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Seed propagation of nutritionally rich selected underutilized tropical fruit species

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ABSTRACT

Tropical countries harbor numerous fruit species with unexplored commercial potential. To reintroduce these species into cultivation, it is crucial to establish effective propagation systems to ensure continuous supply. This research aims to determine the suitability of seeds for the mass propagation of 10 underutilized yet nutritionally rich fruit species in Sri Lanka. We investigated key seed characteristics of the 10 fruit species, including viability, water imbibition, germination, and the necessity of dormancy-breaking methods, to develop standard seed propagation protocols. Seed viability was assessed using three different concentrations of triphenyl tetrazolium chloride (TTC) solution, and viability percentages were calculated. Water imbibition, moisture content, and in vitro and in vivo germination percentages were measured for all species. Except for Antidesma ghaesembilla, all seeds were viable. The highest water absorption was recorded for Syzygium caryophyllatum over 48 hours. Microcos paniculata exhibited the highest seed moisture percentage (75.1±0.66 %), while Ziziphus oenoplia had the lowest (6.8±0.03 %). Under in vitro conditions, seeds of S. caryophyllatum and Cynometra cauliflora showed 100 % germination, followed by Antidesma alexiteria with 13.3 %. In vivo, S. caryophyllatum, A. alexiteria, Baccauria motleyana, C. cauliflora, and Phoenix pusilla exhibited more than 50 % germination. However, dormancy-breaking methods were unsuccessful for ungerminated seeds both in vivo and in vitro conditions. The propagation of S. caryophyllatum, A. alexiteria, B. motleyana, C. cauliflora, and P. pusilla through seeds can be recommended as a suitable method for large scale propagation and commercialization.

Keywords: Seed germination, seed propagation, seed viability, underutilized

INTRODUCTION

South, Southeast, and East Asia are rich in diverse tropical fruits and their wild relatives (Sebastian and Prasad, 2014). Many of these fruits are considered "underutilized" because their full potential has not been realized, despite their commercial development prospects (Tontisirin, 2014). These fruit crops can be described as having value but are not widely grown, rarely found in the market, and not cultivated commercially (Hare Krishna *et al*., 2019).

Sri Lanka boasts a rich diversity of around 230 fruit species spanning 57 plant families, many of which are classified as underutilized (Pushpakumara and

Heenkenda, 2007). Over 60 varieties of such fruits have been identified, which are predominantly cultivated in forests, marginal lands, and home gardens (Dahanayake, 2015; Malkanthi, 2017). Recognizing indigenous tropical fruits with commercial potential is crucial for researchers, farmers, and industries seeking opportunities to promote and commercialize these fruits (Dahanayake, 2015; Ratnayake *et al*., 2019). However, the unchecked harvesting of these fruits from the wild poses a threat to their survival in their natural habitats. Additionally, most underutilized fruits lack standardized vegetative propagation methods tailored to various agro-climatic zones, hindering their mass-scale commercialization.

Seed propagation is known to enhance genetic diversity within a species, which is crucial for both conservation and crop improvement efforts (Keerthika *et al.,* 2020; Bohra *et al.,* 2021). Despite this, the sexual propagation of underutilized fruit plants by seeds remains largely unexplored globally (Waman and Bohra, 2019). Moreover, there is a lack of extensive research on the quality, germination, viability and dormancy of seeds from these underutilized fruits (Maldonado-Peralta *et al.,* 2016). Ten underutilized fruit plants which have received little scientific attention in recent years, were studied under the research project “Smart Village as a Strategy for Socio-economic Development of Rural Communities in Sri Lanka” funded by Accelerating Higher Education Expansion and Development)AHEAD(Development Oriented Research)DOR(grants. To address these issues, in this research, seed characteristics such as viability, water imbibition, germination, and the need for dormancy-breaking methods were studied for 10 selected underutilized fruits plants to develop standard seed propagation systems.

MATERIALS AND METHODS

Selection of underutilized fruit plants and collection of seeds

The fruit plants for the research were selected following a reconnaissance survey of Sri Lanka. The fruit species examined in this research included *Syzygium caryopyllatum*, *Microcos paniculata*, *Antidesma ghaesembilla*, *Antidesma alexiteria*, *Baccaurea motleyana*, *Cynometra cauliflora*, *Phoenix pussilla*, *Psidium guineense*, *Ziziphus oenoplia* and *Elaeocarpus angustifolius.* Seeds were collected from mature, ripe fruits of a healthy, fully grown single mother plant. The extraction process involved manually chopping the flesh around the seeds. The seeds were then screened using the floating method. The floating seeds were discarded. The remaining seeds were chosen for further experiments and were stored at room temperature (25±2 ºC) until needed.

Viability of seeds

The seeds were soaked in 50 ml of water for 16 hours at room temperature (25±2 ºC). After soaking, they were sectioned longitudinally with a sharp blade and stained with 0.01 %, 0.05 %, and 1 % 2,3,5-triphenyl tetrazolium chloride (TTC) solution for 5 hours at 38 ºC. Three seeds from each species were stained at each TTC concentration, with three replicates for each treatment. Following staining, the TTC solution was discarded, and the seeds were rinsed with tap water. Seeds that turned red were considered viable, and the viability percentage was calculated following Sourabh, (2020).

$$\text{Viability percentage} = \frac{\text{Number of viable seeds}}{\text{Total number of seeds}} X\ 100$$

Seed water imbibition

Ten seeds of each species were placed in a petri dish lined with filter paper. A volume of 10 ml of distilled water was added onto the filter paper, and the setup was maintained at room temperature. Each species had three replicate petri dishes. At intervals of 0, 3, 6, 9, 12, 24, 36, and 48 hours each replicate seed was gently blotted to dry, weighed, and returned to its respective petri dish. The water uptake by each seed after each soaking period was then determined using the formula outlined by Shalimu *et al*. (2012).

$$\text{Imbibition percentage} = \frac{\text{Mass of the imbibed seeds}}{\text{Mass of the nonimbibed seeds}} X\ 100$$

Moisture content of the seeds

Ten seeds from each species were weighed and then subjected to drying in an oven at 80 °C for 48 hours. The percentage of seed moisture was determined using the equation provided by Shalimu *et al*. (2012).

$$\text{Seed moisture percentage} = \frac{\text{Fresh mass of the seeds}}{\text{Dry mass of the seeds}} X\ 100$$

Germination percentage of seeds

Germination tests were conducted both *in vitro* and *in vivo*. A hundred seeds of each species were soaked in water, and any floating seeds were discarded. The remaining seeds were then utilized to assess germination percentages both *in vivo* and *in vitro*.

***In vivo* seed germination and breaking seed dormancy**

For the experiment, three sets of eight seeds from each plant species were employed. These seeds were sown in seed trays filled with topsoil and watered three times weekly. Germination was determined by observing radicle protrusion from the seeds (Dinesh and Sushma, 2012). The count of germinated seeds was recorded over a period of 12 weeks. The *in vivo* seed germination percentage was calculated using the equation provided by Dinesh and Sushma (2012).

$$\text{Seed germinaton percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} X\ 100$$

Seed dormancy breaking techniques were applied to the non-germinating seeds of *M. paniculata*, *A. ghaesembilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius*. For these experiments, three sets of eight seeds from each of the mentioned plants were utilized. The seeds were first soaked in distilled water for 48 hours and then planted in trays filled with topsoil (Hossain *et al*., 2011). Mechanical scarification of the seeds was performed by rubbing them with sandpaper before planting in similar trays (Dinesh *et al*., 2019). Additionally, the seeds were treated with freshly squeezed lime (*Citrus aurantifolia*) juice for 15 hours to break dormancy, followed by planting in topsoil-containing trays (Eşen *et al*., 2009). The trays were watered three times weekly, and seed germination was monitored for a period of 12 weeks.

***In vitro* seed germination and breaking seed dormancy**

Fifteen seeds from each species underwent surface sterilization using ethanol (70 %) for 1 minute, followed by soaking in chlorox (5 %) for 10 minutes. Subsequently, the seeds were rinsed with sterilized distilled water. Five seeds were then placed in each Petri dish containing a moistened filter paper, with three replicate petri dishes utilized for each treatment. Germination was determined by observing radicle protrusion from the seeds, and the count of germinated seeds was recorded over an 8-week period (Elhindi *et al*., 2016). The *in vitro* seed germination

percentage was calculated using the equation provided by Elhindi *et al.* (2016).

$$\text{Seed germinaton percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} X\ 100$$

Economical and readily available techniques for seed dormancy breaking were applied to the surface-sterilized seeds that failed to germinate from *M. paniculata*, *A. ghaesembilla*, *P. pussilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius*. Following the method outlined by Elhindi *et al.* (2016), the seeds underwent surface sterilization. Subsequently, the seed dormancy breaking methods; soaking in water for 48 hours, mechanical scarification, and soaking in lime juice were applied as detailed earlier. Five seeds from each species were placed in petri dishes containing moistened filter paper, with three replicates used for each treatment. After 8 weeks, the seed germination percentage was calculated.

Analysis of data

One-way ANOVA and Tukey's pairwise comparison tests were conducted to assess significant differences among the means of seed viability percentages, seed imbibition percentages, seed moisture contents (Shalimu *et al.*, 2012), as well as *in vivo* and *in vitro* seed germination percentages.

RESULTS AND DISCUSSION

Viability of seeds

Seeds of all fruit plants except *A. ghaesembilla* were viable. The seeds of the other nine fruit species showed viability in at least one TTC treatment. The viability of the seeds of *A. alexiteria*, *B. motleyana*, *C. cauliflora*, *P. pussilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius* ranged from 83.33% to 100%. The viability of the seeds of *S. caryophyllatum* was 66.8%. The lowest seed viability was observed in *M. paniculata* at 16.67%. There were significant differences in seed viabilities among TTC concentrations for *S. caryophyllatum*, *A. alexiteria*, *C. cauliflora*, *P. pussilla*, *P. guineense*, and *Z. oenoplia* (Table 1).

Table 1. Viability percentages of seeds for each fruit plant species.

Fruit species	0.01% TTC*	0.05 % TTC*	1 % TTC*
S. caryophyllatum (A)	66.8 ^{a} ± 0	50.0 ^{b} ± 0	5.56 ^{c} ± 4.54
M. paniculata (B)	16.67 ± 0	16.67 ± 0	16.67 ± 0
A. ghaesembilla (C)	0	0	0
A. alexiteria (D)	22.22 ^{c} ± 4.54	72.22 ^{b} ± 4.54	100.00 ^{a} ± 0
B. motleyana (E)	94.44 ± 4.54	100.00 ± 0	100.00 ± 0
C. cauliflora (F)	0 ^{b}	0 ^{b}	100.00 ^{a} ± 0
P. pussilla (G)	100.00 ^{a} ± 0	77.78 ^{b} ± 4.54	0.00 ^{c} ± 0
P. guineense (H)	0.00 ^{c} ± 0	61.11 ^{b} ± 4.54	94.44 ^{a} ± 4.54
Z. oenoplia (I)	16.67 ^{b} ± 9.86	83.33 ^{a} ± 9.86	0.00 ^{b} ± 0
E. angustifolius (J)	72.22 ± 9.07	88.89 ± 9.07	83.33 ± 0

*Means sharing the same letters are not significantly different at $P < 0.05$ (n=3).

In this study, three concentrations of TTC (0.01 %, 0.05 %, and 1 %) were used to enhance the accuracy in determining the germination potential of seeds. Interestingly, different TTC concentrations resulted in varying seed viability percentages within the same species indicating that the concentration of TTC can significantly influence the results of viability tests. Notably, seeds of *A.*

ghaesembilla exhibited 0% viability across all three TTC concentrations tested. This lack of viability could suggest either the inherent non-viability of the seeds or potential inadequacies in the testing conditions, TTC concentration, staining period and temperature that might affect the effective staining of the embryo (Victoria, 2006). These findings emphasize the importance of optimizing TTC test conditions for each species to obtain accurate assessments of seed viability. Further investigation is needed to determine whether the lack of viability in *A. ghaesembilla* seeds is due to true non-viability or suboptimal test conditions.

Seed water imbibition

Water absorption by seeds of *P. guineense* (H) and *M. paniculata* (B) ceased after 6 and 12 hours, respectively (Figure 1). Seeds of *A. alexiteria* (D) and *E. angustifolius* (J) stopped absorbing water after 24 hours. Seeds of *A. ghaesembilla* (C), *B. motleyana* (E), *C. cauliflora* (F), *Z. oenoplia* (I), and *P. pussilla* (G) ceased water absorption after 36 hours. Seeds of *S. caryophyllatum* (A) ended absorbing water after 48 hours.

Moisture contents of seeds

There was a significant difference in seed moisture percentages among the 10 fruit plant species (Table 2). The highest seed moisture percentage was recorded in *M. paniculata* (B) at 75.1±0.66 %, followed by *P. guineense* (H) at 73.8±2.40 %. The lowest values were observed in *B. motleyana* (E) at 18.1±1.90 % and *Z. oenoplia* (I) at 6.8±0.03 %. The seed moisture contents of the remaining fruit species ranged between 29.2±0.02 % and 50.6±0.42 %.

Germination of seeds *in vivo* and breaking seed dormancy

Under *in vivo* conditions, only seeds of *S. caryophyllatum*, *A. alexiteria*, *B. motleyana*, *C. cauliflora*, and *P. pussilla* exhibited germination rates of more than 50 % (Table 2). The highest seed germination percentage was observed in *P. pussilla* (79.2 ±4.2 %), while the lowest was recorded in *B. motleyana* (50.0 ±0.0 %). Seeds of *M. paniculata*, *A. ghaesembilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius* did not germinate successfully under *in vivo* conditions. Furthermore, the applied seed dormancy breaking methods were not effective for any species under *in vivo* conditions.

Germination of seeds *in vitro* and breaking seed dormancy

Only seeds of *S. caryophyllatum*, *A. alexiteria*, and *C. cauliflora* germinated under *in vitro* conditions (Table 2). The germination percentage for *S. caryophyllatum* and *C. cauliflora* seeds was 100 %, while for *A. alexiteria* seeds, it was 13.3 %. Seeds of *M. paniculata*, *A. ghaesembilla*, *B. motleyana*, *P. pussilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius* did not successfully germinate under *in vitro* conditions. Furthermore, the applied seed dormancy breaking methods were ineffective for any species under *in vitro* conditions.

Based on the TTC test conducted in this study, the seeds of all fruit plant species were viable except for *A. ghaesembilla*. Consequently, seed propagation of *A. ghaesembilla* is not recommended at this time; however, further study is needed. Seeds from five species: *S. caryophyllatum*, *A. alexiteria*, *C. cauliflora*, *B. motleyana*, and *P. pussilla*, successfully germinated *in vivo*, suggesting seed propagation as a viable method for large-scale cultivation of these species. Among these, only three species (*S. caryophyllatum*, *A. alexiteria*, and *C. cauliflora*) also showed germination *in vitro*. Additionally, our previous research demonstrated successful propagation of *A. alexiteria* and *S. caryophyllatum* though stem cuttings using media commonly

employed by farmers indicating the suitability of both seeds and stem cuttings for large-scale propagation of these two species (Somasiri *et al.,* 2023). None of the seed dormancy breaking methods applied was successful for any species under both *in vivo* and *in vitro* conditions. Seed germination is influenced by seed genetics, hormones, and environmental factors during seed maturation (Abubakar and Attanda, 2022). Seed dormancy can be viewed as a barrier preventing germination of intact, viable seeds under favorable conditions. In this study, all seeds except those of *A. ghaesembilla* were viable despite not germinating. This highlights the need for further research into methods for breaking seed dormancy, as well as exploring various potting media and environmental conditions such as light intensity, temperature, and soil moisture, to enhance the germination potential of these species.

CONCLUSIONS

The fruit plant species studied exhibited different levels of seed viability. Propagation of *S. caryopyllatum*, *A. alexiteria*, *B. motleyana*, *C. cauliflora* and *P. pusilla* can be promoted by employing seed propagation for large scale production. However, the selected seed dormancy breaking methods were not successful in promoting germination of the seeds of the other tested fruit plant species indicating the need of further research to identify effective dormancy breaking techniques to enhance their germination and propagation potential.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2. Moisture and germination percentages of seeds for each fruit plant species.

Fruit species	Seed moisture percentage*	Seed germination *in vivo* (%)*	Seed germination *in vitro* (%)*
S. caryophyllatum (A)	28.3 [c] ± 0.69	58.3 [ab] ± 11.0	100.0 [a] ± 0.0
M. paniculata (B)	75.1 [a] ± 0.66	0.0 [c] ± 0.0	0 [c] ± 0.0
A. ghaesembilla (C)	29.2 [c] ± 0.02	0.0 [c] ± 0.0	0 [c] ± 0.0
A. alexiteria (D)	48.8 [b] ± 0.49	54.2 [b] ± 4.2	13.3 [b] ± 0.0
B. motleyana (E)	18.1 [d] ± 1.90	50.0 [b] ± 0.0	0 [c] ± 0.0
C. cauliflora (F)	50.6 [b] ± 0.42	66.7 [ab] ± 4.2	100 [a] ± 0.0
P. pussilla (G)	30.4 [c] ± 0.22	79.2 [a] ± 4.2	0 [c] ± 0.0
P. guineense (H)	73.8 [a] ± 2.40	0.0 [c] ± 0.0	0 [c] ± 0.0
Z. oenoplia (I)	6.8 [e] ± 0.03	0.0 [c] ± 0.0	0 [c] ± 0.0
E. angustifolius (J)	34.3 [c] ± 4.70	0.0 [c] ± 0.0	0 [c] ± 0.0

* Means sharing the same letters are not significantly different.

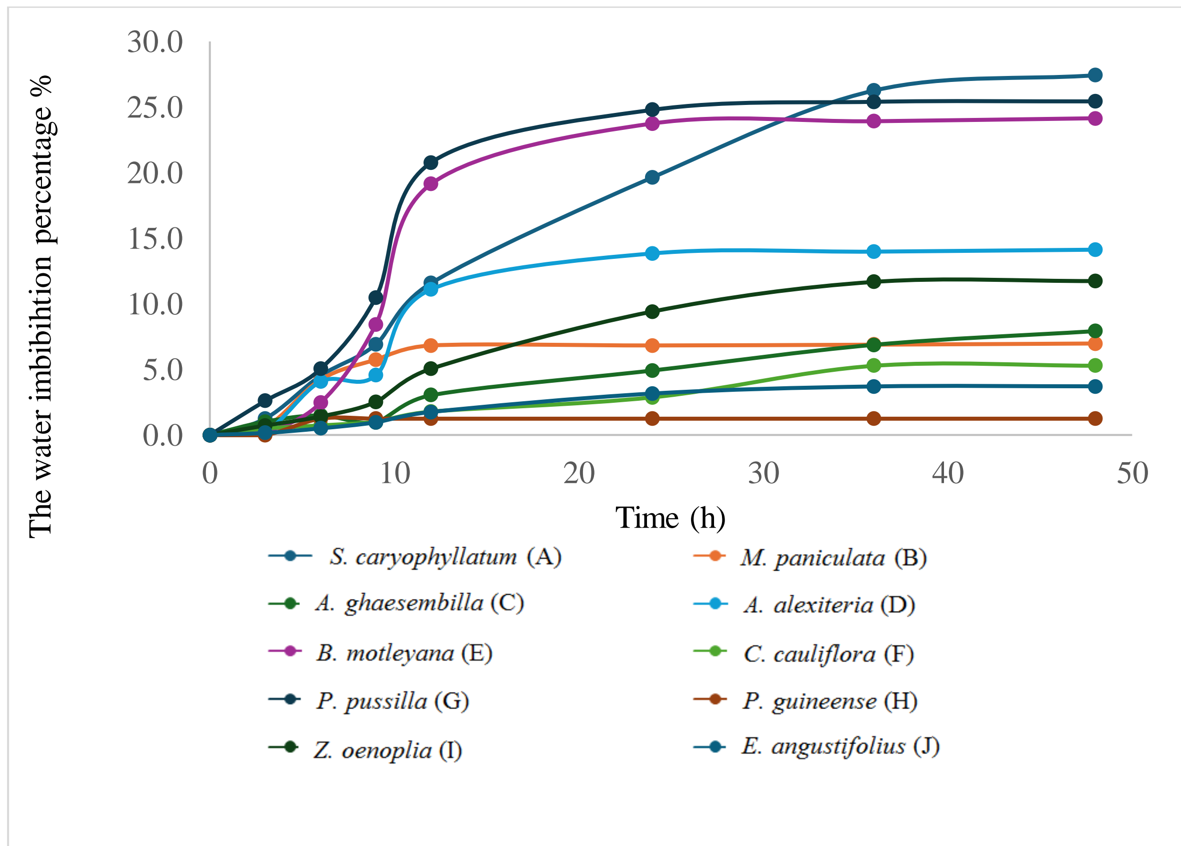

Figure 1: **The 48-hour water imbibition percentages of the 10 seed species.**