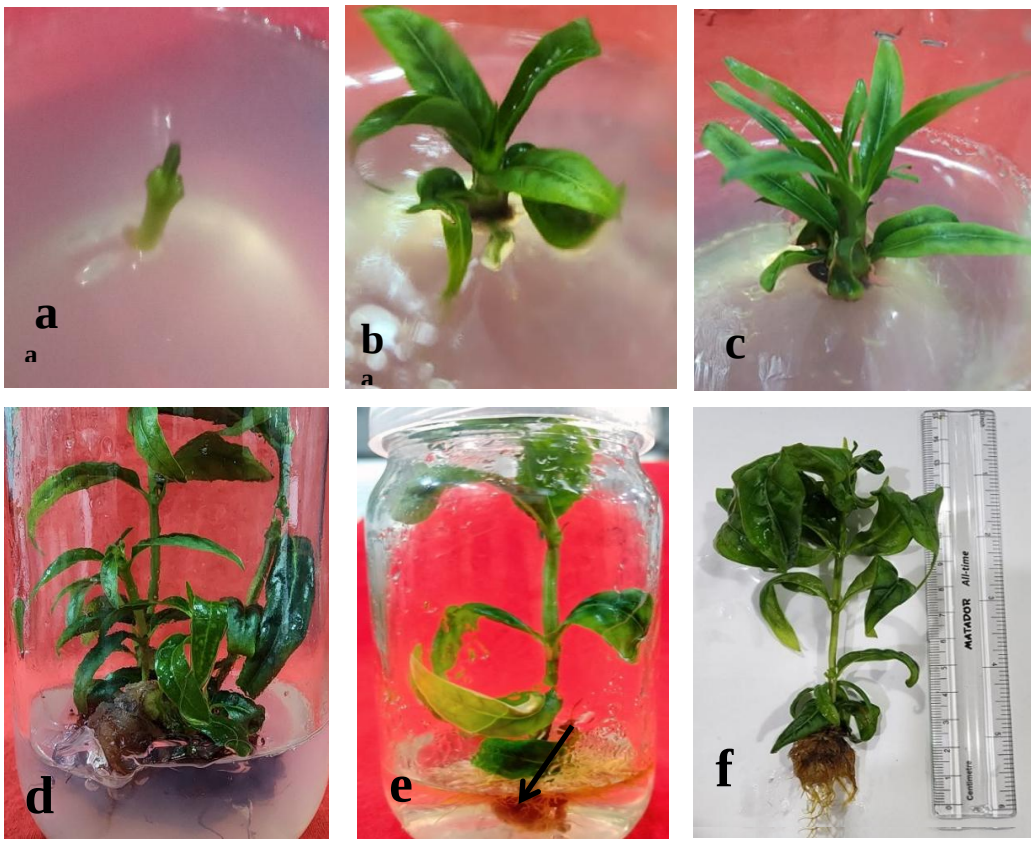

Fig. 1 (a-f): *In vitro* micropropagation of Noni from shoot tips. a) Initiation of shoot tip, b-c) Induction and development of shoots, d) Formation of multiple shoots, e) Well rooted shoots, f) Measurement of shoot heights with roots.

Table 2: Effects of MS and ½MS culture media with different concentrations of PGRs (IBA, IAA, NAA) on percent of shoots roots and root development after 21 and 42 days of culture initiation in Noni.

PGRs (mg/l)	% of shoots induced roots	Roots per shoot (days after inoculation, Mean±SE)		Length (cm) of roots (days after inoculation, Mean±SE)	
		Days			
MS + IBA	-	21	42	21	42
0.5	64.0	12.8±0.25^{b}	25.9±0.38^{a}	0.82±0.02^{b}	1.4±0.16^{a}
1.0	65.0	12.9±0.28^{b}	26.7±0.50^{a}	0.84±0.02^{b}	1.5±0.17^{a}
1.5	73.5	13.2±0.20^{b}	27.1±0.43^{a}	0.85±0.02^{b}	1.6±0.16^{a}
2.0	82.5	15.2±0.36^{a}	31.3±0.72^{a}	0.96±0.05^{a}	1.8±0.13^{a}
2.5	64.0	12.7±0.21^{b}	25.7±0.40^{a}	0.84±0.02^{b}	1.3±0.15^{a}
MS + IAA					
0.5	38.5	11.4±0.31^{c}	23.2±0.53^{a}	0.74±0.02^{b}	1.2±0.13a
1.0	41.5	11.7±0.26^{bc}	24.3±0.56^{a}	0.79±0.03^{ab}	1.4±0.16^{a}
1.5	49.5	12.6±0.22^{a}	26.1±0.41^{a}	0.83±0.03^{a}	1.6±0.16^{a}
2.0	44.0	12.1±0.18^{abc}	25.1±0.35^{a}	0.81±0.03^{ab}	1.4±0.16^{a}
2.5	39.5	12.2±0.20^{ab}	24.6±0.43^{a}	0.80±0.03^{ab}	1.3±0.15^{a}
MS + NAA					
0.5	33.5	10.9±0.23^{c}	22.2±0.55^{a}	0.71±0.03^{b}	1.1±0.10^{a}
1.0	36.5	11.8±0.20^{b}	23.2±0.45^{a}	0.72±0.04^{b}	1.3±0.15^{a}
1.5	42.0	12.5±0.22^{a}	25.2±0.52^{a}	0.79±0.02^{ab}	1.4±0.16^{a}
2.0	46.5	11.6±0.31^{bc}	23.6±0.73^{a}	0.84±0.02^{a}	1.5±0.17^{a}
2.5	38.0	11.2±0.20^{bc}	22.9±0.31^{a}	0.78±0.02^{ab}	1.2±0.13^{a}

Contd. Table 2

½ MS + IBA					
0.5	70.5	13.3±0.21^{b}	26.2±0.29^{a}	0.85±0.02^{b}	1.5±0.17^{b}
1.0	73.0	13.6±0.27^{b}	26.9±0.41^{a}	0.87±0.02^{b}	1.7±0.15^{b}
1.5	78.0	13.8±0.33^{b}	27.3±0.37^{a}	0.89±0.03^{b}	1.8±0.13^{a}
2.0	86.0	16.2±0.39^{a}	32.5±0.40^{a}	0.98±0.05^{a}	1.9±0.07^{a}
2.5	74.0	13.2±0.25^{b}	25.8±0.36^{a}	0.84±0.02^{b}	1.3±0.15^{c}
½MS + IAA					
0.5	42.5	11.6±0.27^{b}	23.4±0.45^{a}	0.76±0.02^{b}	1.3±0.15^{a}
1.0	46.5	11.9±0.28^{b}	24.5±0.50^{a}	0.81±0.03^{ab}	1.4±0.16^{a}
1.5	52.5	13.1±0.35^{a}	26.6±0.48^{a}	0.85±0.02^{a}	1.7±0.15^{a}
2.0	42.0	12.2±0.13^{b}	25.4±0.34^{a}	0.81±0.03^{ab}	1.3±0.15^{a}
2.5	43.0	12.3±0.21^{b}	25.1±0.31^{a}	0.80±0.03^{ab}	1.4±0.16^{a}
½MS + NAA					
0.5	37.5	11.3±0.21^{c}	22.5±0.45^{a}	0.74±0.02^{c}	1.2±0.13^{a}
1.0	38.5	11.7±0.21^{bc}	24.1±0.35^{a}	0.75±0.03^{bc}	1.3±0.15^{a}
1.5	41.0	12.3±0.26^{ab}	25.7±0.40^{a}	0.80±0.02^{bc}	1.4±0.15^{a}
2.0	47.5	12.5±0.34^{a}	23.9±0.48^{a}	0.86±0.02^{a}	1.6±0.16^{a}
2.5	37	11.3±0.15^{c}	23.1±0.31^{a}	0.81±0.02^{ab}	1.2±0.13^{a}
MS0 (Control)	21	5.1±0.18	7.5±0.27	0.66±0.06	0.75±0.05

PGRs = Plant growth regulators; Values were expressed in Mean±SE (Standard Error). Every treatment was repeated in three times with ten culture vessels. Means in a column with the different letter (superscript) are significantly different according to least significant difference (LSD) at $p<0.05$ level.

Here, the highest percentage of shoots induced roots (86.0%), the highest number of roots (32.5±0.40) and the highest length of roots (1.9±0.07 cm) per plantlet were found on ½MS medium with IBA (2.0 mg/l) after 42 days in this experiment. According to the findings of some researchers IBA in different concentrations was the best PGR for root induction, increasing the length of roots in plantlets in the *in vitro* condition (Anis et al. 2003, Rani et al. 2006, Pérez-Tornero et al. 2010, Boonerjee et al. 2013, Parvin et al. 2013, Sarkar et al. 2021, Bhattacharjee and Islam 2014). On the contrary Das et al. (2013) reported that IAA was the best PGR for rooting in *Dioscorea alata*.

Conclusion

Now-a-days micropropagation is the best method for the rapid reproduction of many plants which are not able to produce seedlings from seeds due to the lack of germination viability or germination takes much longer than usual or not propagate with the help of vegetative reproduction method in a short time . We have done this experiment on Noni (*Morinda citrifolia* L.) and got the best result for induction of shoots, increased of shoot length (5.2±0.15) , increased number of shoots (3.2±0.13) on MS medium with BAP (4.0 mg/l) and Kinetin (2.0 mg/l) using nodal segments as explants. Highest no of roots (32.5±0.40) and root length (1.9±0.07) were found on MS medium with IBA (2.0 mg/l). We have established the suitable methods on acclimatization of propagated plants through this experiment. So, the achievement of this experiment is to establish the *in vitro* micropropagation method to enhance the propagation of Noni to meet demand of this plant in medicinal and commercial purposes.

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Author's contribution: SMSI designed the experiment, supervised the study, and corrected the manuscript. MW conducted experiments, collected data statistical analysis procedure and completed the draft of the article.

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Data availability: Data generated in the study are reported in the manuscript, and unprocessed data is with the corresponding author and available upon request.

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