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Identifying methods for rapid and uniform germination and storage conditions for seeds of kithul (*Caryota urens* L.)

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Abstract

This experiment was conducted to identify methods to get rapid and uniform germination and packaging material for storing Kithul seeds. The study was conducted at the University seed testing laboratory, Dodangolla, Kundasale. Pericarp removed seeds were subjected to nine treatments: removal of portion of endosperm (t_2), rubbing of seed coat using a sand paper (t_3), hot water treatment 80^{o}C for 5 seconds (t_4), for 30 seconds (t_5), oven heating at 60^{o}C for 10 seconds (t_6), for 2 minutes (t_7), for 5 minutes (t_8), concentrated sulfuric acid for 10 seconds (t_9), 0.5M sulfuric acid for 10 seconds (t_{10}) and untreated seeds (t_1). Numbers of seeds germinated at weekly intervals were recorded. Seeds stored with and without pericarp in sealed glass bottles, polythene and paper bags were tested for viability at monthly intervals. Germination percentage was evaluated after keeping Kithul seeds in sealed polythene bags filled with moist sand for two months. Results showed that the removal of pericarp completely and rubbing of seed coat overlying the embryo was the most effective treatments to break the dormancy. However, oven heating or H_2SO_4 acid treatments are better suited for large scale operations. Use of sealed containers is the best method to store seeds. Keeping seed mixed with moist sand in sealed polybags for germination is a promising method in handling large quantities of seed. Planting seeds as soon as collection is essential as viability of seeds were reduced to 85.5% from 92.2% after one month in the storage.

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Introduction

Kithul (*Caryota urens* L.) belongs to the family Arecaceae and is considered as a multipurpose tree in Sri Lanka. The species naturally inhabits the understorey of lowland rainforests in Sri Lanka (World Agroforestry Center, 2018). It is an evergreen tree growing to a height about 12m and takes about 15 years to reach the maturity. It prefers moist and well-drained soil conditions. It can be established on wide range of pH conditions, very acidic to very alkaline soils (PFAF, 2018). Similarly Kithul thrives on wide range of light levels, full shade, semi-shade or even under open conditions (PFAF, 2018; World Agroforestry Center, 2018). This versatile species is native to Sri Lanka (World Agroforestry Center, 2018) and is popular among the rural communities. It is known as jaggary palm or toddy palm as the sap extracted from the inflorescence is used to make sugar and alcoholic beverages. The tree also has many medicinal uses.

Recently Kithul gained importance as a commercial species in floriculture industry. It is a commonly marketed as an ornamental tree and leaves are sold as cut foliage. Low germination rates, uneven germination, long duration taken for germination due to hard seed coat and recalcitrant nature of seeds are major problems in large scale commercial propagation of species. Most palm seeds are short-lived and viability gets reduced rapidly within a few days, months or at most a year following harvesting (Hartmann *et al.*, 2010). Meanwhile Marcus and Banks (1999) have reported that fresh seed, good sanitation, proper medium, proper hydration, and adequate heat as the most important conditions for successful germination of palm seeds. General belief among rural farmers in Sri Lanka is that Kithul seeds should pass through the digestive system of either polecat or palm civet, the two major distributing agents of Kithul seed.

The Kithul seeds consist of endosperm and this is covered with endocarp (inner hard layer) which is attached to the seed, fleshy mesocarp and smooth epicarp. The embryo is embedded in the endosperm at one end. Mesocarp contains an irritant, which may cause skin damage if get contacted directly.

It is assumed that the pericarp (seed coat) of Kithul contain germination inhibitory substances (Taiz and Zeiger, 2010).

This experiment was conducted to identify suitable techniques to get rapid and uniform germination, and to identify suitable method for storing Kithul seed. Further a bag technique that is keeping seeds in sealed polythene bags filled with moist sand was evaluated as a method for germinating Kithul seeds.

Materials and methods

This experiment was conducted at the seed testing laboratory of the Agricultural Experimental Station, Faculty of Agriculture, University of Peradeniya located at Dodangolla, Kundasale in the IM_3 agro ecological region of Sri Lanka (Punyawardena, 2008). The daytime temperature varied from 26-34°C and relative humidity from 72-90% during the experimental period. Fresh fully ripen (i.e. blackish purple colour) fruits of *Caryota urens* were plucked from a strong and healthy trees.

Experiment 1: Identifying seed treatments to induce germination

Seeds were collected from three mature and healthy trees. Each replicate comprised of fifty seeds and there were 10 replicates for each treatment. Total of 500 seeds were collected from each tree and 1500 seeds were used for the whole experiment. Seeds were soaked in water for two weeks and they were cleaned by removing the pericarp. They were subjected to nine treatments: removal of portion of endosperm (t_2), rubbing of seed coat using a sand paper (t_3), hot water at 80°C treatment for 5 seconds (t_4) and for 30 seconds (t_5), oven heating at 60°C for 10 seconds (t_6), for 2 minutes (t_7) and for 5 minutes (t_8), concentrated sulfuric acid for 10 seconds (t_9), 0.5 M sulfuric acid for 10 seconds (t_{10}) and untreated seeds (t_1). Numbers of seeds germinated at weekly intervals were recorded. Treated seeds were planted on seed trays filled with sterilized sand and coir dust media mixed at 2:1 ratio. These trays were kept inside a propagator made using polythene to facilitate germination. Germination rates under different seed treatments were analyzed using CATMOD (Categorical Data Module) procedure in a SAS system.

Experiment 2: Identifying seed storage conditions

Viability of seeds stored with and without pericarp using different packaging materials namely, glass bottles, black polythene, transparent polythene and paper bags were examined for three months. Stored seeds were subjected to tetrazolium test and numbers of viable seeds were recoded.

Kithul seeds for the experiment was collected fresh fully ripen fruits harvested from strong and healthy mother tree from Peradeniya area. Half of the seeds collected were soaked in water for two weeks and pericarp was removed. Other half was kept without removing the pericarp. Then the damaged seeds by pest and other means, oversized and undersized were removed. A large proportion of seeds were found damaged by seed weevil. Low weight and presence of external cavities were the signs of weevil damage. Seeds were treated with a fungicide and a pesticide before applying storage treatments. Seeds were then stored in glass bottles, polythene bags and paper bags, separately. Each storage condition was replicated thrice and each replicate contained hundred seeds. All seeds were stored in room temperature. Seeds were subjected to tetrazolium test after one, two and three months in storage to test the viability.

To prepare for the tetrazolium test, pericarp was removed in seeds that were stored with it. Then the non-essential part of the hard seed was damaged using a secateur to facilitate water absorption. After that the seeds were fully immersed in water and left two days until completely imbibed. Water absorbed, swelled seeds were selected for the test. They were split into two using a sharp secotier to expose embryo. These segments were transferred to tetrazolium solution (1% 2.3.5 Triphenyl tetrazolium chloride) in petri dishes. They were covered and kept in dark for six hours to allow staining of embryos. Then the seeds were washed with water and segments were classified into four groups (Highly viable, medium viable, low viable and non-viable) based on staining pattern, using a magnifying glass.

Experiment 3: Evaluation of bag technique for germinating Kithul seeds

The objective of this experiment was to evaluate the "bag technique" that is keeping seeds in sealed polythene bags filled with moist sand as a method of germinating a large quantity of Kithul seeds. The pericarp removed Kithul seeds were mixed thoroughly with moist sand sealed in a sturdy polythene bag. Moist sand was used as a moisture retaining medium. The bag was then placed in a warm shady position (i.e. under a bench in a plant house) as palm seeds require prolonged exposure to high temperature and high humidity for successful germination (Jones, 1995; Marcus and Banks, 1999). Each bag contained fifty seeds and was replicated three times. Germination percentage was evaluated after two months.

Results and discussion

Experiment 1: Identifying seed treatments to induce germination

Statistical analysis also revealed that block effect is significant (P=0.05) indicating high tree-to-tree variation in relation to the quality of seed produced as each tree was considered as a block. Seeds of treatment 1 and 4 did not germinated during the experimental period. Hence they were not considered during data analysis. Seeds of the treatment 1 fail to germinate due to presence of pericarp. Gunaratne *et al,* (1996) showed that seeds of Kithul planted without removing the pericarp did not germinate in a short period as pericarp created an inhibitory effect on germination. Hard seed coat of palm species act as a physical barrier for absorbing water to initiate germination (Jones, 1995). Seeds subjected to hot water treatment (80^{o}C for five minutes) did not germinate because of loss of viability.

"Z" values calculated for germination percentages at third, fifth and eighth week using estimated value and standard error are presented in the Table 1. When "Z" value is more than 1.96 then the differences of the means of two treatments compared is considered to be significant (P=0.05) whereas differences of means are non-significant (P=0.05) when "Z" value is less than 1.96. The obtained "Z" values were grouped in to three.

Table 1. "Z" values estimated after comparing the means of different treatments.

Group	3rd week		5th week		8th week	
	Compared treatments	"Z" Value	Compared treatments	"Z" Value	Compared treatments	"Z" Value
1	2-6	0.18	2-7	0.00	6-7	0.49
	2-7	0.86	2-8	1.43	6-8	0.62
	2-8	1.93	2-9	0.88	6-9	1.77
	2-10	1.21	2-10	0.74	6-10	0.66
	6-7	1.06	6-8	0.95	7-8	0.12
	6-10	1.45	7-10	0.74	9-10	1.81
	7-8	1.08	8-10	1.26		
	7-10	0.02				
	8-10	1.56				
2	2-5	3.35	2-6	2.37	2-3	2.35
	2-9	4.37	3-9	5.70	2-6	5.17
	3-7	5.68	5-6	4.02	2-7	5.62
	3-8	4.74	5-8	4.87	2-8	5.73
	3-9	2.26	6-7	2.37	2-9	3.52
	5-6	3.22	6-9	3.24	3-6	4.60
	5-7	3.91	6-10	2.54	3-7	5.06
	5-8	4.55	8-9	2.31	3-8	5.18
	5-9	5.92	9-10	2.01	3-9	2.91
	5-10	4.27			3-10	2.91
	6-8	2.21			7-9	2.27
	6-9	4.52			8-9	2.39
	7-9	3.60				
	8-9	2.57				
	9-10	5.63				
3	2-3	6.35	2-3	6.51	2-5	10.78
	3-5	7.00	2-5	6.10	2-10	6.09
	3-6	6.49	3-5	11.25	3-5	10.30
	3-10	9.07	3-6	8.56	5-6	6.63
			3-7	6.51	5-7	6.18
			3-8	7.77	5-8	6.07
			3-10	9.35	5-9	8.15
			5-7	6.10	5-10	9.20
			5-9	6.84		
			5-10	6.94		

Treatment 9 (10 seconds in conc. H_2SO_4) obtained higher germination rate at the beginning. H_2SO_4 acid softens the seed coat and facilitates the germination. Kelly and Van Staden (1985) have reported that H_2SO_4 acid could directly increase the permeability of seed to water. Treatment 5 (hot water treatment for 10 seconds) seems to have reduced the viability and also the germination rate.

After 8 weeks the differences between means of treatments 6, 7, 8, 9 and 10 were non-significant (P=0.05). Differences between means of treatment 2 and others (3, 5, 6, 7, 8, 9 and 10), 3 and others (2, 5, 6, 7, 8, 9 and 10) and 5 and others (2, 3, 6, 7, 8, 9 and 10) were significant (P=0.05). After 8 weeks, highest germination (82%) was obtained by treatment 2 that is removal of portion of the endosperm followed by treatment 3 that is rubbing the seed coat with a sand paper (79%).

The germination rates for the treatments 6, 7, 8, 9 and 10 were 56%, 53%, 53%, 65% and 53%, respectively. Other than treatment 1 and 4 lowest germination (21%) was recorded with treatment 5 (hot water treatment at 80°C for 30 seconds).

The results of the present study shows that mechanical treatments are the best for breaking the seed dormancy as reported by other authors (Jones, 1995; Copeland and McDonald, 2001; Hartmann *et al.*, 2010). These treatments will allow gradual absorption of water (Copeland and McDonald, 2001) and will initiate the enzymatic activities resulting seed germination (Mayer and Maber, 1982). However, both treatment 2 and 3 are difficult to apply at commercial scale but can be used at small scale. Hence oven heating or H_2SO_4 acid treatments has to be considered for large scale operations.

Ranasinghe *et al.* (2008) have found that the complete removal of fleshy pericarps of ripe *C. urens* fruits is the most effective way to achieve a higher rate of germination. Rodrigo *et al.* (2012) when *C. urens* seeds treated with 50% HNO_3 for 5 minutes recorded high germination rate (85%) followed by 30% HNO_3 for 5 minutes (77.5%) and tepid water for 24 hours (75%) as compared to untreated control (10%).

Experiment 2: Identifying seed storage conditions

General Linear Model conducted to analyze the results of tetrazolium test indicated that the effect of pericarp and packaging material interaction (P=0.7854) was non-significant on seed viability. But pericarp and time interaction (P=0.0021) and packaging material and time interaction (P=0.0042) showed significant effect on seed viability. The effect of interaction between above three factors were significant at P=0.0024 level.

Table 2. Summary of treatment effects on viability of Kithul seeds.

Treatment interactions	Treatment effects (P=0.05)
Pericarp x Packaging material	Non-significant
Pericarp x Time	Significant
Packaging material x Time	Significant
Pericarp x Packaging material x time	Significant

Mean comparison by Duncan multiple range test indicated that after one month, seed viability is high and not different between seeds stored with pericarp and without pericarp when stored in glass bottles and polythene bags. But when stored in paper bags viability has reduced significantly than when stored with other two types of containers especially when stored without pericarp. It can be concluded from the viability results obtained after two and three months of storage of Kithul seeds that if seeds are going to be stored beyond a month then significantly higher viability can be obtained when they are stored with pericarp than without it. Overall results of the experiment shows that glass bottles and polythene bags are equally good and are significantly better than paper bags for storing Kithul seeds to obtain high viability.

Table 3. Viability percentage after storage.

Packaging material	Time	Viability %	
		With Pericarp	Without Pericarp
Glass bottle	After 1 Month	85.6[a]	81.1[a]
Polythene bag		85.6[a]	81.1[a]
Paper bag		70.0[b]	56.8[d]
Glass bottle	After 2 Months	81.1[a]	64.4[c]
Polythene bag		80.0[a]	62.1[c]
Paper bag		40.0[e]	34.4[f]
Glass bottle	After 3 Months	71.1[b]	58.9[d]
Polythene bag		71.1[b]	57.8[d]
Paper bag		26.7[g]	16.7[h]

Key: Difference of means denoted by same letter are non-significant.

Both high and medium viable seeds have a good potential for germination and perform well under field conditions whereas seedlings produced by the low viable seeds would not survive under adverse conditions and also would fail to produce vigourous seedlings even under favourable field conditions (AOSA, 1976).

The present study confirms the recalcitrant (Unorthodox) behavior of seeds of Kithul. It is reported elsewhere that at room temperature Kithul seeds could remain viable for 30 - 90 days, depending on storage conditions (PFAF, 2018). Exposure of seeds to direct sunlight for 6 hours prior to sowing inhibits germination (World agroforestry center, 2018). Satisfactory germination could be obtained by placing seeds in a moist, dark environment (PFAF, 2018; World agroforestry center, 2018). Wood *et al*, (2006) have showed that *C. urens* is a desiccation-sensitive species as it failed to germinate after drying. When a significant amount of moisture is lost (i.e. the cut-off limit is considered as 20% loss of moisture), the kernel which contains the embryo will shrink away from the walls of the endocarp. Such seeds will lose their viability and will not germinate.

Experiment 3: Evaluation of bag technique for germinating Kithul seeds

The results shows that bag techniques (i.e. storing seed mixed with moist sand in a sealed polythene bag) is a highly successful method in germinating Kithul seeds. The germination initiated within two weeks while in the bag and germination percentage reached 84% by two months (Table 1). The seedlings were vigorous and healthy and were large enough to plant in a pot after two months.

This shows that this technique has provided suitable conditions that is adequate moisture and temperature for successful germination of Kithul seeds.

Table 4. Germination percentage after two months in the sealed polythene bags.

Replicate number	Germination percentage (%)
1	84
2	88
3	80
Mean	84

Conclusions

The results of the study show that removals of hard seed coat by mechanical methods are more successful for inducing germination of Kithul seeds. However, both mechanical treatment experimented under the present study are difficult to apply at commercial scale. Hence oven heating or H_2SO_4 acid treatments has to be considered for large scale operations. Use of sealed (air tight) containers is a very effective method to store Kithul seeds. Glass bottles are better for storing of Kithul seeds. Also it appears that use of paper bags and black polythene is not suitable for storing Kithul seeds. Seeds with pericarp can maintain viability for longer period than seeds stored without pericarp. Planting seeds as soon as collected is essential to obtain high percentage of germination. Germination of *C. urens* commences less than two weeks from planting of seeds. The bag technique is a very useful method for handling large quantities of seed. Further, there is a tree-to-tree variation in relation to quality of seeds produced.

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