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Research Article

INFLUENCE OF VARIOUS CONCENTRATIONS OF PLANT GROWTH REGULATORS ON IN VITRO NICKED SEED GERMINATION OF MORINDA CITRIFOLIA L.

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ABSTRACT
A study was carried out to investigate the effect of MS media supplemented with various concentrations of plant growth regulators (PGRs) in combination with nicking on the *in vitro* germination of Noni *(Morinda citrifolia L.).* Freshly extracted Noni seeds were nicked first and then treated with various concentrations (0.1- 2mg/l) of Gibberellic acid (GA_3), Indole-3-Butyric acid (IBA), Naphthalene-1-acetic acid (NAA), 6-Benzyl-amino-purine (BAP), Kinetin and nicking alone along with control (MS media without PGRs). The highest germination percent, shoot length, root length, fresh and dry weights were recorded in seeds treated with MS media fortified with Gibberellic acid at 1mg/l concentration (97.37%, 8.67 and 5.78cm, 1.512 and 0.192g); followed by MS media with 1.75mg/l Indole-3- Butyric acid (86.15%, 6.64 and 5.61cm, 1.498 and 0.167g); Naphthalene-1-acetic acid (75.44%, 5.56 and 3.91cm, 1.410 and 0.0.162g); 6-Benzyl-amino-purine (69.20%, 5.93 and 2.56cm, 1.401 and 0.158g ; and Kinetin (56.66%, 4.94 and 2.49cm, 1.397 and 0.154g) at MS media with 2mg/l concentration; followed by nicking alone (32.26%, 1.70 and 1.48cm,1.060 and 0.025g). Whereas poor response was observed in control with low germination percent (16.40%), shoot length (1.55cm), root length (1.01cm), fresh weight (1.025g) and dry weight (0.015g).

Key Words: *Morinda Citrifolia L, Nicking, in vitro Seed Germination, MS Media and Plant Growth Regulators*

INTRODUCTION
Morinda citrifolia L commonly called as Noni is one of the most important medicinal plants belongs to the family Rubiaceae. Its various vernacular names are: ‘‘Indian mulberry’’, ‘‘nuna’’, or ‘‘ach’’ on the Indian subcontinent, ‘‘mengkudu’’ in Malaysia, ‘‘nhau’’ in Southeast Asia, ‘‘painkiller bush’’ in the Caribbean, or ‘‘cheese fruit’’ in Australia**.** Noni is native from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, Central and northern South America. *Morinda citrifolia* is a bush or small tree, 3–10m tall, with abundant wide elliptical leaves (5–17 cm length, 10–40 cm width). The small tubular white flowers are grouped together and inserted on the peduncle. The petioles leave ring-like marks on the stalks and the corolla is greenish white (Cardon, 2003). The Noni fruit (3–10 cm length, 3–6 cm width) is oval and fleshy with an embossed appearance. It is slightly wrinkly, semi-translucent, and ranges in colour from green to yellow, to almost white at the time of picking. It is covered with small reddish-brown buds containing the seeds. The ripe fruit exhales a strong butyric acid-like rancid smell (Morton, 1992). The pulp is juicy and bitter, light dull yellow or whitish, gelatinous when the fruit is ripe; numerous hard triangular reddish-brown pits are found, each containing four seeds (3.5 mm) (Dittmar, 1993). It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections; it is also used to prevent the formation and proliferation of tumors, including malignant ones. Noni juice is also claimed to relieve inflammation. Mostly Noni is consumed as juice, although leaves, flowers, bark and roots can also be used (Wang *et al.,* 2002). Noni has recently been the object of many claims concerning its nutraceutic properties. The propagation of plants by seeds

is comparatively easy, fast and reliable. Seed can be considered as the starting structure in the life of seed plants. Successful seed germination depends on numerous internal and external factors. Seed germination involves the protrusion of embryonic axis from the seed to resume plant growth (Park *et al.,* 2011)**.** However, many seeds exhibit dormancy and fail to germinate even in favorable conditions. Seed germination is influenced by internal factors controlling dormancy, including phytohormones inducing dormancy (ABA), and seed coat factors (Linkies and Leubner – Metzger, 2012). Depending on the plant species and type of dormancy, various methods like scarification, stratification, removal of inhibitor and treatment with growth regulators are used to break dormancy (Hidayati *et al.,* 2012). Phytohormones represent a group of organic molecules that are produced by plant tissues and translocated to some other tissue where they influence much diverse developmental process (Bakrim *et al.,* 2007). Phytohormones regulate and integrate the overall growth, development and reproduction in plants by acting as chemical messengers for the communication among cells, tissues and organs (Kucera *et al.,* 2005). Specific endogenous hormones and its levels are directly involved in the control of seed development, dormancy and germination (Hartman *et al.,* 1997). Correlations of concentration of endogenous level of hormone with specific developmental stages, effects of applied hormones, and the relationship with metabolic activities suggests an involvement of hormones in these metabolic activities (Pedroza –Manrique *et al.,* 2005). The Noni seeds have a problem of seed dormancy/hard seed coat (water repellant) thus limiting its commercial cultivation. So untreated seeds need several months to a year before natural germination takes place but their dormancy can be reduced to a month by using heat or chemical scarification. . Germination time for scarified Noni seeds was 20-120 days depending upon temperature, environment and variety/genotype (Nelson, 2002). This necessitates a non-conventional *in-vitro* rapid propagation system for the production of planting material locally. Therefore, this study was carried out to study the effect of MS (Murashige and Skoog, 1962) media supplemented with various concentrations of plant growth regulators on *in-vitro* seed germination and growth performance of nicked seeds of *Morinda citrifolia.*

MATERIALS AND METHODS

The present investigation was conducted at Department of Biotechnology, Acharya Nagarjuna University, Guntur and Andhra Pradesh, India. Noni seeds were brought from Tirumala hills, lie between 79° 19^1 to 79º 23^1 East and 13º 37^1 to 13º to 43^1 North latitude in the Chittoor District of Andhra Pradesh, India. Fully ripened, soft and white colored Noni fruits were collected. The pulp of the Noni fruit was washed thoroughly with water to obtain Noni seeds. Noni seeds were dried under shade for 2 to 3 days. Seeds were stored in the airtight container at room temperature. Noni seeds surface sterilized with 0.1% (w/v) aqueous $HgCl_2$ (Qualigens, Mumbai, India) were then nicked using an ordinary sterile nail clipper to create an opening in the tough seed coat, so that water and air may enter and contact the embryo. The nicked seeds were then inoculated aseptically in culture tubes (25×150 mm) containing 15-20ml of MS (Murashige and skoog) media supplemented with 3% (w/v) sucrose and various concentrations plant growth regulators along with nicked seeds and control where MS media was supplemented with no hormones. pH of the media was adjusted to 5.7±0.1 with 0.1N NaOH or 0.1N HCl prior to the addition of 0.8% Agar-agar (gelling agent) and autoclaved at 121°C for 15 min. All cultures were incubated at 25.0º± 3.0ºC under white florescent light (3,000 lux) with a photo period cycle of 16 hours light and 8 hours darkness. The experiment was conducted with nine treatments like MS media with various plant growth regulators like Gibberellic acid (GA_3), Indole-3-Butyric acid (IBA), Naphthalene-1-Acetic acid (NAA), 6-Benzyl-amino-purine (BAP) and Kinetin (KN) with various concentrations (0.1, 0.5, 1.0, 1.25, 1.50, 1.75, 2.0 mg/l) and nicking alone along with control (MS media without any hormones) with 20 seeds per each treatment with three replications in a completely randomized design (CRD). The number of seeds germinating everyday in each treatment was counted for calculating the final germination percent in each treatment and same temperature and humidity are maintained throughout the growth of the seedlings for a period of six weeks. The fresh weight was calculated, root and the shoot length of the seedlings were measured using a transparent plastic ruler. The seedlings were placed in an oven at 80ºC for 24 hours and

dry weights were recorded. Data on germination (%); shoot length (cm); root length (cm); fresh weight (g) and dry weight (g) was collected and were presented as mean and standard error (SE).

RESULTS AND DISCUSSION

Seed Germination (%)

The data on the effect of MS media fortified with various concentrations of plant growth regulators on in vitro nicked seed germination of *Morinda citrifolia* were presented in table.1.The *in vitro* nicked seed germination response of Noni with various concentrations of plant growth regulators were presented in Figure 1-5. The mean germination percent (%) was found to be superior in MS media supplemented with 1.0mg/l GA_3 (97.37) followed by MS media with 1.75mg/l IBA (86.15), MS media with 2mg/l NAA (75.44), MS media with 2mg/l BAP (69.20) and in MS media with 2.0mg/l KN (56.66). least percent of germination were observed in nicking alone (32.26) and in control treatment (16.40) where MS media was not supplied with any plant growth regulators.

Table 1: Effect of MS media with various concentrations of Gibberellic acid, Indole- 3- Butyric acid, Naphthalene-1- acetic acid, 6-Benzyl-amino-purine, Kinetin and nicking on *in vitro* germination (%) of *Morinda citrifolia* seeds

Concentration of PGRs(mg/l)	GA3	IBA	NAA	BAP	KN
Control	16.40±0.20	16.40±0.20	16.40±0.20	16.40±0.20	16.40±0.20
Nicking alone	32.26±0.11	32.26±0.11	32.26±0.11	32.26±0.11	32.26±0.11
0.1	34.27±0.14	32.97±0.03	32.91±0.05	32.49±0.23	32.29±0.18
0.5	48.19±0.03	38.89±0.05	33.00±0.04	32.59±0.02	32.52±0.20
1.0	97.37±0.14	41.35±0.15	35.50±0.23	35.36±0.30	33.56±0.40
1.25	80.40±0.35	52.31±0.14	39.98±0.03	39.26±0.09	35.48±0.19
1.50	78.24±0.14	60.93±0.02	44.47±0.20	41.19±0.40	37.60±0.20
1.75	77.88±0.06	86.15±0.08	56.78±0.8	54.78±0.20	40.08±0.60
2.0	71.26±0.2	79.89±0.06	75.44±0.4	69.20±0.1	56.66±0.22

Mean of three replications, mean ± standard error.

Shoot Length and Root Length

The data on the effect of MS media fortified with various concentrations of plant growth regulators on shoot and root length of *in vitro* raised seedlings of *Morinda citrifolia* were presented in table. 2. The mean shoot length and root length of *in vitro* raised seedlings of Noni was maximum in MS media fortified with 1mg/l GA_3 (8.67; 5.78) followed by MS media with 1.75mg/l IBA (6.64; 5.61), in MS media with 2.0mg/l NAA (5.56; 3.91), in MS media with 2.0mg/l BAP (5.93; 2.56) and in MS media with 2.0mg/l KN (4.94; 2.49). The mean shoot length and root length was found to be very low in nicking alone (1.70; 1.48) and in control treatment (1.55; 1.01).

Fresh and Dry Weight

The data on the effect MS media supplemented with various concentrations of plant growth regulators on the fresh and dry weights of *in vitro* nicked Noni seeds are presented in Table - 3. Fresh weight and dry weight in MS media supplemented with 1.0 mg/l GA3 (1.512; 0.192) was found superior over other treatments like MS media with 1.75mg/L IBA (1.498; 0.167), MS media with 2.0mg/l NAA (1.410; 0.162), MS media with 2.0mg/l BAP (1.401; 0.158) and in MS media with 2.0mg/l KN (1.397; 0.154) than nicking alone (1.060;0.025). Control treatment recorded least fresh weight and dry weight (1.025; 0.015). Biotechnological tools can be applied to achieve rapid multiplication of many medicinal plants including forest tree species in a short time (Nikam and Barmukh, 2009). Regeneration from seeds is the most often used and cheapest method of propagation in several plants (Willenborg *et al.,* 2005). Micro propagation is an advanced technique for producing a large number of genetically uniform and pathogen free plants in limited time and space**.** *In vitro* propagation of species through tissue culture has been

frequently based on the successful adjustment of the type and combinations of plant growth hormones (Uranbey *et al.*, 2005).

Figure 1: GA3

Figure 2: IBA

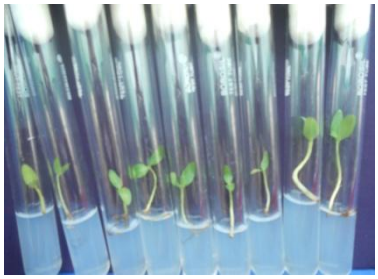

Figure 3: NAA

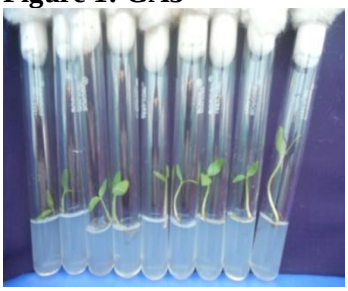

Figure 4: BAP

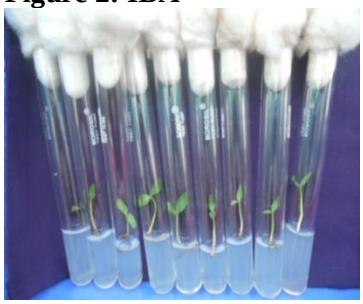

Figure 5: KN

Figure 1-5: Effect of various concentrations of GA3, IBA, NAA, BAP and KN on *in vitro* seed germination of *Morinda citrifolia L.*

Seed dormancy and germination are complex traits of higher plants that are influenced by a large number of genetic and environmental factors. Present study on Noni seeds indicated that MS media with Gibberellic acid (GA_3) at 1 mg/l induced higher germination percent, shoot length and root length, fresh and dry weight when compared with other treatments like MS media supplemented with Indole-3-Butyric acid, Naphthalene-1-acetic acid, 6-Benzyl-amino-purine, Kinetin and nicking alone. Least germination percent was observed in control treatment.

These results confirm the findings of Nikolic *et al.*, (2006), who reported that different hormones and hormonal concentrations on seeds of *Lotus coniculatus* stimulated the percentage of seed germination at least two fold in optimum concentrations. Response of cultures to the different media protocols agrees with the work of Mahmoodzadeh *et al.*, (2010), investigated the effect of hormones and sucrose level on *Zinnia elegans* plants *in vitro*, proving that it is possible to improve the production of plantlets from seeds using different plant growth hormones and carbon sources. It also confirms the study conducted by Rashmi *et al.*, (2010) in which concentration of various PGRs increased the root and shoot length of plantlets germinating from bamboo seeds as compared to control. Dormant seeds can be stimulated to germinate using treatments that emulate natural conditions or satisfy certain physiological requirements. Stratification leaching, scarification, light and plant growth regulators [especially gibberellic acid & cytokinins] are effective dormancy releasing treatments (Rahman *et al.*, 2006). Several germination stimulators have been used to improve the seed germination, e.g., GA3 (Vijaya *et al.*, 1996). The role of GA3 in promoting seed germination has been described by several authors (Karssen, 1995). The promoting effect of GA3 treatment is often attributed to the mobilization of stored reserves (Soyler & Khawar, 2007) and acceleration of the disappearance of abscisic acid (ABA)-regulated polypeptides, which are abundant in dormant seeds**.** The evidence for hormone involvement comes from correlation of hormone concentration with specific development stages, effects of applied hormones and the relationship of hormones to metabolic activities. Sometimes response on growth or differentiation is inhibited by hormones, especially Abscisic acid. This inhibition is removed by the use of certain growth regulators

Table 2: Effect of MS media with various concentrations of Gibberellic acid, Indole – 3- Butyric acid, Naphthalene-1- acetic acid, 6-Benzyl-amino-purine, Kinetin and nicking on shoot length (SL)and root length (RL) of *in vitro* raised *Morinda citrifolia* seedlings

Concentration of PGRs(mg/l)	GA3		IBA		NAA		BAP		KN	
	SL (cm)	RL (cm)	SL(cm)	RL(cm)	SL(cm)	RL (cm)	SL (cm)	RL (cm)	SL (cm)	RL(cm)
Control	1.55±0.18	1.01±0.22	1.55±0.18	1.01±0.22	1.55±0.18	1.01±0.22	1.55±0.18	1.01±0.22	1.55±0.18	1.01±0.22
Nicking alone	1.70±0.3	1.48±0.1	1.70±0.3	1.48±0.1	1.70±0.3	1.48±0.1	1.70±0.3	1.48±0.1	1.70±0.3	1.48±0.1
0.1	2.30±0.12	1.94±0.2	1.84±0.1	1.54±0.2	1.74±0.2	1.51±0.1	1.73±0.3	1.50±0.5	1.71±0.5	1.49±0.1
0.5	2.58±0.8	2.29±0.36	2.06±0.2	1.72±0.36	1.78±0.15	1.52±0.2	1.83±0.2	1.51±0.28	1.80±0.4	1.51±0.5
1.0	8.67±0.7	5.78±0.5	2.09±0.4	2.03±0.5	1.86±0.12	1.62±0.34	1.92±0.4	1.57±0.2	1.90±0.6	1.54±0.2
1.25	7.08±0.2	5.05±0.4	2.19±0.1	2.10±0.5	2.96±0.2	1.70±0.4	2.94±0.5	1.61±0.3	2.82±0.1	1.57±0.2
1.50	6.26±0.28	4.95±0.5	3.34±0.28	3.01±0.05	3.49±0.2	2.06±0.5	3.09±0.1	1.63±0.33	3.01±0.2	1.62±0.05
1.75	6.07±0.11	3.53±0.2	6.64±0.1	5.61±0.2	4.10±0.4	3.08±0.3	3.15±0.1	2.03±0.7	3.11±0.1	1.84±0.4
2.0	6.00±0.5	3.29±0.05	5.99±0.5	4.96±0.1	5.56±0.3	3.91±0.4	5.93±0.2	2.56±0.3	4.94±0.05	2.49±0.05

Mean of three replications, mean ± standard error.

Table 3: Effect of MS media with various concentrations of Gibberellic acid, Indole-3-Butyric acid, Naphthalene-1-acetic acid, 6-Benzyl-amino-purine, Kinetin and nicking on fresh weight (FW) and dry weight (DW) of *in vitro* raised seedlings of *Morinda citrifolia*

Concentration of PGRs(mg/l)	GA3		IBA		NAA		BAP		KN	
	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
Control	1.025±0.17	0.015±0.4	1.025±0.17	0.015±0.4	1.025±0.17	0.015±0.4	1.025±0.17	0.015±0.4	1.025±0.17	0.015±0.4
Nicking alone	1.060±0.20	0.025±0.1	1.060±0.20	0.025±0.1	1.060±0.20	0.025±0.1	1.060±0.20	0.025±0.1	1.060±0.20	0.025±0.1
0.1	1.245±0.57	0.090±0.05	1.110±0.42	0.060±0.2	1.108±0.3	0.047±0.1	1.102±0.32	0.041±0.1	1.100±0.4	0.031±0.13
0.5	1.250±0.3	0.155±0.33	1.196±0.14	0.071±0.14	1.185±1.1	0.060±0.25	1.144±0.12	0.046±0.5	1.136±0.1	0.044±0.2
1.0	1.512±0.05	0.192±0.1	1.250±0.5	0.080±0.3	1.249±1.2	0.065±0.3	1.196±0.14	0.060±0.25	1.190±0.05	0.057±0.9
1.25	1.490±0.4	0.175±0.3	1.310±0.3	0.096±0.21	1.300±0.5	0.072±0.17	1.256±0.5	0.044±0.12	1.247±0.52	0.051±0.24
1.50	1.420±05	0.164±0.2	1.376±0.2	0.110±0.32	1.305±0.7	0.080±0.36	1.301±0.2	0.057±0.1	1.298±0.41	0.064±0.31
1.75	1.400±0.12	0.168±0.14	1.498±0.5	0.167±0.05	1.400±0.05	0.0142±0.41	1.307±0.4	0.123±0.14	1.305±0.22	0.120±0.42
2.0	1.390±0.17	0.161±0.2	1.360±0.7	0.131±0.5	1.410±0.14	0.162±0.1	1.401±0.8	0.158±0.2	1.397±0.34	0.154±0.61

Mean of three replications, mean ± standard error.

like Gibberellin and Auxins. This investigation with MS media with growth hormones will help in determining that the hormonal concentration are suitable for rapid seed germination and seedling growth of *Morinda citrifolia.*

Nelson (2006) reported that nicking helps the seed to imbibe; causing seed coat to rupture hence increases the seed germination rate uniformly. The role of plant growth regulators in overcoming the harmful effects on growth may be due to the change in the endogenous growth regulators. Although varied in seed germination and root- shoot elongation by different treatments, the nicking with different treatments evident that nicked seed with various plant growth regulators especially Gibberellic acid could improve in germination and seedling establishment and this observation was found equivalent to the observation of Singh *et al.,* (2005) which revealed that Noni seeds treated with GA significantly increased the height of seedling and number of leaves per seedling. Studies have revealed that seed dormancy and germination are under hormonal control.

Conclusion

Multiplication of medicinal plants is very much essential for obtaining valuable phytochemicals and there is clear need to formulate a procedure that will facilitate quick and reliable germination using in vitro techniques. If properly planned, growth regulator can bring in very quick, rapid and distinctive changes in the target plants, and show appreciable improvement with high commercial and aesthetic value; which no other technology can offer in such a short span of time. It has been proven in several studies including the present one that plant growth regulators exerts far reaching effects on plant growth, the precise action depends on the concentrations of the substances present and the sensitivity of the concerned organ. *In vitro* seed culture is a method for producing improved regenerants under controlled condition, especially under conditions where seeds are scarce and expensive to purchase. Conservation and multiplication can however be more easily carried out on plantlets grown *in vitro* through seed culture by circumventing problems of exposure to disease and contaminants usually associated with plants grown under field conditions.

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