Research Article

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Dormancy breaking studies and seed germination in *Arenga wightii* Griffith

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Abstract

Arenga wightii Griffith, an endemic palm, of Western Ghats and south and central Sahyadris having multiple uses is currently under threat due to habitat loss and over exploitation. Since seeds are the major propagules of this palm, seed study was carried out with a view to conserve this highly promising tree, in both *ex-situ* and *in- situ* conditions. The study included seed viability, germination, desiccation and dormancy breaking tests. The results of the study suggest desiccation, GA_3/ acid treatment enhance germination, and dormancy observed is of both physiological and mechanical. The cost efficient means of germination induction is desiccation which could aid the farmers and common people in cultivating the species at large extent enabling reintroduction of the species.

Keywords: *Arenga wightii*, Desiccation, Dormancy, Seed germination, Storage, Viability

Introduction

Arenga wightii, (Arecaceae) commonly known as Wight's Sago Palm or Wild Coconut is a solitary monoecious palm of India, frequently found distributed in the evergreen forests of Western Ghats and south and central Sahyadris at an elevation of 300-1000m,where moisture content and humus are abundant (Renuka *et al,* 1996). The palm is pleonanthic as it starts flowering after 5-10 years of planting and perishes after producing a series of inflorescences. Male and female spadices are found distinct. At present due to habitat destruction and anthropogenic activities the population of the species is diminishing in its natural habitats and is categorized as vulnerable in IUCN Red List of Threatened plants (Johnson, 1998**).** The palm has medicinal properties and is widely used in folk medicine by the ethnic groups of Kerala and Tamil Nadu as a source of food, medicine and building material. Rajasekharan *et al*, (1990), reported that Kani tribe of Kerala has a custom of using fresh toddy from the peduncle of *Arenga wightii* internally for the treatment of Jaundice. Renuka, (1999), in her book "Palms of Kerala" stated that the starch from the trunk of *Arenga wightii* has medicinal properties and is used in the treatment of body rashes. Asha *et al*,(2002), described that Paniyan and Kani tribes of Malappuram and Wayanad districts of Kerala

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consume fresh toddy from the young inflorescence and juice of fruit husk as a cure for Jaundice. While documenting the wild edibles consumed by paniya tribe of Kerala, Narayanan *et al*, (2003), recorded that the tribe used the pith of *Arenga wightii* against venereal diseases. Sasidharan *et al*, (2006) stated that Malampandaram tribe of Periyar Tiger Reserve use the stem pith of *Arenga wightii* as a remedy for Jaundice. Similarly, Augustine *et al*, (2010), documented that Malampandaram tribe of Periyar Tiger Reserve, Western Ghats has a practice of serving little quantity of fresh toddy from the inflorescence of *Arenga wightii*, hardened with the roots of *Thottea siliquosa* to pregnant women to prevent abnormal child birth. This palm is also used for making brooms, palm wine and is a unique source of starch.

A. Habit, **B.** Male inflorescence, **C.** Infrutescence, **D.** Tricarpellary fruits, **E.** Seeds, **F.** Tetrazolium test, **G.** Seed germination

Materials and methods

Fruit/ Seed collection

Mature and ripened fruits were hand harvested from the natural population at Ponmudi as well as from the Palmatum of JNTBGRI, Thiruvananthapuram during the hot and humid months of April-May in two consecutive years, 2017 and 2018. The fruits were sorted, so that infected and mechanically damaged fruits were discarded. Seeds were extracted from fruits by depulping and washed in water to extract the immiscible seeds. Purity of the seeds was determined on the basis of pure seed number / water floating method. Morphological characters of fruits and seeds were documented along with photographs. Seeds were provided accession number and few seeds were curated as active and reference collection of JNTBGRI seed bank.

Moisture content determination

Seed moisture content was determined through high constant hot air oven method (ISTA, 2008). Seeds were surface dried on blotting paper at laboratory conditions (28 ± 2°C, 70% RH). For dry weight determination, the samples were taken in a pre-weighed bottle and weighed in an electronic balance, then dried in a hot air oven at 130^{0}C for 1 hour or until the weight became constant. For each test, 5 replicates of samples were used. Dry weight of each sample of each test was recorded after cooling to room temperature in a desiccator. Moisture content was determined by the following formula and was expressed as percentage.

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight- Dry weight}}{\text{Fresh weight}} \times 100$$

Seeds kept open in laboratory conditions served as control.

Germination test

Thirty seeds in three replicates were tested for germination. Germination test was conducted in wet rolled, acid-free, germination paper towels placed in a seed germinator without light maintained at 30 ± 2^{0}C/80% RH.

Seed viability tests

Seed viability was determined by conducting germination test as well as supporting viability tests. Both destructive (tetrazolium Test) and non-destructive methods (conductivity Test) were carried out.

Tetrazolium test (Moore, 1985)

Seeds were at first pre-soaked in sterile distilled water for 2 hours at room temperature

conditions (28 ± 2^{0}C/70% RH). Three replicates of 10 seeds each were used for a treatment. After pre-conditioning, the seeds were bisected longitudinally without damaging and exposing the embryo, and immersed in1% tetrazolium solution for 24 hours. The tetrazolium solution, after incubation, was decanted and seeds were rinsed thoroughly with water. The staining pattern of embryo, endosperm and cotyledon were noted.

Conductivity test

Three replicates of 10 each fresh as well as desiccated seeds were soaked in 50ml distilled water in a beaker. The beaker was covered to minimize evaporation and contamination by dust and was kept in open laboratory conditions (28 ± 2^{0}C/70% RH) for 24 hours. The leachate was collected after 24 hours soaking and conductivity test was performed using Systronics Conductivity meter (306) (Vieira *et al*, 2001). Specific conductance was expressed in µs cm^{-}1g^{-}1.

Desiccation

Seeds of one lot were desiccated to different moisture contents until lowest moisture content having substantial seed viability was attained. Seeds were subjected to both slow and fast desiccation. Slow desiccation was attained with seeds kept opened at laboratory conditions (28 ± 2^{0}C/70% RH). For fast desiccation about 100 seeds, tied in muslin cloth bags, in four replicates, were placed over freshly regenerated silica gel in a desiccator for different intervals. The silica gel in the desiccator was changed after every 24 hours. In both the conditions moisture content of the seeds were recorded at regular intervals. Germination tests were also done at each moisture content levels. Desiccated seeds were conditioned at 80% RH for every 24 hours prior to germination test to avoid imbibitional injury (Ellis *et al*., 1990). Germination data were recorded consequently.

Dormancy breaking treatments

The following pre-treatments were carried out for breaking the dormancy. All treatments were carried out in fresh seeds with 39 ± 1.32 % moisture content.

(a).GA_3 treatment: -Seeds were pre-treated with GA_3 solution of different concentrations (0, 50, 100, 250, 500, 1000, 2000, 3000, 4000 and 5000 ppm). For this 30 numbers of seeds in triplicates were immersed in GA_3 solutions of respective concentrations for 24 hours and after that seeds were tested for germination. Seeds immersed in distilled water for 24 hours served as control.

(b). GA_3 treatments were carried out in operculum removed seeds and also in scarified seeds.

(c). Acid scarification: - Seeds were pre-treated with concentrated Sulphuric Acid for different time durations (1, 3, 5, 7, 10, 12 and 15 minutes),followed by seeds washed in running tap water for 24 hours and tested for germination.

(d). Hot water treatment: - Seeds were pre-treated with 70^{0}C hot water for different durations (1, 3, 5, 7, 10 minutes) and tested for germination.

Speed of germination was calculated using the following formula (Czabator, 1962).

Speed of germination= n1/d1+n2/d2+n3/d3+----------

Where, n = number of germinated seeds, d= number of days.

Results and Discussion

Fruit is a globose – sub globose greenish black berry, tricarpellary syncarpus and sessile. Each fruit with three persistent green, small, triangular and

Table 1. Seed viability tests

Moisture content %	Germination %	Tetrazolium Test	Conductivity test
39 ± 1.32	20 ± 0.9	Embryo, endosperm and cotyledons not stained	8.35 ± 0.16µs
35 ± 1.4	70 ± 0.7	Embryo deeply stained, endosperm and cotyledons stained red	5.13 ± 0.02 µs
30.2 ± 0.96	68 ± 1.4	Embryo, endosperm and cotyledons stained red	5.67 ± 0.05 µs
27.4 ± 0.93	65 ± 1	Embryo, endosperm and cotyledons stained red	6.16 ± 0.09 µs
24 ± 1.7	60 ± 1.2	Embryo, endosperm and cotyledons stained feebly	6.49 ± 0.07 µs
22.4 ± 0.87	48 ± 0.5	Very slight colour change, embryo turned slight red	7.36 ± 0.3 µs
17 ± 0.71	Nil	No colour change	8.79 ± 0.5 µs

Data Mean ±SD

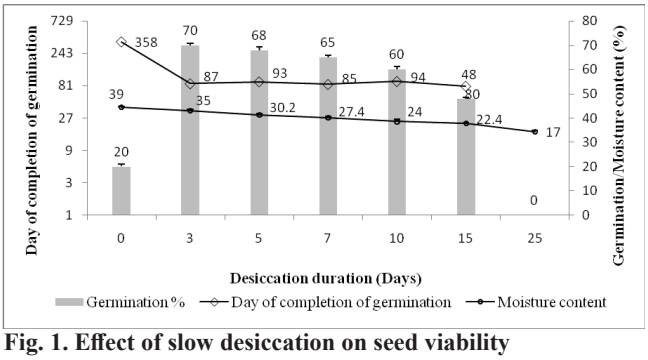

Fig. 1. Effect of slow desiccation on seed viability

valvate petals and three greenish yellow triangular acrescent sepals enclose 2-3 seeds having dimension [2.41 ± 1.1 x 2. 22 ±0.9 cm (LxB)] and weight (20.37 ± 0.7 g). Seeds are blackish brown, compressed or planoconvex, longitudinally striated with triangular apex and base. Seed coat is hard and embryo is lateral. Seeds have 2.12 ± 0.5 x 1.47 ± 1.3cm (LxB) dimension, 0.95 ± 0.5 cm thickness, and 2.31 ± 0.5gm weights.

Initial moisture content of the seeds was 39 ± 1.32 %. Fresh seeds registered only 20 ± 1.3 % germination, that too after an incubation period of 11 months. Seeds have dormancy, which has to undergo dormancy breaking treatments.

Among the two types of germination in palms (remote and adjacent), in *Arenga wightii* adjacent type of germination was observed. A small portion of the cotyledon emerges from the seed which appears as a swollen body abutting the seed surface and is called 'button'. The first seedling root or radicle is usually narrow and very short lived and is quickly replaced by roots formed at the seedling stem base (adventitious roots), the haustorium remains inside the seed absorbing food from the

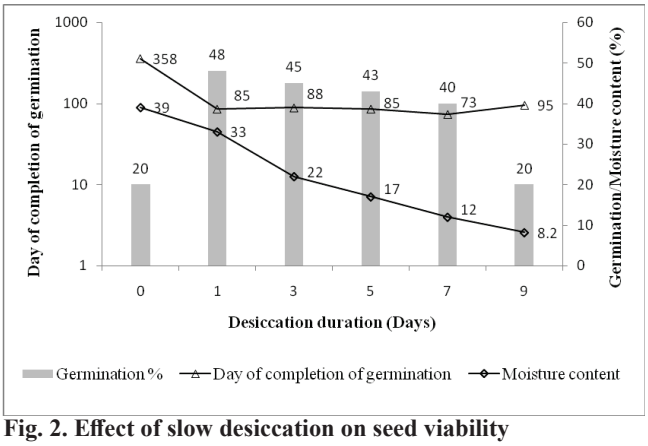

Fig. 2. Effect of slow desiccation on seed viability

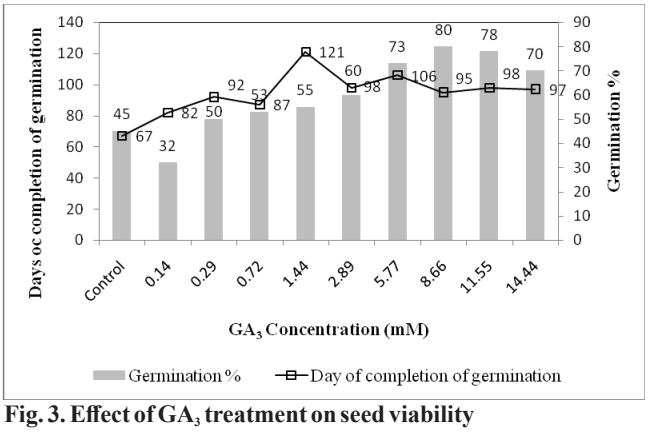

Fig. 3. Effect of GA_3 treatment on seed viability

endosperm. However, in remote type germination, the cotyledonary petiole close to the seedling root emerges early from the seed. The cotyledonary petiole grows downwards into the soil and swells at its base from which radicle and plumule emerges. The actual cotyledon or seed leaf remains inside the seed which is haustorium and it transfers nutrients from the endosperm to the young seedlings. Here radicle persists for some time and produces lateral roots

Viability is one of the most important aspects of seed quality because it is crucial that seeds conserved in a gene bank can develop in to normal plants for future use. In tetrazolium test, seeds with 35 ± 1.4% moisture content exhibited high germination % (70 ± 0.7) maximum staining intensity and low conductivity value. Staining intensity was found to decrease with the reduction of germination %, while conductivity value increases with the reduction of germination % (Table – 1). This is in conformity with the fact that seed viability is directly proportional to staining

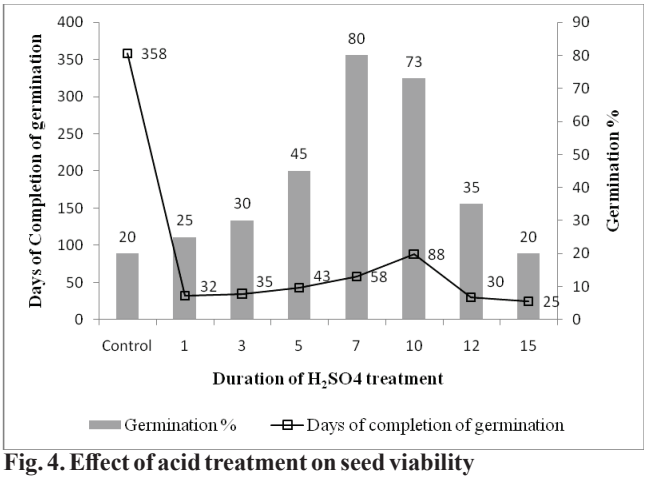

Fig. 4. Effect of acid treatment on seed viability

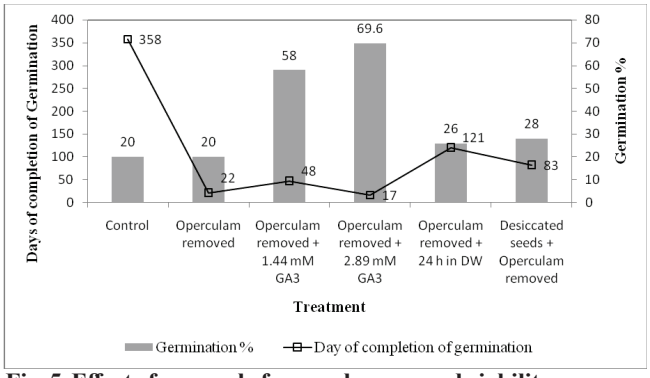

Fig. 5. Effect of removal of operculum on seed viability

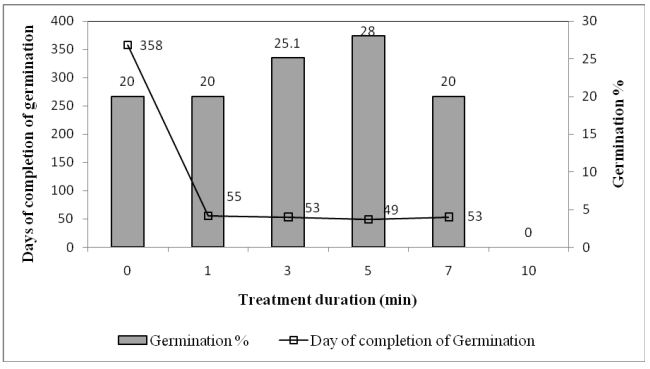

Fig. 7. Effect of hot water treatment on seed viability

intensity and inversely proportional to conductivity values. Fresh seeds of *Arenga wightii* have low germination %, very low staining intensity and high conductivity value. Similar results of tetrazolium test were reported in *Jatropha curcas* seeds (Kak *et al*, 2009) and also in *Baliospermum montanum* seeds (Gupta *et al*, 2010). Simon and Raja-Harun (1972) and Halloin (1975) carried out conductivity test in the seeds of *Pisum sativum* and *Gossypium hirsutum*, respectively and observed a negative correlation between viability and leachate conductivity.

When seeds are subjected to slow desiccation, within 3 days, moisture content is reduced to 35 ± 1.4% with 70 ± 0.7% germination. Germination % is retained up to 60 ± 1.2% with the reduction of moisture content to 24 ± 1.7% within 10 days. Within 25 days, moisture content is reduced to 17 ± 0.71% and seeds lost viability completely (Fig. 1). In fast desiccation, the rate of loss of viability is more rapid, as germination % could not be increased considerably. Within a week moisture content is reduced to 12 ± 0.67 with a corresponding germination % of 40 ± 1.4 (Fig.2). This may be due to the desiccation stress and hence it can be interpreted that desiccation may enhance germination process to some extent. When speed of germination was calculated, peak values are with seeds with higher germination % (Fig.1 and Fig.2). Since speed of germination varies with day of completion of germination it did not show correlation with germination percentage.

Higher concentration of GA_3 enhanced germination percentage. Pre-treatment with 8.66 mM (3000ppm) solution enhanced germination % to 80 ± 0.7 within 95 ± 1.3 days followed by 11.55 mM (4000ppm) solution which resulted 78 ± 0.51% germination within 98 ± 1.4 days. Control seeds showed only 45 ± 0.13 germination within 67 ± 0.5 % days (figure – 3).

When fresh seeds were pre-treated with concentrated H_2SO_4 for different durations (1 to 15 minutes), seeds pre- treated for 7 minutes showed enhanced germination percentage to 80 ± 0.7 within 58 ± 0.5 days followed by 10 minutes pre-treatment with 73 ± 0.2% germination within 88 ± 0.6 days (figure – 4).

Seeds when tested for germination, after removing the operculam, showed no effect, but seeds pre-treated with GA_3 2.89 mM (1000ppm) solution after removing the operculam, showed germination of 69.6 ± 1.2% within 17 ± 0.54 days. Same seeds pre-treated with GA_3 1.44 mM (500ppm) solution after removing the operculum showed 58 ± 0.6% germination within 48 ± 0.4 days (figure – 5).

Scarification also did not improve germination significantly. Scarified seeds pre-treated with GA_3 2.89 mM (1000ppm) solution recorded 65 ± 1.1% germination within 83 ± 1.4 days. Same seeds pre-treated with GA3 1.44 mM (500ppm) solution after

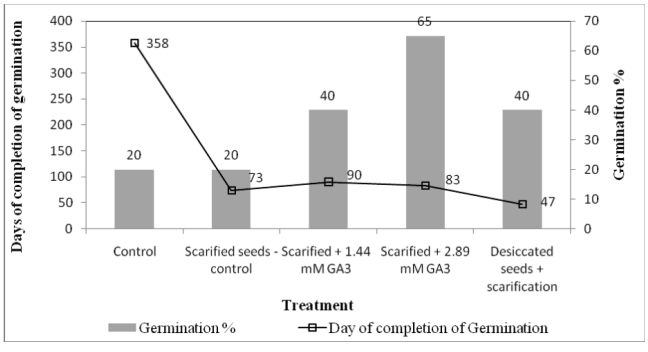

Fig. 6. Effect of scarification on seed viability

scarification show 40 ± 0.7% germination within 90 ± 0.6 days (figure- 6).

Hot water treatment had no effect on increasing seed germination. Seeds showed a maximum of 28 ± 1.3% germination within 49 ± 1.7 days (figure – 7). Seeds are found to decay after the germination and germinated seeds also did not survive.

Both the GA_3 and acid treatments enhanced germination percentage, but more speedy germination was found during acid treatment. Since 80% germination was registered within 98 ± 1.4 days in GA_3 treatment, as against 58 ± 0.5 days in acid treatment, dormancy may be both mechanical and physiological.

GA_3 has been established as an effective dormancy breaking growth regulator in many plant species including maize (Rood *et al*, 1990), in Macaw palm (Oliveira *et al*, 2013), in *Gentiana rigescens* seeds (Yang *et al*, 2011), in five species of Cacti (Rojas- Arechiga *et al*, 2011) and in *Penstemon digitalis* (De Mello *et al*, 2009).

Mechanical dormancy in palms is due to restriction of embryo protrusion during germination by operculum of the seeds as reported in *Elaeis guineensis* (Myint *et al*, 2010), in *Pritchardia remota* (Perez *et al*, 2008) and in Macaw palm (Reberio *et al*, 2011). GA_3 treatment after removing the operculum was found effective in *Pritchardia remota* seeds (Perez *et al*, 2008).

Pre-treatment with conc. H_2SO_4 found to enhance seed germination in legume seeds (Argel and Paton, 1999), in three species of *Stylosanthes* (Nan *et al*, 1998), in *Santalum album* (Nagaveni and Srimathi, 1980) and in *Koelreuteria paniculata* (Rehman and Park, 2000).

It can be concluded that the dormancy in *Arenga wightii* seeds was due to the combined effect of physiological and mechanical parameters and it can be overcome by desiccation / GA_3 and acid treatment. The highest germination percentage was recorded when seeds are pre-treated with GA_3 8.66 mM (3000 ppm) solution and also pre-treated with conc. H_2SO_4 for 7 minutes. Speedy germination was found in acid treatment. When operculum removed seeds were pre-treated with GA_3 2.89 mM (1000ppm) solution, recorded 69.6 ± 1.2% germination within 17 ± 0.54 days. The most cost effective method to improve germination was found to desiccate seeds in open laboratory condition (28 ± 2^0C/70% RH, slow desiccation) for 3 days to decrease the moisture content to 35 ± 1.4% and when such seeds were tested for germination 70 ± 0.7% germination was occur within 87 ± 0.8 days.

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