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INFLUENCE OF NICKING IN COMBINATION WITH VARIOUS PLANT GROWTH SUBSTANCES ON SEED GERMINATION AND SEEDLING GROWTH OF NONI (*Morinda citrifolia* L.*)*

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ABSTRACT

A study was carried out to investigate the effect of various concentrations of plant growth substances in combination with nicking on the germination of Noni (*Morinda citrifolia* L.). Freshly extracted Noni seeds were nicked first and then treated with various concentrations (100-1000ppm) of Gibberellic acid, Indole-3-Butyric acid, Naphthalene-1-acetic acid, 6-Benzyl-amino-purine, Potassium nitrate, Kinetin and nicking along with control (seeds soaked in water) for a period of 24 hours. The highest germination percent, shoot length, root length, fresh and dry weights were recorded in seeds treated with Gibberellic acid at 800 ppm concentration (96.33%, 7.92 and 3.34cm, 1.408 and 0.076g); followed by Indole-3- Butyric acid (89.26%, 6.53 and 2.97cm, 1.312 and 0.068g); Naphthalene-1-acetic acid (73.61%, 5.36 and 2.84cm, 1.293 and 0.066g); 6-Benzyl-amino-purine (65.33%, 5.28 and 2.62cm, 1.255 and 0.061g; Potassium nitrate (55.86%, 3.92 and 2.14cm, 1.201 and 0.041g) and Kinetin (54.16%, 4.11 and 2.38cm, 1.221 and 0.043g) at 1000ppm concentration; followed by nicking alone (39.42%, 1.98 and 1.14cm,1.050 and 0.015g). Whereas poor response was observed in control with low germination percent (34.28%), shoot length (1.72cm), root length (1.11cm), fresh weight (1.020g) and dry weight (0.011g).

Key words: *plant growth substances, germination, Morinda citrifolia* L.

INTRODUCTION

Morinda citrifolia L. commonly known as Indian Noni, is one of the most significant sources of traditional medicine [1, 2]. *Morinda citrifolia* L. belongs to the family Rubiaceae [3]. Noni is also known as Indian mulberry. Various parts of the plant, including its leaves, fruit, bark and roots, have been used for over 2000 years to treat several diseases such as high blood pressure and diabetes, and to cure eye problems, skin wounds, throat problems, respiratory ailments, constipation, and stomach pains [4]. About 160 valuable phytochemicals have been identified in this plant, and the major compounds are polyphenols, organic acids and alkaloids [5].

Dormancy is a condition in which seeds do not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination [6, 7]. The Noni seeds have a problem of seed dormancy thus limiting its commercial cultivation. Noni seeds are buoyant and hydrophobic due to air chamber and durable, water repellant, fibrous seed coat. Noni is easy to propagate from seeds and the primary disadvantage of seed propagation is that without seed treatment and applied heat, uniformity of seed germination may be unreliable and seedling production may take 6-12 months for germination and 9-12 more months before they are ready to transplant.

Hence, untreated seeds need several months to a year for germination but their germination period can be reduced to a month using techniques such as trimming, heating or chemical scarification etc. Pre sowing chemical treatments have generally been used to enhance seed germination and seedling vigor. Various methods have been used by seed scientists and technologists to break seed dormancy. Stratification plays an important role as a stimulator that helps to break dormancy [7]. In order to accelerate this method, it can be combined with some treatments such as chemical applications or mechanical seed coat removal [8]. Many investigators have studied the effects of exogenous growth regulators on seed germination. Gibberellins are believed to be important in controlling the germination of seeds in nature [9].

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Scarifying the hard seed coat by nicking or puncturing significantly reduces the germination time, improves germination percentage and promotes uniform sprouting. Scarifying can be done by blending machine, nail cutter etc. [10]. Hence, present research was carried to study the effect of various plant growth substances along with nicking to improve the germination percent in Noni seeds.

MATERIALS & METHODS

The present investigation was conducted at Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. Noni seeds were brought from Tirumala hills, lie between 79° 19' to 79º 23' East and 13º 37' to 13º to 43' both 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.

The shade dried Noni seeds were nicked using an ordinary fingernail clipper to create an opening in the tough seed coat, so that water and air may enter and contact the embryo. This clipping can increase the germination percent and also reduce the time required for germination from several months to only 4 weeks or more. The nicked seeds were treated with different concentrations (100-1000ppm) of Gibberellic acid (GA), Indole -3-Butyric acid (IBA), Naphthalene-1-Acetic acid (NAA), 6-Benzyl-amino-purine (BAP), Potassium nitrate (KNO_3), Kinetin (KN) and nicking alone along with control (seeds soaked in water) for a period of 24 hours. Ten imbibed seeds were placed in petri dishes lined with two layers of Whatmann No.1 filter papers, moistened with sterile distilled water to provide humidity. Petri dishes were placed in an incubator at 25°C with 70-80% humidity. 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.

The experiment was conducted with eight treatments like Gibberellic acid (GA), Indole-3-Butyric acid (IBA), Naphthalene-1-Acetic acid (NAA), 6-Benzyl-amino-purine (BAP), Potassium nitrate (KNO_3), Kinetin (KN) with various concentrations (100-1000ppm) and nicking alone along with control (seeds soaked in water) for a period of 24 hours in three replications in a completely randomized design (CRD). Data on germination percent (%); shoot length (cm); root length (cm); fresh weight (g) and dry weight (g) was collected and ANOVA was carried out using IRRISTAT software package.

RESULTS AND DISCUSSION

Seed germination (%)

The treatments differed significantly for the character studied. The data on the effect of various concentrations of growth substances on the nicked Noni seed germination are presented in Table -1. At 800 ppm, Gibberellic acid was found to be significantly superior over other treatments and control for seed germination percent (96.33), followed by Indole-3-Butyric acid (89.26) and Naphthalene-1-Acetic acid (73.61) at 1000ppm concentration, where as other growth hormones like 6-Benzyl-amino-purine (65.33), Potassium nitrate (55.86) and Kinetin (54.16) were significantly superior at 1000ppm concentration over nicking alone (39.42) and control treatment (34.28%).

Shoot length and root length (cm)

The data on the effect of various concentrations of growth substances on the length of the shoot and root are presented in Table - 2. The hormone Gibberellic acid recorded maximum shoot length (7.92) and root length (3.34) at 800ppm concentration whereas Indole – 3- Butyric acid (6.53 and 2.97) and Naphthalene-1-Acetic acid (5.36 and 2.84), 6-Benzyl-amino-purine (5.28 and 2.62), Potassium nitrate (3.92 and 2.14) and Kinetin (4.11 and 2.38) were found to be significantly superior for root length and shoot length at 1000ppm concentration over nicking alone (1.98 and 1.14) and control (1.11 and 1.72). In general, it was observed that shoot length was more when compared to root length for all the treatments tested, might be due to application of hormones which had a physiological effect on plant growth.

Fresh weight and dry weight (g)

The data on the effect of various concentrations of growth substances on the fresh and dry weights of nicked Noni seed are presented in Table - 3. Fresh weight and dry weight were found to be superior at 800ppm concentration when tested with Gibberellic acid (1.408 and 0.076) which was significantly superior over other treatments like Indole- 3-Butyric acid (1.312 and 0.068), Naphthalene-1-Acetic acid (1.293 and 0.066), 6-Benzyl-amino-purine (1.255 and 0.061), Potassium nitrate (1.201 and 0.041) and Kinetin (1.221 and 0.043) at 1000ppm concentration than nicking alone (1.050 and 0.015). Control treatment recorded the least fresh weight and dry weights (1.02 and 0.011).

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 application of Gibberllic acid (GA) induced higher germination percent, shoot length and root length, fresh and dry weight when compared with soaking of seeds for 24 hours in Indole – 3- Butyric acid, Naphthalene-1-acetic acid, 6-Benzyl-amino-purine, Kinetin, Potassium nitra**te**, nicking alone and control treatments. From the above investigations it was observed that *Morinda citrifolia*. L. seed germination and other parameters studied were highest in 800 ppm concentration of Gibberellic acid (GA) along with nicking followed by Indole-3- Butyric acid (IBA), Naphthalene-1-acetic acid (NAA), 6-Benzyl-amino-purine(BAP), Kinetin and Potassium nitra**te**; this is due to the effect of Gibberellic acid on seed germination along with scarification or nicking. Kockemann [11] reported that growth substances serve to regulate the germination of seeds. These growth regulators when applied exogenously or naturally occurring exert either stimulatory or inhibitory effects under different conditions and concentrations either as germination stimulators or germination inhibitors. Davies [12] mentioned that Gibberellins (GA) is an essential phytohormone induces many aspects of plant development including seed germination. Pandey and Sinha [13] reported that GA induced the synthesis of α-amylase and other hydrolytic enzymes during the early stages of seed germination. Similarly, Raliton [14] also found the accelerating effect of Gibberellic acid in seed germination of *Morus indica*.

Table - 1 Effect of Gibberellic acid, Indole- 3- Butyric acid, Naphthalene-1- acetic acid, 6-Benzyl-amino-purine, Potassium nitrate, Kinetin and nicking on germination of Noni seeds.

Concentration (ppm)	Germination (%)					
	GA_3	IBA	NAA	BAP	KNO3	KN
Control	34.28	34.28	34.28	34.28	34.28	34.28
Nicking alone	39.42	39.42	39.42	39.42	39.42	39.42
100	46.6	42.53	42	40.66	40.66	41.96
200	50.16	47.06	43.2	41.91	44.41	42.93
300	51.9	51.41	48.78	46	46.9	44.86
400	58.3	53	52.26	50.58	47.55	46.5
500	62.21	62.61	60.66	55.45	52.83	47.83
600	75.36	72.16	62.91	60.73	52.16	51.61
700	83	78.13	65.62	63.5	55.1	52.26
800	96.33	84.43	69.25	63.76	55.66	52.66
900	95.83	85.4	73.5	65.16	54.63	53.66
1000	94.5	89.26	73.61	65.33	55.86	54.16
Mean	68.05	63.67	56.92	53.40	49.10	47.52
CD (5%)	2.36	2.53	2.45	2.74	2.50	3.05
CV (%)	2.0	2.3	2.5	3.0	3.0	3.8

Table - 2 Effect of Gibberellic acid, Indole – 3- Butyric acid, Naphthalene-1- acetic acid, 6-Benzyl-amino-purine, Potassium nitrate, Kinetin and nicking on shoot and root length of Noni seedlings**.**

Concentration (ppm)	GA_3		IBA		NAA		BAP		KNO_3		KN	
	SL(cm)	RL (cm)	SL (cm)	RL (cm)	SL (cm)	RL (cm)	SL (cm)	RL (cm)	SL (cm)	RL (cm)	SL (cm)	RL (cm)
Control	1.72	1.11	1.72	1.11	1.72	1.11	1.72	1.11	1.72	1.11	1.72	1.11
Nicking alone	1.98	1.14	1.98	1.14	1.98	1.14	1.98	1.14	1.98	1.14	1.98	1.14
100	4.11	1.72	3.41	1.45	2.13	1.47	2.25	1.35	1.88	1.15	1.96	1.14
200	4.20	1.73	3.92	1.52	2.52	1.51	2.52	1.61	1.92	1.33	2.07	1.22
300	4.42	1.83	4.06	1.75	2.81	1.84	2.91	1.85	2.12	1.46	2.14	1.35
400	4.86	2.04	4.31	2.11	3.42	1.91	3.32	2.02	2.53	1.65	2.41	1.57
500	5.15	2.14	5.02	2.33	3.86	2.01	3.42	2.07	2.61	1.61	2.64	1.71
600	5.23	2.21	5.21	2.42	4.15	2.21	4.11	2.15	2.82	1.71	2.80	1.85
700	5.58	2.37	5.36	2.51	4.33	2.36	4.33	2.21	3.06	1.75	3.06	1.92
800	7.92	3.24	5.71	2.56	4.36	2.42	4.48	2.45	3.54	1.86	3.53	2.03
900	7.42	3.31	5.82	2.61	4.61	2.51	4.71	2.58	3.63	1.91	3.60	2.04
1000	7.32	3.34	6.53	2.97	5.36	2.84	5.28	2.62	3.92	2.14	4.11	2.38
Mean	5.27	2.28	4.64	2.12	3.57	2.02	3.55	2.00	2.71	1.61	2.73	1.67
CD (5%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02
CV (%)	0.2	0.5	0.3	0.6	0.3	0.5	0.3	0.7	0.5	0.7	0.3	0.7

Table - 3 Effect of Gibberellic acid, Indole-3-Butyric acid, Naphthalene-1-acetic acid, 6-Benzyl-amino-purine, Potassium nitrate, Kinetin and nicking on fresh and dry weight of Noni seeds.

Concentration (ppm)	GA_3		IBA		NAA		BAP		KNO_3		KN	
	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
Control	1.020	0.011	1.020	0.011	1.020	0.011	1.020	0.011	1.020	0.011	1.020	0.011
Nicking alone	1.050	0.015	1.050	0.015	1.050	0.015	1.050	0.015	1.050	0.015	1.050	0.015
100	1.239	0.036	1.146	0.024	1.099	0.035	1.121	0.031	1.051	0.011	1.107	0.011
200	1.244	0.039	1.151	0.025	1.149	0.040	1.131	0.033	1.099	0.014	1.116	0.015
300	1.289	0.045	1.159	0.028	1.201	0.042	1.141	0.035	1.107	0.015	1.151	0.015
400	1.319	0.049	1.171	0.031	1.210	0.045	1.181	0.036	1.131	0.018	1.181	0.019
500	1.322	0.058	1.181	0.035	1.234	0.050	1.189	0.041	1.139	0.020	1.189	0.020
600	1.325	0.064	1.188	0.038	1.238	0.051	1.196	0.043	1.156	0.023	1.196	0.024
700	1.351	0.066	1.201	0.041	1.259	0.055	1.201	0.049	1.160	0.025	1.199	0.034
800	1.406	0.074	1.241	0.045	1..262	0.058	1.206	0.053	1.181	0.028	1.206	0.038
900	1.407	0.075	1.291	0.047	1.271	0.059	1.221	0.056	1.191	0.038	1.211	0.040
1000	1.408	0.076	1.312	0.068	1.293	0.066	1.255	0.061	1.201	0.041	1.221	0.043
Mean	1.303	0.034	1.187	0.036	1.203	0.047	1.169	0.041	1.131	0.022	1.163	0.025
CD (5%)	0.014	0.002	0.013	0.02	0.014	0.002	0.014	0.02	0.014	0.002	0.013	0.002
CV (%)	0.6	1.9	0.7	2.7	0.7	2.0	0.7	2.5	0.7	4.6	0.7	4.0

Nelson [10] reported that nicking helps the seed to imbibe, causing seed coat to rupture hence, increases the seed germination rate uniformly and similarly Gibberellic acid (GA) enhances the shoot and root length simultaneously which was found comparable with the other treatments. These results were similar to the results of Singh *et al.* [15] which revealed that Noni seeds treated with GA significantly increased the height of seedling and number of leaves per seedling. Similar studies by Ponnaiyan and Vezhavendan [16] reported that hulling of seed coat or scarification of tough seed coat of Noni seeds reduce the time required for germination.The germination percent shoot length, root length, fresh and dry weight of the seeds treated with Indole- 3- Butyric acid (IBA) and other plant growth substances like NAA. BAP, KN and KNO_3 were low when compared to Gibberellic acid treatment. This might be due to the seeds not withstanding more concentrations. Potassium nitrate is most widely used chemical for promoting seed germination. It acts synergistically with Gibberellic acid in inducing germination in tobacco seeds. However, the actual role of potassium nitrate in inducing the germination rate is not clearly known [7], potassium nitrate was found to be effective in breaking seed dormancy of many species [17]. Studies have revealed that seed dormancy and germination are under hormonal control [18, 19].

As a conclusion, the methods used in this study are practical and easily applicable for experimental studies and in the field conditions for rapid germination of Noni seeds and are useful in commercial rapid seedling production in large scale which would be essential for anti-desertification.

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