Research Article

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Effect of desiccation on viability of recalcitrant arecanut (*Areca catechu* L.)

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Abstract

Arecanut is propagated only by its seeds and the seed storage behaviour was obscure. The influence of desiccation on the seed viability and germination were evaluated in the arecanut varieties. Various methods like germination recording, indirect tests for viability such as tetrazolium (TTC) staining and conductivity of embryo leachate were employed to understand the phenomenon. Maximum germination of 92% was found in fresh nuts of Mohitnagar, whereas it was minimum (74.7%) for South Kanara Local. The germination percentage decreased in all the varieties during storage and reached zero by the fourth week. Conductivity of the embryo solute leachates was shown to be sigmoidal in all the varieties. Local variety showed less TTC staining in the beginning and did not decrease sharply. But in all other varieties it decreased sharply in the first week itself and remained almost constant thereafter. The Local variety is found to be superior in terms of viability followed by Mohitnagar but the percent germination remained lowest in the Local during the early stages which was maintained till the third week indicating that it can be stored up to two weeks without much damage. However, in general arecanut is evidenced to be recalcitrant from this study. Storage of the nuts sequentially decreased germination and hence sowing the seeds immediately after harvest is the only successful method for obtaining maximum germination. The maximum period for which areca seed nuts can be stored without loss of viability below 50% is up to two weeks after harvest. Future strategies for storage of arecanut are discussed.

Keywords : Areca catechu, germination, viability, recalcitrant seed, storage.

Introduction

Areca is a graceful perennial palm tree species grown in plantations, the nuts of which are used mainly as a masticatory. The palm is propagated only by its seeds but the seed storage behaviour of the plant remains obscure. Arecanut growers follow traditional practices of drying the seed nuts after harvest from two days upto one week before sowing. Palm seeds in general, lose viability after 2 weeks to 3 months of storage (De Leon, 1958). Effective storage methods have been determined for seeds of *Elaeis guineensis* (Rees, 1963). Bronschat and Donselman (1988) have shown that fungicide treated seeds of *Roystonea regia* sealed in polythene bags remained viable up to nine months. Palm seeds exhibit a variety of storage behaviours such as orthodox, intermediate as well as recalcitrant characters. Ellis *et al.* (1985) suspected Areca species may possibly be orthodox despite the observations of Bhat (1956) and Parameshwar (1962) in South Kanara Local variety of arecanut on the effect of drying in loss of viability. Orthodox seeds are those, which remain viable and can be stored for a long time, at very low moisture content. In the recent past four high yielding varieties of arecanut have been released from CPCRI, Regional Station, Vittal, Karnataka. Physiological or biochemical studies on viability of the seeds of any of these varieties have not been understood so far.

In the present study, an attempt has been made to understand the process of drying during storage and the influence of desiccation on the seed viability and germination, in arecanut varieties. Apart from

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germination recording, indirect tests such as tetrazolium staining and conductivity of embryo leachate were employed for testing the viability.

Materials and Methods

Plant Material

Nuts of *Areca catechu* L. of family Arecaceae was used for the study. Five varieties: 'South Kanara Local', VTL-3 ('Mangala' of Peking origin), VTL-11 ('Sumangala' of Indonesian origin), VTL-17 ('Sreemangala' of Philippines origin) and Mohitnagar were selected for investigations.

The arecanut seed materials were collected in the month of December 1995, for sowing. Nuts of Local variety were collected from CPCRI Regional Station, Vittal, Dakshina Kannada, Karnataka and the other four varieties were collected from CPCRI Seed Farm, Kidu, Dakshina Kannada, Karnataka. Fully ripe arecanut of equal sizes were harvested from mature palms. They were brought to the experimental field immediately after harvest and used for experiments.

Seed Sowing

Seed beds were prepared in sandy loam soil. The seed nuts were sown in a completely randomized design (CRD) with three replications for each treatment and consisted of 50 nuts each. There were five time-course desiccation treatments with control - seed nuts sown immediately after harvest (T1) and four storage treatments (T2 to T5) of seven days interval each, up to 28 days.

Viability

Viability was assessed by two analytical parameters, viz., electrolyte leakage and tetrazolium staining reaction. Electrolyte leakage was assessed by measuring the conductivity of the deionized water in which the excised embryos were put for imbibition for an hour (Becwar *et al.*, 1982). The conductivity of the leachates in water was measured in milli-Siemens (mS) using a conductivity meter.

Tetrazolium staining was done as described by Steponkus and Lamphear (1967) with slight modifications. 1% triphenyl tetrazolium chloride (TTC) solution was prepared in 0.1 mM potassium phosphate buffer, pH 7.0. Ten excised embryos preconditioned by imbibing deionized water for one hour were incubated in darkness for 16 hours in 10 ml of TTC. The embryos were washed thrice in distilled water, the reduced red coloured formazan was estimated by extracting in 90% ethanol at 60°C for two hours and the absorbance was measured at 570 nm using a Shimadzu UV 160A recording spectrophotometer.

Field evaluation of viability was done by taking germination count.

Results and Discussion

Morphological observation indicated the shrinking of embryo, separation from the surrounding tissues of seed coat and the endosperm due to desiccation. Gradual decrease in weight in samples was noticed due to moisture depletion.

Fresh weight of the embryos showed sequential decrease in all the varieties except the variety Mohitnagar where it was stable up to the second week, after which it decreased sharply. The fresh weight of the whole nuts decreased with time, which was rapid in the first week but was gradual and sequential there after. Loss of moisture in 'Mangala' variety was faster compared to the other varieties. Moisture loss from Local variety was the least among the group. The decrease of weight from the whole nuts, husk and kernel are given in Table 1.

Table 1. Varietal difference in the fresh weight of ten whole nuts, husk and kernels of areca seeds under different storage periods

	0 day			7 days			14 days			21 days			28 days		
Var.	Nut	Husk	Kernel	Nut	Husk	Kernel	Nut	Husk	Kernel	Nut	Husk	Kernel	Nut	Husk	Kernel
V1	324.33 ±3.3	171.7 ±0.8	151.9 +0.6	262.3 ±2.2	120.2 ±0.6	141.2 ±0.7	197 ±3.0	59.4 ±0.7	136.1 ±2.6	182.3 ±3.3	53.5 ±1.2	126.1 ±4.1	173 ±5.5	47.7 ±0.4	124.5 ±1.0
V2	348 ±12.5	222.9 ±3.4	123.1 ±1.6	245 ±3.5	130.9 ±0.3	114.1 ±2.1	167 ±6.0	59.8 ±1.2	107.2 ±1.7	154.3 ±3.2	54.6 ±0.9	100.2 ±0.7	145.4 ±4.2	45.5 ±1.0	100.4 ±1.1
V3	269.3 ±5.3	177.5 ±4.0	92.9 ±1.6	208 ±3.0	120.7 ±1.3	86.7 ±1.2	159 ±8.0	74.3 ±0.7	83.9 ±4.4	153 ±1.5	73.0 ±1.0	79.124 ±0.5	138.3 ±4.2	60.522 ±2.1	77.808 ±1.0
V4	318 ±5.5	216.05 ±0.8	101.95 ±1.0	245.3 ±5.7	150.207 ±2.6	95.123 ±0.5	189.7 ±2.3	97.805 ±0.7	91.865 ±1.0	182.6 ±2.3	96.983 ±1.5	85.687 ±1.6	169 ±10.0	85.391 ±0.6	83.609 ±1.6
V5	319.8 ±5.6	206.28 ±1.3	110.6 ±0.9	244.7 ±4.3	141.77 ±0.2	102.9 ±0.5	175.7 ±3.2	77.695 ±1.2	97.975 ±1.1	164.7 ±2.7	79.878 ±1.1	92.452 ±0.4	159 ±6.0	67.466 ±0.7	91.534 ±2.4

Maximum germination was found in 'Mohitnagar' variety (92%) and minimum in Local variety (74.7%), in the seeds sown immediately after harvest. The germination percentage decreased in all the varieties during storage (Fig. 1). There was no sharp decrease in germination in the 'Local' variety up to the second week where it was 72%, but there after it decreased sharply and reached 14.7% by the fourth week. But in all others it was sequential and sharp. In Mangala variety it decreased from 79.3% through 55.33% in the second week to 2% in the fourth week. In Sumangala variety it was 87.33%, 53%, and 0.67% whereas in 'Sreemangala' it was 80%, 56%, 0% and in 'Mohitnagar' the germination decreased from 92%, 62.4% and 2.67% respectively for 0, 2 and 4 weeks.

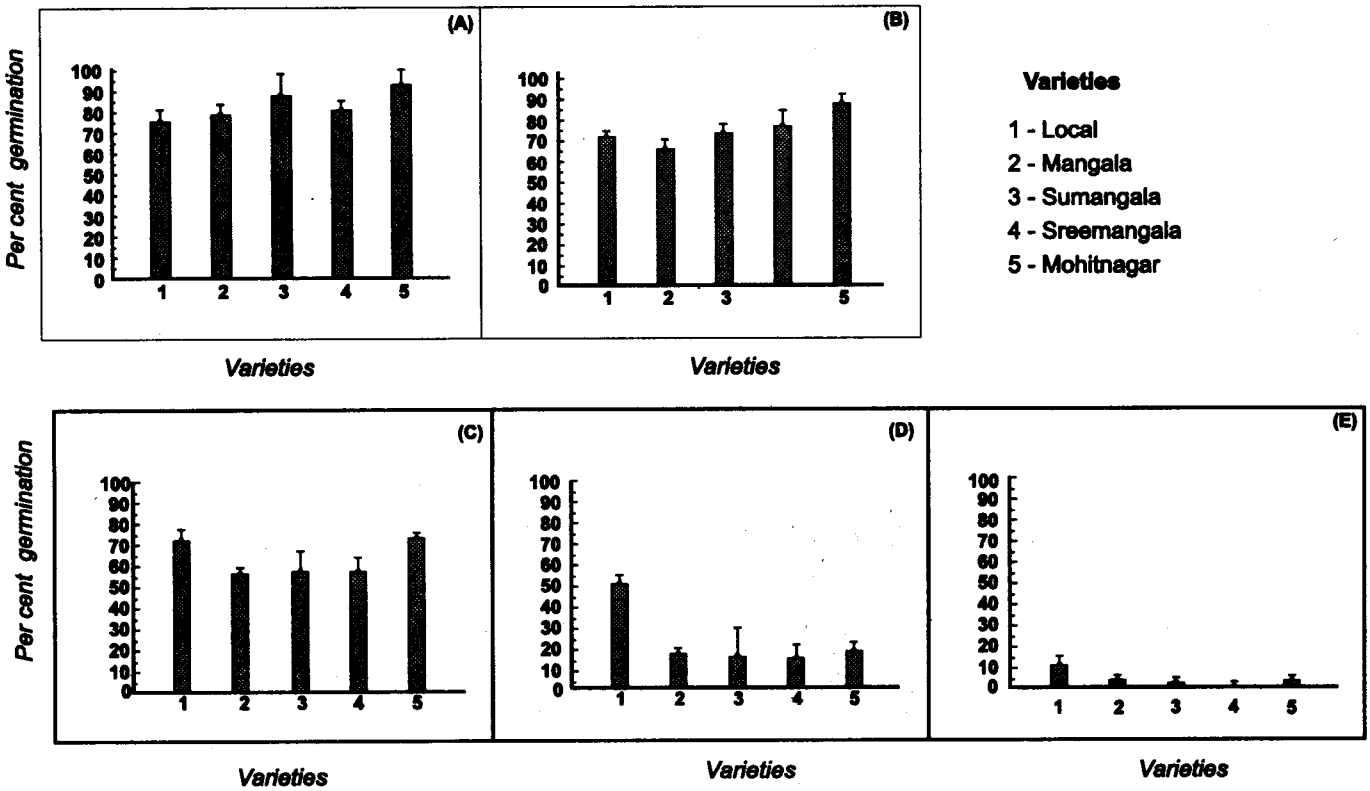

Fig. 1. Varietal difference in the germination of areca seeds under different storage periods (A-T1, B-T2, C-T3, D-T4 and E-T5)

Electrolyte leakage

Conductivity of the embryo solute leachates was shown to be sigmoidal in all the varieties (Fig. 2). Conductivity of the embryo leachates remained high in the Local from the beginning till end except during second week. Conductivity of the leachates immediately after harvest was found to be least in the variety 'Mangala' (138.7 mS/sec.) followed by 'Sumanagala' (145.3 mS/sec.), 'Sreemangala' (183.0 mS/sec.) and 'Mohitnagar' (184.3 mS/sec). But it was maximum for 'Local' variety (209.0 mS/sec). In the fourth week also higher conductivity was recorded in the 'Local' (662.3 mS/sec) than 'Mangala' (430.3 mS/sec), 'Sumangala' (437.3 mS/sec), 'Sreemangala' (455.3 mS/sec) and 'Mohitnagar' (563.3 mS/sec). In Mangala, 'Sumangala' and 'Mohitnagar', the conductivity showed a sharp increase up to second week, then it became negligible, but it continued to increase sharply up to the third week in 'Sumanagala'. In the other varieties the increase was negligible up to third week. Conductivity decreased slightly in 'Sumanagala', 'Sreemangala' and 'Mohitnagar' from third to fourth week, whereas it remained slightly higher in 'Local' and 'Mangala' varieties.

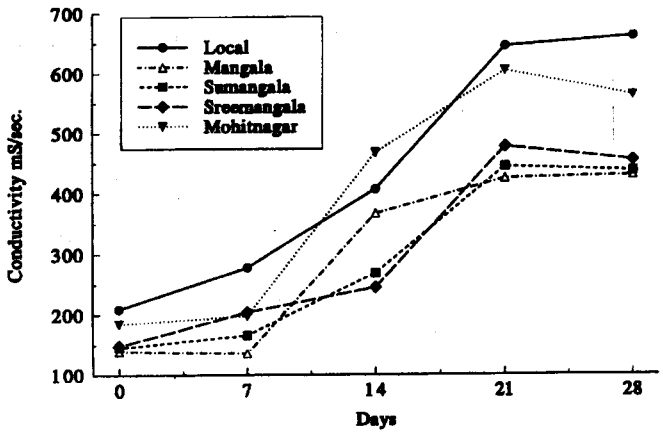

Fig. 2. Varietal difference in embryo leachates of areca seeds under different storage periods.

Tetrazolium test

The tetrazolium staining of embryo during the time course of observations is shown in Fig. 3. In 'Local' variety it stained less in the beginning and did not decrease sharply. But in all other varieties it decreased sharply in the first week itself and remained almost constant thereafter.

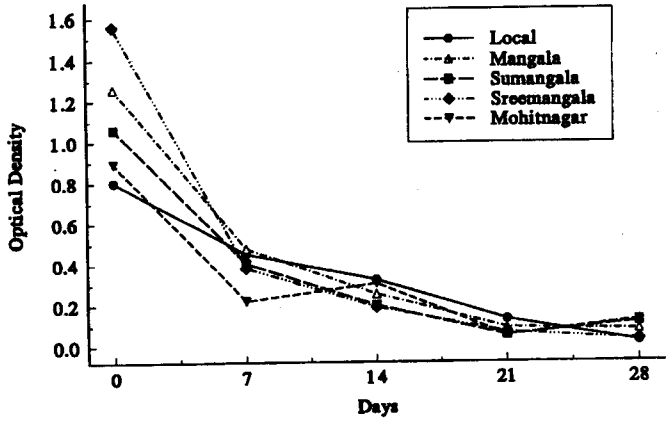

Fig. 3. Varietal difference in the viability of embryos (Formazan produced - OD values) of areca seeds under different storage periods

According to Roberts (1973), seeds of certain species which suffer from damage on desiccation below a critical level cannot be stored for long periods. Such seeds are called recalcitrant. Das and Ray (1980) observed retention of germinability of arecanut even after half an year under cold climate and high levels of humidity.

A high germination percentage in Areca seeds immediately after harvest is in agreement with the observation of Tompsett (1987) where 100% germination was observed in *Dipterocarpus obtusifolius* and about 90% in *D. alatus* and *D. tuberculatus*. In *Hopea odorata*, Huang and Villanueva (1993) observed almost 100% germination.

Since the previous studies (Bhat, 1956; Parameshwar, 1962; Das and Ray, 1980) were not supported by sufficient number of samples and moisture depletion data, it was not possible to use them for understanding the nature of seed behaviour. Berjak *et al.* (1992) pointed out that the recalcitrant seeds when shed have high water contents and are sensitive to desiccation. They have used electrolyte leakage to assess the membrane damage. The extent of electrolyte leakage is proportional to the degree of vacuolation and content of lipids and starch in the tissue. Electrolyte leakage in *Chrysalidocarpus lutescens* embryos increased in response to dehydration and the rates of leakage were non-linear during the initial phase after which it became linear and steady. Relatively low levels of leakage indicates that cellular membranes are semi-permeable and high levels of leakage indicates damage to membranes (Becwar *et al.* 1982). The intensity of staining with tetrazolium salt decreased with time and desiccation; and conductivity of the leachates in water increased with loss of weight in all the varieties. Time-course germination study showed gradual decrease in germination percentage. Below 50% germination was observed in the third week. Highest viability was found for the Local variety followed by the Mohitnagar variety. This corresponded with the increase in conductivity of the water leachates and tetrazolium staining of the embryos. In arecanut the three fold increase of electrolyte leachates indicate membrane denaturation as the cause for loss of viability. The loss of viability was well indicated by the decrease in tetrazolium staining of the embryos.

Effect of desiccation on germination of the nuts seems to be directly correlated with the data on germination. Gradual decrease in weight of samples was noticed due to moisture depletion (Fig.4). Moisture depletion was faster in the whole fruits in the early stages, contributed by the quick loss of moisture from the husk whereas, that of nuts (kernel) appeared to be gradual and linear. Sequential but quick loss of moisture content in the whole fruits stored under normal laboratory conditions was noticed in the present experiment.

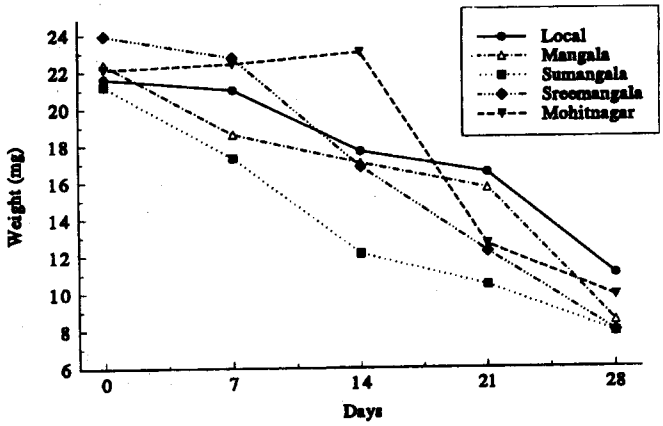

Fig. 4: Varietal difference in the whole nut weight under different storage periods

Germination percentage decreased below 60% after the second week of storage and reached zero by the fourth week with a loss of 52 - 67% moisture content in embryos. The results of the present

experiment corroborate with the observations of Tompsett (1987). The same author observed the loss of moisture content to a magnitude of 40-60% among the species of *Dipterocarpus* with a consequential loss of viability. The reasons for loss of viability due to desiccation was elaborately discussed in *Shorea robusta* by Nautiyal and Purohit (1985). The sequential loss of moisture content damages the cellular membranes resulting in the loss of semi-permeable character of the membranes as evidenced by the increase in solute leachates and it is observed that the damage to the membranes once happened is irreperable with further hydration. Studies on the maintenance of moisture content and rehydration of the seed to protect the structural and biochemical integrity are relevant to extend the viability of the species.

The Local variety is found to be superior in terms of viability followed by 'Mohitnagar' variety but the percent germination remained lowest in the Local variety during the early stages, which was maintained till the third week. This indicates that arecanut can be stored up to two weeks without much damage. However, in general arecanut is evidenced to be recalcitrant from this study. In all other varieties storage sequentially decreased germination and hence germinating the seeds immediately after harvest is the only successful method for obtaining maximum germination. The result of the observations clearly support that arecanut exhibits recalcitrant character.

One of the possible storage conditions may be by preserving the moisture content without microbial damage at normal temperature. Cryopreservation and embryo culture will help to understand the possibilities of *in vitro* preservation of the embryos. An understanding of the behaviour of abnormal embryos may be obtained through embryo culture. To answer other questions such as whether desiccation to a moderate extent helps to build up seedling vigour and faster rate of germination, further studies in this line will prove useful.

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