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GERMINATION EFFICIENCY OF MORINDA CITRIFOLIA SEEDS: IMPACT OF PRE-TREATMENT AND SUBSTRATE TYPE

Tunde Emmanuel Akinola
The Department of Crops & Horticultural Sciences, Faculty of Agriculture, University of Ibadan, Nigeria
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Abstract: Noni is a useful tropical fruit shrub, difficult to propagate due to seeds dormancy. Hence, a two-stage experiment was conducted to develop easily applicable rapid germination method for the crop. Firstly, seeds were subjected
to different pretreatments [nicking, soaking in 50% H_2SO_4 for 5 min., 800 ppm GA_3 for 24 h, hot-water at 40 °C for 24 h and control (untreated)]. Secondly, the most suitable pre-treatment method was applied to another set of seeds and sown in six sowing media [topsoil, river-sand (reference), sawdust, topsoil+river-sand (1:1), topsoil+sawdust (1:1) and river-sand+sawdust (1:1)]. A total of 2000 and 2400 seeds were sown in first and second stages, respectively. Each set of treatments was laid in completely randomised design with four replicates. Data collected were used to determine germination percentage (GP), mean germination time (MGT), germination index (GI) and mean germination rate (MGR). The results showed that there were significant
differences ($p \leq 0.05$) in the effects of the treatments for all the parameters considered at each of the stages. Nicking elicited highest GP (64.8%), MGR (0.026 sprouts/day), GI (1.79) and shortest MGT (38 days) at 12 weeks after sowing. Subsequently, the results of Morinda citrifolia seeds sown in different media indicated that topsoil/river-sand mixture significantly increased GP (81.5%) and GI (3.08) while topsoil gave highest MGR (0.038 sprouts/day) and shortest MGT (26.4 days). Hence, for commercial large scale seedling production, Noni seeds should be nicked and sowed directly in topsoil or a mixture of topsoil and river-sand.
Key words: germination index, germination percentage, nicking, river-sand, topsoil

INTRODUCTION

Morinda citrifolia L., commonly called Noni, belongs to the family Rubiaceae and is believed to have originated from South-East India and Asia to Australasia (Nelson and Elevitch, 2006; Muhammad *et al.*, 2017). The species is a shrub and primarily valued for its edible fruit as well as various medicinal benefits (Abbott, 1992). The seeds, fruit pulp, leaves, roots and bark of the shrub contains several macro and micro elements and phytochemicals that gives the shrub its high food, drink and health values (Ayunda *et al.*, 2020; Nwakanma and Akujuobi, 2022). Globally, Tahiti, India, US, Thai and Malaysia are currently leading the table in terms of cultivation, utilization and derivation of its economic benefits (Assi *et al.*, 2015). Unlike many of these countries, the commercial exploitation of Noni in Nigeria is restricted by several factors. These include's limited understanding of the appropriate methods of propa- gation, transplanting, fertilizer application, irrigation, pruning, shading, harvesting, post-harvest handling, processing and packaging (Antônio *et al.*, 2018). Hence, for Nigeria to fully utilize

the economic and industrial potentials of Noni, technical knowledge of the agricultural practices required for optimum crop performance has to be established.

Among the different propagation means available for Noni (seeds, stem or root cuttings and air layering) the preferred method of germination is by seeds (Rajamani, 2015). However, this is faced with the problem of seed dormancy (Elakkuvan and Manivannan, 2010; Natarajan et al., 2023). Noni seeds are triangular, brown in colour and have air sac attached to one end which makes the seeds buoyant (Bhoomika, 2015). In addition, the seeds is characterized by hard coat which imposed dormancy. Although dormancy helps in long term seed storage, it makes seeds becomes unreliable thus limiting its germination and commercial cultivation (Chandra and Sagar, 2013). While many of the reported researches on Noni has been directed on the compositional, nutritional and medicinal aspect of the crop, very little information is available on overcoming Noni seed dormancy and means of decreasing its period of germination.

Before now, it has been reported that untreated Noni seeds can take several months to a year before natural germination takes place (Nelson and Elevitch, 2006). Attempt to reduce the prolong period to a month resulted to the use of heat and chemical scarification (Chandra and Sagar, 2013). Some other reported methods with appreciable results include the use of sea weed extract (*Ascophyllum nodosum*), *Trichoderma* and gibberellic acid (Muthu *et al*., 2006; Singh *et al*., 2006; Singh and Rai, 2005). Nonetheless, these methods are expensive and not readily accessible to farmers in Nigeria willing to establish the crop. Consequently, there is need to develop a more appropriate propagation technique for the species to be able to meet demand. The use of different sowing media for propagation of seeds has been reported by several authors (Majekodunmi *et al*., 2021; Purwantoro, 2016; Ekwu and Mbah, 2007). Topsoil, river-sand and sawdust are generally used for seed propagation in the nursery because of their availability. Differently blended sowing media often vary in physico-chemical properties (Adubasim *et al*., 2018), and such variations could influence the germination of dormant seeds (Santosa *et al*., 2019; Ugwu *et al*., 2020). Yet, reports on *Morinda citrifolia* seed propagation in different sowing media are scanty. Hence, the present study sought to assess the germination of Noni seeds subjected to different pretreatments and sowing media.

MATERIALS AND METHODS

Study Area

The two-stage experiments were conducted at the Screenhouse of the Biotechnology Department, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. The site is located on longitude 07°23'18□□ N to 07°23'43□□N and latitude 03°51'20□□E to 03°23'43□□E. The weather reports of the study area during the period of investigation (February to April, 2022) indicated that average temperature was steady from February to March having the highest value (29 °C), while the temperature decreased in April with an average of 27 °C. On the contrary, average rainfall and relative humidity increased gradually towards April which also had the highest records for both parameters (27.61 mm and 77%, respectively).

Fruit Collection and Processing

Matured ripened Noni fruits were collected from FRIN arboretum. The seeds were extracted by pressing the fruits against a basket and washed under running tap water to remove the pulp. The seeds

were sun dried for 30-60 min., while those with good appearance and similar in size were sorted out and used for the study.

Sowing Media Collection and Analysis The sowing media used were top soil (0-15 cm depth), river sand and sawdust. The top soil was collected with shovel from the arboretum of FRIN, air dried, sieved through 2-mm mesh screen and sterilized at 100 °C for 1 h. The river sand was collected along Asanmagbe river bank in FRIN, thoroughly washed and drained. The river sand was sieved to remove large particles above 10 mm and then sterilised at same condition used for soil.

Sawdust was collected from Sango sawmills in Ibadan, Nigeria. The sawdust was soaked in water for 72 h to leach toxins and before being drained (Waddington *et al*., 1967; Bobo and Jackson, 2024). Each of the media was contained in 25 cm × 8.5 cm plastic basket. The sub-samples of the media used were taken to the Soil Laboratory of the Department of Soil Resources Management, University of Ibadan, to analyse for their water holding capacity (WHC), pH, total nitrogen (N), available phosphorus (P) and exchangeable potassium (K). The WHC of the media was determined by a method described by Bernard (1963). The pH was determined using pH meter in 1:2 soil/water suspension (McLean, 1982). Total N was determined using micro-Kjeldahl procedure (Bremner, 1996). Available P was extracted with Mehlich-III solution and analysed colorimetrically (Murphy and Rilley, 1962; Mehlich, 1984). Exchangeable K was determined using flame photometer (Jackson, 1973).

Experimental Design and Procedure Seed pre-treatment

The completely randomized design (CRD), involving five treatments with four replicates was used for the study. The pre-germination treatments involved nicking, soaking in 50% H_2SO_4 for 5 min., 800 ppm GA_3 for 24 h, hot-water at 40 °C for 24 h and control (untreated). A total of 2000 seeds were used for the experiment at 400 seeds per treatment and 100 per replicate. The seeds were sown in plastic baskets, filled with washed and sterilized river-sand to prevent damping off. The baskets were monitored and watered in the morning thrice a week to maintain adequate moisture content under screenhouse.

Sowing media

The CRD involving six sowing media with four replicates was used for the study. The most suitable pretreatment method (nicking) was used to pretreat seeds of *Morinda citrifolia* before sowing in different sowing media. Treatments were topsoil, river-sand (reference), sawdust, topsoil + river-sand (1:1), topsoil + sawdust (1:1) and river-sand + sawdust (1:1). A total of 2400 seeds were used for the experiment at 400 seeds per treatment and 100 seeds per replicate. Pre-treated seeds were sown in the plastic basket filled with the sowing media and the baskets were watered three times a week under screenhouse.

Data collection

Germination count was taken daily for 12 weeks after which the experiment was terminated. Germination was taken to have occurred when the plumule emerged from the sowing media surface. Data on germination were used to calculate germination percentage (GP), mean germination time (MGT), germination index
(GI) and mean germination rate (MGR) as follows:

$$GP = \left(\frac{\text{Total germinated seeds}}{\text{Total seeds sown}}\right) \times 100;$$

$$MTG = \frac{\sum(t_i \times n_i)}{\sum n_i};$$

$$GI = \left(\frac{G1}{1}\right) + \left(\frac{G2}{2}\right) + \cdots + \left(\frac{Gx}{x}\right); \text{ and}$$

$$MGR = \frac{1}{MTG};$$

where t_i is the number of days starting from the date of sowing and n_i is the number of seeds germinated at each day (Bewley and Black 1994); G is the number of seeds germinated in day 1, 2..., and x represents the corresponding day of germination (Botsheleng et al., 2014).

Data Analysis

Data collected on sprouted seeds were analysed using GenStat Discovery (Edition 4, VSN Int. Ltd., Hemel Hempstead, UK). One-way analysis of variance was used to determine variation and F value was signifi- can't at $p \leq 0.05$. Duncan multiple range test was used to compare means and indicate levels of difference.

RESULTS

Physical and Chemical Properties of Sowing Media Results of some initial physical and chemical properties of the sowing media used showed that their pH (H_2O) ranged from 4.0 (extremely acid) in sawdust to 6.6 (neutral) in river-sand (Table 1). Sawdust only had the highest value for total N (31.0 g kg^{-1}), available P (11.76 mg kg^{-1}), exchangeable K (1.13 cmol kg^{-1}) and WHC (97.10%), while river-sand had the lowest values (0.9 g kg^{-1}, 0.48 mg kg^{-1}, 0.04 cmol kg^{-1} and 22.50%, respectively). Moreover, higher values were obtained for total N, available P, exchangeable K and WHC in sawdust-containing media compared to others, except total N in topsoil/river-sand mixture.

Germination Attributes of Noni Seeds as Affected by Pre-Treatments

The effects of pre-treatments were assessed on the germination of Noni seeds under screen-house conditions. The results of the analysis of variance conducted on the germination percentage (GP), germination index (GI), mean germination time (MGT) and mean germination rate (MGR) of the seeds indicated that there were highly significant differences ($p \leq 0.05$) between the treatments for the germination attributes at 12 weeks after sowing (WAS) (Table 2). Nicking elicited highest GP (64.8%), which was significantly higher than control (untreated seeds) and other treatments. Moreover, GP for the untreated seeds was higher than the values obtained for each of H_2SO_4, GA_3 and hotwater treated seeds. The lowest GP (1.3%) was obtained from GA_3-treated seeds at 12 WAS.

Nicking also resulted in the shortest MGT (38 days) and highest rate of germination (0.026 sprouts day^{-1}) at 12 WAS (Table 2). These results were closely followed by those due to the control treatment (41.4 days and 0.024 sprouts day^{-1}, respectively) and H_2SO_4 (45.2 days and 0.022 sprouts day^{-1}, respectively) whereas, these three treatments had significantly ($p \leq 0.05$) lower MGT and higher GR than each of the hot-water and GA_3 treatments. The longest MGT (72.4 days) was obtained from GA_3 treated seeds throughout the investigation period. It was also evident that nicking positively influenced GI, having a value of 1.79 which was significantly higher ($p \leq 0.05$) than control and each of the other treatments. Moreover, the GI of the control (0.35) was higher than that of H_2SO_4 (0.06), GA_3 (0.02), and hot water (0.05) treatments during the same period.

Germination Attributes of Nicked Noni Seeds as Affected by Sowing Media

The results of the effects of sowing media on the germination of nicked Noni seeds indicated that there were highly significant differences ($p \leq 0.05$) among sowing media for germination percentage (GP), mean germination time (MGT), mean germination rate (MGR) and germination index (GI) (Table 3).

Noni seeds sown in topsoil/river-sand mixture had highest germination (81.5%), which was comparable to GP of seeds sown in topsoil (79.8%), but significantly different from that of the other media (Table 3 and Figure 1). There was similarity in the GP obtained for seeds sown in riversand (65.5%) and topsoil/sawdust mixture (59%), while GP for both media were higher than GP of seeds sown in each of sawdust only (15.5%) and river-sand/sawdust mixture (15%) at 12 WAS.

The results also indicated that the use of topsoil alone elicited the shortest mean germination time of 26.4 days which was similar to 26.7 days obtained for seeds sown in topsoil/river-sand mixture. However, the MGT of seeds sown in each of these two media had significantly lower ($p \leq 0.05$) germination time than seeds sown in each of the other media. The seeds sown in sawdust alone had the longest MGT of 72.4 days (Table 3). Similarly, the use of topsoil alone gave highest germination rate (0.038 seedlings day^{-1}), which was closely followed by topsoil/river-sand mixture (0.037 sprouts day^{-1}). Nonetheless, these two media significantly enhanced germination more than the other media, while sawdust alone gave the least mean germination rate of 0.014 seedlings day^{-1} at 12 WAS (Table 3). The results also revealed that nicked Noni seeds sown in topsoil/river-sand mixture had highest GI (3.08), which was similar to GI of the seeds sown in topsoil alone (3.05) whereas, the GI for either of these two media were significantly higher than the GI for each of the other media (Table 3). Moreover, the GI for seeds in river-sand only (1.8) and topsoil/sawdust mixture (1.79) were comparable, while GI values for each of these media were higher than that of river-sand/sawdust mixture (0.26) and sawdust alone (0.23), which had the least GI under the periods of investigation.

DISCUSSIONS

The highest germination percentage (GP) and germination index (GI) observed in nicked Noni seeds compared with other pre-treatments (soaking in 50% H_2SO_4 for 5 min., 800 ppm GA_3 for 24 h and hot-water at 40 °C for 24 h) and control (untreated) indicated that nicking was the best pre-treatment for seeds of *Morinda citrifolia*. These results could be attributed to the small opening created through nicking of the seeds coupled with favourable weather conditions. Noni seeds is said to require heat and optimum temperature for sprouting (Malik *et al.*, 2009). The degree of hotness during the time of conducting this experiment was slightly warm as indicated by the maximum average temperature (29 °C) recorded. These factors might have facilitated quick water imbibition and gaseous exchange, which might have led to quick initiation of germination processes thereby giving the nicked seeds an edge over other treatments. The performance of nicked seeds in this experiment is in agreement with the report of Nelson, (2005) that scarification particularly clipping or nicking can reduce germination time while promoting uniform germination in Noni seeds. This report is further supported by the shortest mean germination time (MGT) and mean germination rate (MGR) observed from the nicked seeds, though comparable to values from control and H_2SO_4 treatments for each of the attributes under the periods of investigation. These results obtained from nicked seeds of *Morinda citrifolia* in the present study were an improvement over that of Oliveira *et al.* (2011) who obtained best germination from Noni seeds cut and soaked in water for 48 h. Moreover, unlike nicking, the poor germination results obtained from H_2SO_4 treatment in this study, differ from that of some authors who obtained better germination from the use of H_2SO_4 (50% for 5 min.) alone or in combination with Biozyme (Ponnaiyan and Vezhavendan, 2005; Muthu *et al.*, 2006).

The highest GP and GI obtained from seeds sown in topsoil/river-sand mixture (1:1), which comparable to values for topsoil, indicated that both media were most suitable for the optimum germination of *Morinda citrifolia* seeds. This outcome was further established by the shortest MGT and highest MGR obtained from seeds sown in topsoil, which were similar to the values obtained for topsoil/river-sand

mixture. These results demonstrated that Noni seeds have preference for suitable sowing medium. Similar results were reported by Sudomo and Swestiani, (2018) that *Syzygium cumini* seeds pretreated with coconut water had highest GP and GR when sown in soil and sand mixture. These results could also be related to that of Mahmoud *et al*. (2019), when loamy soil, sandy soil and vermiculilte mixture (2:1:1) enhanced maximum germination of pistachio seeds.

The observed better results from topsoil only and topsoil/river-sand mixture in contrast to other growth media might be attributed to the moderate level of their water holding capacity (WHC), which could have allowed free drainage and good aeration. Seeds only require moisture, air and favourable weather conditions for germination (Stivers, 2017). The percentage of WHC in sawdust, topsoil/sawdust and river-sand/sawdust mixtures used for this study ranged between 72 to 97%. This means much water could have been retained by those media which might have prevented gaseous exchange thereby suffocating the seeds and slowed down germination. This explains the better results obtained from topsoil and topsoil/river-sand mixture with lower WHC compared with the sawdust containing media. However, the lower results obtained from river-sand unlike topsoil and topsoil/river-sand mixture could be attributed to its lowest level of WHC among the three media. The water level might not be enough to sufficiently penetrate the nicked Noni seeds within the short time thereby preventing comparable higher number of germinations as obtained in the other two media. Although, information regarding similar experiment on nicked Noni seeds germination is scanty nonetheless, these results could be related to that of Srigandha *et al*. (2021), when coffee (of the same family) seeds germination was greatly influenced by media used as red-soil, sand and vermicompost mixture recorded maximum germination percentage at 45 DAS.

CONCLUSIONS

This study has demonstrated the importance of seed pre-treatment and suitable growth medium for Noni seeds propagation. The results indicated that nicking was the best pre-treatment to overcome Noni seeds dormancy among others while topsoil alone and mixture of topsoil and river-sand (1:1) provided optimum support for best germination of nicked Noni seeds under screenhouse. Hence, in order to speed up and obtain uniform germination and seedlings growth, it is recommended that Noni seeds should be nicked and sowed directly either in topsoil alone or topsoil/river-sand mixture.

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