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In vitro Propagation of Noni (*Morinda citrifolia* L.) Through Embryo Culture

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Abstract

The chances of shoot-regeneration from embryo culture of *Morinda citrifolia* L. seeds was investigated. Germination on different strengths of MS and control (Sterile distilled water) started by two weeks after inoculation (WAI). At 6 WAI, 90% of the embryo had germinated from 25% MS followed by 80% in control, 70% from 50% MS and 40% each from 100 and 75% MS. Similarly, the same MS media strengths with basal application of 2.0/1.0 mg/l BAP/Kn affected the growth of regenerated Noni-plantlets. The longest shoot length (3.46 cm) and the number of nodes (1.75) were obtained from 75% MS while the highest number of leaves (7.25) was obtained in 100% MS between 4 and 12 WAI. The lowest value for these parameters were observed in 25% MS. This showed that mature zygotic embryo is good explant for the establishment of highly viable and re-generable plantlets of Noni.

Introduction

Morinda citrifolia L. is a tropical, evergreen, medicinal and fruit-bearing shrub. The species commonly called Noni or Indian mulberry belongs to Rubiaceae. Different parts of the plant were used in the treatment of about 40 known ailments and diseases because of its abundant phytochemical constituents (Singh et al. 2012). Commercial products including Noni tea, Noni powder and Noni fruit juice made from the species leaves, pulp and fruits, respectively are sold and consumed worldwide for their health benefit (Potterat and Hamburger 2007, Cassileth 2010).

To date, a lot of reports on phytotherapeutic effects of extracts from different parts of Noni have been documented (Srinivasan et al. 2015, Almeida et al. 2019). Nonetheless, research on its method of propagation and nursery technique has not been extensive. The dormant nature of Noni seeds which required stringent measures including the use of sulphuric acid, hot water, gibberellic acid and biozyme (*Ascophyllum nodosum*) to break is

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a limitation to commercial exploitation of the species (Muthu et al. 2006, Chandra and Sagar 2013). Whereas, other traditional means of propagating the species through the stem and root cuttings as well as air layering are inefficient for massive production of its seedlings (Nelson 2001). This necessitates the use of micropropagation technique as a reliable alternative. Yet, this method has not been fully explored for large-scale and rapid multiplication of the species. Initial efforts to develop and refine the method of micropropagation for Noni employed the use of seeds and explants collected from the wild and stocks under management (Selvaraj et al. 2006, Sreeranjini and Siril 2014, Jayaprakash et al. 2017). Explants from such sources are usually infected with plant pathogens and take rigorous efforts to disinfect (Jan et al. 2013). The challenges of establishing clean culture could delay or completely hindered achieving the set mass production objectives in due time. Consequently, fast and efficient means of propagation techniques is then required. Given the above, the present study employed the use of mature embryo culture to generate clean plantlets for subsequent propagation of Noni species.

Materials and Methods

This study involved two stages of tissue culture; initiation and shoot regeneration. The culture initiation was designed with five treatments which include - A: Control (Sterile distilled water), B: 25% MS basal medium (BM), C: 50% MS BM, D: 75% MS BM and E: 100% MS BM. The shoot regeneration stage consisted of four treatments including: (i) 25% MS BM, (ii) 50% MS BM, (iii) 75% MS BM and (iv) 100% MS BM, all supplemented with 2.0/1.0 mg/l BAP/Kn (Shekhawat et al. 2015). The first set of treatments was replicated ten times while the second set was replicated eight times. All the treatments in both experiments were laid out in a CRD.

MS powder with vitamins and sucrose supplements (M5501, SXS5501015A) was used for the preparation of the media in each stage. An amount of 34.43 g of the MS powder is standard for 1 litre basal medium. Hence, corresponding quantity of MS powder with respect to percentages in each treatment were weighed and dissolved in distilled water. The media solution were stirred using a magnetic stirrer. In the case of the second set of treatments, BAP and Kn were basally added. All media pH were adjusted to 5.8 and gelled with 10 g of agar. Twenty ml of the media were dispensed in a 50 ml test tube, covered and sterilized at 121°C and 15 psi for 15 min.

The matured zygotic embryos of freshly collected Noni seeds were excised with the aid of a sterile surgical blade. These were disinfected using 70% ethanol for 3 min, rinsed three times and 10% hypochlorite solution for 10 min. These embryos were then rinsed four times with sterile distilled water, drained on sterile filter paper and then inoculated at one embryo per tube. The tubes were covered and sealed with parafilm while placed in the growth room under 16/8 hrs light and dark photoperiod and 20 ± 2°C. Shoot tips

and nodal segments of the germinated embryos were used as explant for shoot regeneration stage.

Data collected on cultured embryos include the rate of germination (%) in terms of radicle and shoot emergence and shoot length (cm) at two weeks interval starting from two weeks after inoculation (WAI). Shoot length (cm), number of leaves and nodes were collected on regenerated shoots at four weeks interval starting from 4 WAI. The qualitative data collected were subjected to analysis of variance using GenStat (3rd edition) while mean separation was conducted with LSD at $p \leq 0.05$.

Results and Discussion

The results of growth of Noni embryos cultured on MS of different strengths and control (gelled sterile distilled water) are presented in Figs 1 and 2. Two weeks after inoculation, germination in terms of radicle emergence had started with 70% from control (Fig. 1). This was sustained until 4 WAI when germination increased from 50 to 70% in 25% MS. However, by 6th WAI, 90% of the embryos germinated from 25% MS followed by 80% in control (Fig. 3). A total of 70 % radicle emergence was observed in 50% MS while 40% germination was obtained individually from 100 and 75% MS.

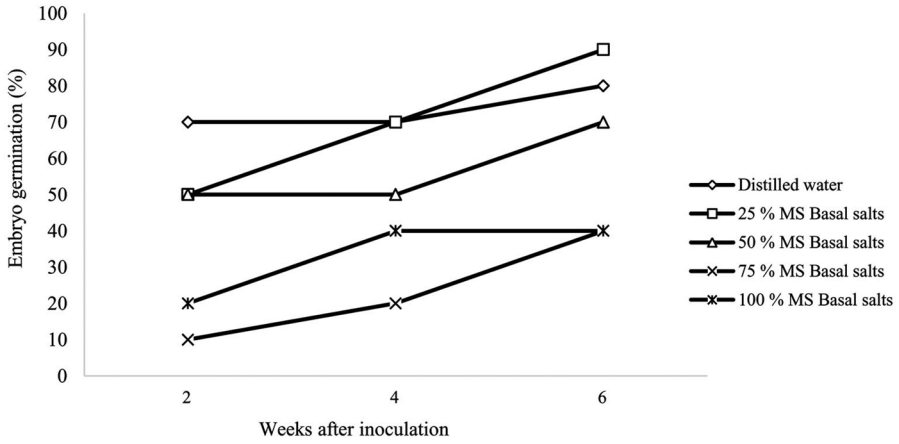

Fig. 1. Radicle emergence of Noni embryos cultured on different strength of MS at successive growth weeks.

Shoot emergence occurred by 4 WAI (Fig. 2). Both control and 25% MS had the highest (70%) shoot emergence each at 4 and 6 WAI. This was followed by 50% in 50% MS, 20 and 10% from media with 100 and 75% MS, respectively.

The germination and growth of the cultured Noni embryos on various MS media strengths (Figs 1 - 3) starting from 2 weeks after inoculation (WAI) indicated that mature zygotic embryos are good explants for the establishment of highly viable and regenerable

plantlets for multiple shoot cultures of the species. Raghavan and Srivastava (1982) had envisioned the immense use of embryo culture in rearing viable seedlings. Present result was similar to that of Qi et al. (2007) who reported that matured Noni embryos germinated within 50 days compared to seeds which did not germinate for over one year

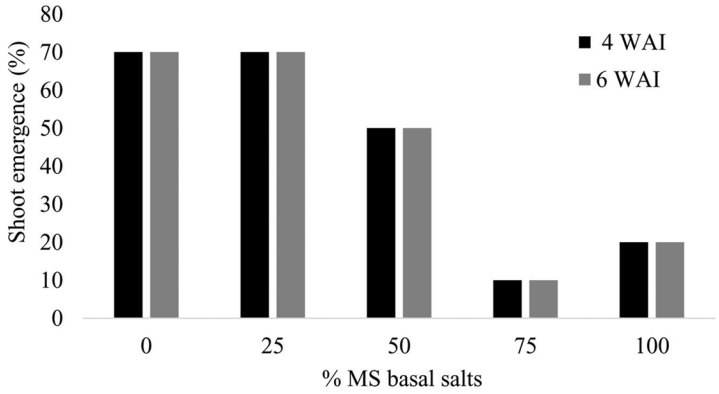

Fig. 2. Shoot emergence of Noni embryos cultured on different MS media strength at successive growth weeks.

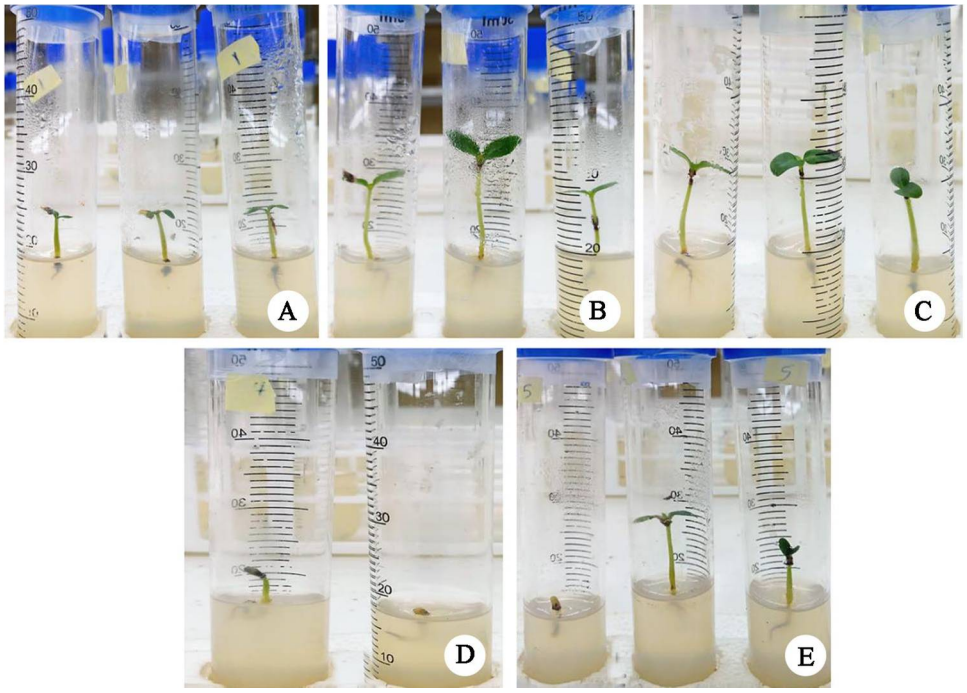

Fig. 3. Growth of Noni embryos inoculated on MS of different strengths at 6 week after inoculation. A: Control (sterile distilled water), B: 25% MS, C: 50% MS, D: 75% MS and E: 100% MS.

after inoculation. In another study, total removal of the seed coat of *P. edulis* seeds was reported to have yielded 100% germination under *in vitro* conditions either on PlantMax® or MS (Rego et al. 2014). Fast germination of Noni embryos within 2 WAI in the present study (Fig. 1) showed that the embryos grown *in vitro* skipped a rest period which is usually observed when it is a part of the intact seed and germinated.

Furthermore, the observed early and higher radicle emergence (70%) from embryos cultured on sterile distilled water (control) at 2 WAI compared with MS nutrient media could be attributed to the nutrient supply from the endosperm of the explant. This showed that growth of the embryo is heterotrophic at the early stage and independent of the media nutrients. Available salt concentrations in other media could have limited fast take-off of the embryos from those media. However, the growth from control could not be sustained beyond 4 WAI when shoot started emerging (Fig. 1). By 6 WAI, 90% of the embryos germinated from 25% MS followed by 80% from control, 70% from half strength and 40% from others (75 and 100% MS). This showed that with increasing growth weeks, the plantlets had transited to autotrophic feeding and nutrient demand had become higher than what could be supplied by endosperm of the explants. This could have resulted in high but comparable shoot emergence (70%) from 25% MS and control (Fig. 2). However, the observed lower results from MS with higher media strengths (50 to 100% MS) showed that mature Noni embryos required low nutrients to have optimum germination and growth.

The growth of regenerated Noni explant as affected by MS media strengths in second stage of the study was evaluated. The results showed that there was a significant difference ($p \leq 0.05$) in shoot length and number of leaves produced by the plantlet at 12 WAI (Table 1). Shoot lengths were similar between the media from 4 to 8 WAI. At 12 WAI, shoot lengths of plantlets (3.46 cm) in 75% MS was comparable to those of 100 and 50% MS whereas, the three media were higher than shoot lengths of plantlets 25% MS (Fig. 3).

Media strength also significantly influenced ($p \leq 0.05$) the number of leaves produced at 8 and 12 WAI (Table 1, Fig. 3). The average number of leaves (6) from 75% MS was greater than those from 50 and 25% MS (4.5 and 3.88, respectively) while similar to that of 100% MS (5.5) at 8 WAI. With an increase in weeks, an average number of leaves (7.25) from 100% MS was comparable to those of 75 and 50% MS while all the three media were higher than 4.75 from plantlets in 25% MS.

The results of the regenerated plantlets from subculture embryo plantlets on MS with varying strength showed that growths in terms of shoot length, number of leaves except nodes were affected by the media strengths. The highest plantlet growth observed in terms of shoot length across successive growth weeks (Table 1) and the number of nodes at 12 WAI (Fig. 4) from 75% MS and number of leaves from 100% MS (Table 1) indicated that Noni shoot regeneration requires high nutrient supply in the range of 50 to 100% with 75% MS as the optimum.

Table 1. Effects of MS strengths on the growth of Noni plantlets at successive weeks.

MS (%)	Shoot length (cm)			Number of leaves		
	4 WAI	8 WAI	12 WAI	4 WAI	8 WAI	12 WAI
25	2.28	2.41	2.49b	3.88	3.88c	4.75b
50	2.46	2.75	3.21a	4.00	4.50bc	6.50a
75	2.35	2.88	3.46a	4.00	6.00a	6.50a
100	2.14	2.70	3.43a	4.12	5.50ab	7.25a
L.S.D @ $p \leq 0.05$	0.54	0.63	0.52**	1.12	1.11**	1.01**

** Means difference was significant at $p \leq 0.01$.

The number of nodes produced by the regenerated Noni plantlets showed that media strength did not account for any significant difference ($p \geq 0.05$) on the parameter at 12 WAI (Fig. 4). Nonetheless, an average of 1.75 number of nodes from 75% MS was the highest followed by 1.63 and 1.5 from 100 and 50% MS while the least (1.13) was from 25% MS (Fig. 4).

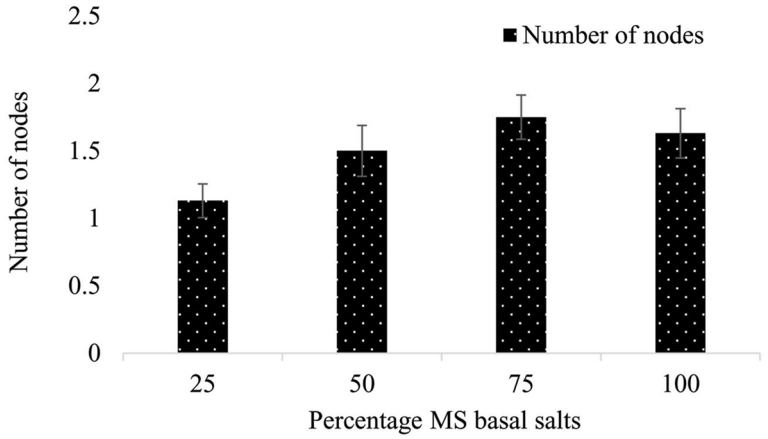

Fig. 4. Effects of MS media strengths on Noni number of nodes at 12 WAI.

However, the lowest level of results observed in 25% MS showed that nutrient concentration in the medium was inadequate to provide optimum support for Noni shoot regeneration and growth. The results could be attributed to the genetic makeup of Noni, as the uptake of nutrients in culture media may vary for different plant species and genotype. The results were in conformity with Gawad et al. (2012) on *Coffea arabica* L. of the same Rubiaceae family with Noni that MS strength affected starting and multiplication stages of the species. They obtained the highest results from full strength MS supplemented with 6 mg/l BA or 6 mg/l Kn which was comparable to three-fourth strength MS in the number of shoots, shoot length and number of leaves. Similarly, the

results related to that of Rezali et al. (2017) on *Typhonium flagelliforme* when maximum shoot number, shoot height, leaf number, root number, fresh weight and dry weight were recorded in full-strength MS followed by half- and quarter-strength MS.

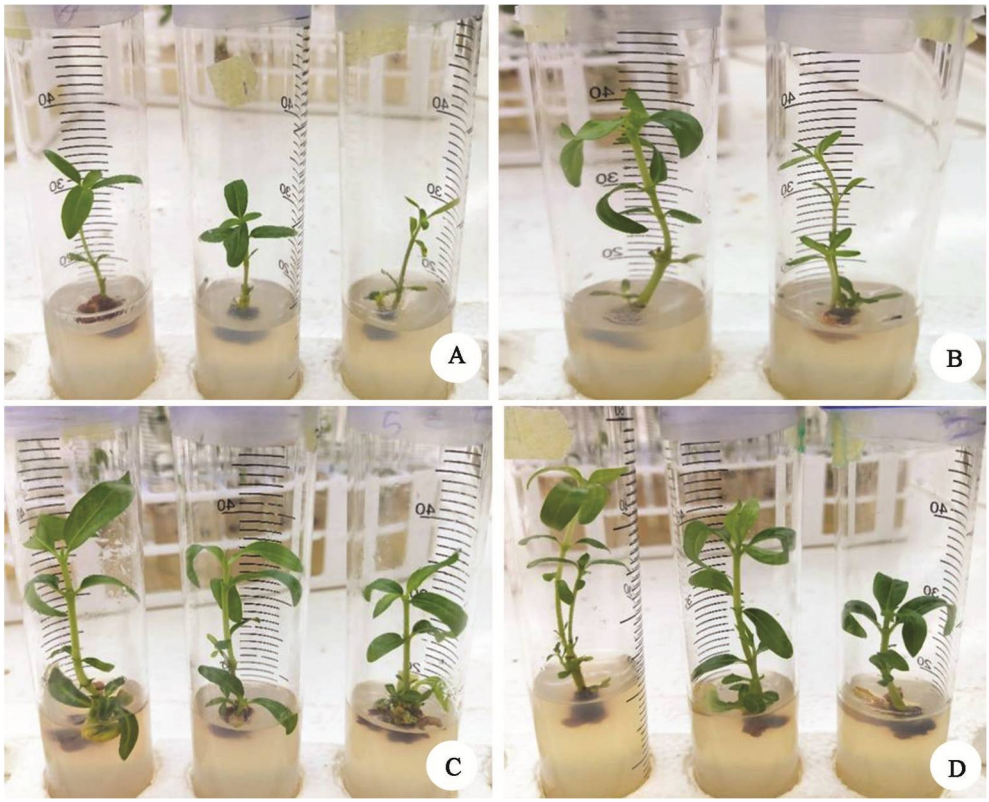

Fig. 5. Regenerated shoots of Noni plant as affected by MS media strength at 12 WAI. A: 25% MS, B: 50% MS, C: 75% MS and D: 100% MS.

Moreover, observation on regenerated Noni shoot in the present study indicated that no root was induced on the plantlets from any of the media except callus formed at the base even at 12 WAI (Fig. 5). This result was against the report of Jayaprakash et al. (2017) that high root induction frequency in Noni was obtained in auxin-free media, particularly in one-fourth MS.

The time taken for *in vitro* germination of dormant seeds and disinfection of explants sourced from the wild could be reduced through embryo culture for rapid clonal multiplication. Consequently, matured embryo of Noni seeds were cultured on MS media of different strengths devoid of growth regulators while its shoot was regenerated on same media with basal application of BAP/Kn.

Considering the fast germination and successful shoot regeneration of Noni plants in this study, it could be inferred that matured zygotic embryo are good explants for the establishment of viable Noni plantlets but required low nutrients; 25 % MS to have optimum germination and growth. Whereas, its optimum shoot regeneration obtained in the presence of 2.0/1.0 mg/l BAP/Kn. This protocol is therefore recommended for establishment of clean Noni plantlets and for subsequent propagation of the species, shoot regeneration requires high nutrient supply in the range of 50 to 100% with 75% MS as optimum.

It may be concluded that *Morinda citrifolia* L. is a commercial multipurpose shrub with great economic importance, highly sought after among other medicinal plants by the pharmaceutical and herbal industry because of its many health benefits. Nonetheless, its propagation through conventional seedling development protocol had proven to be tedious, time-wasting and costly hence, the need for a more efficient propagation technique that would encourage a quick germination of its dormant seed and enhance its seedlings provision.

References

Almeida ES, de Oliveira D and **Hotza D** (2019) Properties and Applications of *Morinda citrifolia* (Noni): A review. Compr. Rev. Food Sci F. **18**(4): 883-909.

Cassileth RB (2010) Noni (*Morinda citrifolia*). Oncology (Williston Park) **24**(11): 1061.

Chandra KJ and **Dasari DGS** (2013) A review on the propagation methods of a miracle fruit *Morinda citrifolia* L. Indian J. Plant Sci. **2**(2): 78-84.

Gawad EA, Nehad MA, Mahdy HA and **Boshra ES** (2012) *In vitro* micropropagation protocol and acclimatization of coffee trees (*Coffea arabica* L.) J. Plant Production. **3**(1): 109-116.

Jan A, Bhat KM, Bhat SJA, Mir MA, Bhat MA, Imtiyaz A, Wani and **Rather JA** (2013) Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for *in vitro* culture. Afr. J. Biotechnol. **12**(39): 5749-5753.

Jayaprakash CM, Babu S, Minoo D, Madhusoodanan PV and **Prakashkumar R** (2017) An efficient *in vitro* propagation protocol for *Morinda citrifolia* L., an important medicinal plant. European J. Biomed. Pharm. Sci. **4**(11): 458-463.

Muthu G, Mathan K and **Karthikeyan R** (2006) Effect of sea weed *Ascophyllum nodosum* extract (Biozyme) along with conventional treatments on seed germination of *Morinda citrifolia.* Intl. J. Noni Res. **1**(2): 10-13.

Nelson SC (2001) Noni cultivation in Hawaii, University of Hawali CTAHR-Co-Operative Extension Service PD-19.

Potterat O and **Hamburger M** (2007) *Morinda citrifolia* (Noni) fruit-phytochemistry, pharmacology, safety. Planta Med. **73**(3): 191-199.

Qi H, Wen-jin H, Ying Y, You-qiang C and **Ru-kai C** (2007) Studies on the micropropagation of Noni (*Morinda citrifolia* L.). J. Fujian Norm. Univ. (Natural Science Edition). **01**: 87-90.

Raghavan V and **Srivastava PS** (1982) Embryo culture. *In:* Experimental embryology of vascular plants. Johri BM (Eds.), Springer-Verlag Berlin Heidelberg, pp. 195-222.

Rego MM, Rego ER, Nattrodt LPU, Barroso PA, Finger FL and **Otoni WC** (2014) Evaluation of different methods to overcome *in vitro* seed dormancy from yellow passion fruit. Afr. J. Biotechnol. **13**(36): 3657-3665.

Rezali IN, Sidik NJ, Saleh A, Osman IN and **Mohd AAN** (2017) The effects of different strength of MS media in solid and liquid media on *in vitro* growth of *Typhonium flagelliforme*. Asian Pac. J. Trop. Biomed. **7**(2): 151-156.

Selvaraj SA, Manju KL and **Subramani J** (2006) Micropropagation of *Morinda citrifolia*. Intl. J. Noni Res. **1**(2): 21-25.

Shekhawat MS, Kannan N, Manokari M and **Ravindran CP** (2015) Enhanced micropropagation protocol of *Morinda citrifolia* L. through nodal explants. J. Appl. Res. Med. Aromat. Plants **2**(4): 174-181.

Singh DR, Singh S and **Shajeeda BV** (2012) High performance liquid chromatography (HPLC) analysis of different parts of *Morinda citrifolia* L. Intl. J. Noni Res. **7**(1): 55-61.

Sreeranjini S and **Siril EA** (2014) Field performance and genetic fidelity evaluation of micropropagated *Morinda citrifolia* L. Indian J. Biotechnol. **13**: 121-130.

Srinivasan TR, Rethinam P and **Pratap PU** (2015) Pharmacological properties and clinical applications of *Morinda citrifolia.* Intl. J. Noni Res. **10**(1 & 2): 1-18

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