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Research Article

Desiccation, Germination and Water Sorption Isotherm of *Garcinia afzelii* Engl. (Clusiaceae) Seeds

¹Joseph Mireku Asomaning and ²Moctar Sacandé

¹National Tree Seed Centre, CSIR-Forestry Research Institute of Ghana, P.O. Box 63, Kumasi, Ghana

²Moctar Sacandé, Forest Resource and Policy Division, Department of Forestry, FAO Viale delle Terme di Caracalla, 00153 Rome, Italy

Abstract

Background and Objectives: Seed desiccation sensitivity is a constraint to handling of *Garcinia afzelii*. A study was thus conducted to determine the effect of drying on germination and vigour of the species in order to help recommend a proper seed handling technique.

Materials and Methods: Matured fruits were harvested in April, 2014 and 2015 from a plantation at Ho in the Volta Region of Ghana. Depulped seeds were mixed with moistened sawdust and placed in white cotton sacks and quickly transported by air to the Seed Conservation Department of the Royal Botanic Gardens in the United Kingdom where a part of the experiment was carried out. The second season's collection was used for another part of the studies in Ghana. Seeds were dried in silica gel and on the laboratory worktable at room temperature (28-31 °C) and evaluated for germination and vigour. Sorption isotherm curve was developed by equilibrating seeds over a series of Lithium Chloride (LiCl) solutions with varying RHs and silica gel with 5% RH also at room temperature.

Results: Seed germination percentage decreased gradually with reduction in seed moisture content until after 25% moisture content when germination percentage reduced drastically to 43%. Seedling dry weight, seed vigour index and speed of germination also decreased as seeds were dried. Electrical conductivity of leachate from seeds increased with desiccation of seeds. Water sorption isotherm curve however, showed the typical sigmoid shape curve. **Conclusion:** The seeds of *G. afzelii* were desiccation sensitive, with 'critical moisture content' of 25%.

Key words: *Garcinia afzelii*, desiccation sensitive, seed vigour, critical moisture content, water sorption isotherm

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Corresponding Author: Joseph Mireku Asomaning, National Tree Seed Centre, CSIR-Forestry Research Institute of Ghana, P.O. Box 63, Kumasi, Ghana
Tel: + 233 24 472 4894

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Genus *Garcinia* L. belongs to the family Clusiaceae (Guttiferae). It is composed of approximately 400-600 species, including dioecious, evergreen trees growing in the tropical parts of the world and only 16 of these species occur in west Africa as reported by Steentoft¹. *Garcinia afzelii* is a species of small to medium tree. It is endemic to the tropical forest of West Africa mostly in the Cameroon, Ghana and the Ivory Coast².

Garcinia afzelii together with *G. kola* are widely exploited as a source of chew stick. Split stems and twigs are used as chewing sticks in many parts of Africa and have been commercialized for many years in the major cities, offering natural dental care, cash revenues and employment to hundreds of people. In Ghana about 90% of the people are reported to be using the chew sticks either often or occasionally for dental care³. The Kumasi markets in Ghana provide the equivalent of between 2000 and 6500 harvested trees of the species every month³. The leaves and flowers of *G. afzelii* are used for their anti-bacterial properties⁴. As with other *Garcinia* species, like *G. cambogia* and *G. virgata*, the species can be exploited and used in cosmetic preparations since the crude extract from it has been found to be a free radical scavenger that exhibits significant anti-oxidant effects⁴.

High demand for the *G. afzelii* coupled with poor control over its exploitation has led to depletion of the species from the natural forest leading to its near economic extinction in Ghana. The species is thus vulnerable and would be facing high risk of extinction in the wild in the medium-term future IUCN⁵. Seeds of most species of the genus *Garcinia* (family Clusiaceae), do not undergo a period of maturation drying and are therefore shed or dropped at high moisture content levels, exhibit low viability are short-lived and are described as recalcitrant and therefore, desiccation sensitive⁶.

Seed desiccation sensitivity may limit the *ex-situ* conservation of plant species and therefore, the first challenge for seed conservation is to determine seed response to desiccation⁷. According to Martins *et al.*⁸, knowledge of the critical and lethal moisture levels of a species is indispensable for planning and execution of seed drying and storage. Critical moisture content is considered to occur when a significant reduction in germination percentage is observed following the drying of seeds considered to be desiccation sensitive and lethal moisture content is determined by a complete lack of germination⁹.

A study was therefore, conducted to determine the effect of drying on germination and vigour of the seed in order to have solid basics for the recommendation of a proper seed handling technique for the species.

MATERIALS AND METHODS

Seed collection and processing: Matured fruits of *Garcinia afzelii* were collected manually from a small plantation in April, 2014 and 2015 at Ho in the Volta Region of Ghana. Fruits were placed in a plastic bowl filled with water for 2 days and manually depulped. Seeds were then cleaned with water as recommended by Seeber and Agpaoa¹⁰. To establish the exact moisture content at which seed and seed parts of the species were shed, samples to be used for the initial moisture contents tests were depulped without fruits being soaked in water. Seeds were then packed in cotton bag filled with moistened sawdust and were immediately sent by air to the Seed Conservation Department of the Royal Botanic Gardens, Kew, in the United Kingdom where part of the laboratory experiment was carried out. Seeds harvested in 2015 were used for the work in Ghana.

Equilibrium relative humidity (eRH) of seed samples: The equilibrium relative humidity (eRH) of *G. afzelii* seeds was measured soon after their arrival in the United Kingdom and again after 11 days of desiccation in silica gel at 20°C using a Rotronic AWVC-DIO sensor manufactured by Rotronic Limited, UK. The control samples were mixed with moistened vermiculite and also kept at 20°C.

Initial moisture content of seed and seed components: Initial determination of moisture content of fruits, seeds and seed components were conducted using 16 whole fruits in four replications; mesocarp extracted from 16 fruits in four replications; 12 whole seeds in three replications and testa extracted from 12 seeds in three replications. Moisture contents were determined gravimetrically by drying fruits, seeds or seed components in an oven¹¹ at 103°C for 17 h ISTA.

Regular seed moisture content determination of bulk seed: Desiccation sensitive species tend to have significant variations between individual seeds in a seed lot in terms of moisture content due to differences in maturity¹² and handling procedures. Hence, to be sure of the moisture status of the seeds stored in the moisten sawdust (i.e., the bulk seed used for the entire study), seed moisture content was determined from time to time and the moisture content value obtained at a particular time was used as the reference point for the next experiment.

Table 1: Relative humidity condition series generated at 28-31 °C by dissolving various quantities of LiCl granules in 100 mL of distilled water

Weight (g) of LiCl granules dissolved in 100 mL of distilled water	*Silica gel	90	70	60	50	40	30	20	10	5
RH (%) generated at 28-31 °C	5	13	47	51	60	67	80	86	95	98

*Well dried silica gel generated 5% RH

Seed drying curve experiments: Thirty five grams of seeds with an initial moisture content of 38.1% placed in a porous bag made of mosquito netting were dried over silica gel at 28-31 °C (1 kg of seeds for 2 kg of silica gel). Seeds were drawn from the desiccating samples at 24 h intervals for moisture content determination¹¹. The data was plotted on a graph to give a drying curve for the seed.

In another experiment, 25 g of seeds also with an initial moisture content of 38.1% in another porous bag were dried over equal weight of silica gel (1 kg seed to 1 kg of silica gel) also kept at a temperature of 28-31 °C. Desiccating seed samples were weighed at 24 h interval to monitor seed weight changes over time. Seed weight in grams was plotted against period of drying in days to construct a drying curve for the seeds¹³.

Seed desiccation and viability assessment: Seeds of *G. afzelii* with initial moisture content of 31.5% were placed in plain polythene bags and desiccated by mixing them with silica gel (1 kg of seeds for 1 kg of silica gel) or dried under shade to various moisture contents. Seed samples were drawn every 2 days for seed moisture contents and seed viability assessment. Seed moisture content was determined gravimetrically¹¹. Germination tests were carried out in heat sterilized river sand (120 °C for 2 h) in plastic boxes with four replicates of 25 seeds per treatment in a constant temperature incubator maintained at 30 °C. Seeds were counted as germinated when plumule attained 1 cm length¹⁴.

Assessment of seed vigour after desiccation: Electrical conductivity measurement of leachates from dried seeds after soaking them in deionized water using the Multiple 100 Cell Conductivity Meter 2.18 (Reid and Associate, South Africa) was used to assess the vigour levels of samples¹⁵. Seed vigour index was calculated by measuring the length of five seedlings from each treatment and multiplying their total length by germination percentages¹⁶. Seedlings used for the measurement of dry weight were dried under shade for 24 h and then dried in hot air oven maintained¹⁷ at 85±2 °C for 48 h. Germination speed was calculated as the sum of the quotients of the number of seeds germinating each day divided by the time in days from the start of the germination¹⁸.

Construction of water sorption isotherm: Water sorption isotherm was developed by equilibrating 10 g seed samples over silica gel and a series of Lithium Chloride (LiCl) solutions ranging in relative humidity from 5-98% at 28-31 °C obtained

by dissolving specified weights of LiCl granules in 100 mL of distilled water MSBP¹⁹ (Table 1). Moisture content of seed samples after equilibration was determined gravimetrically¹¹ and then plotted against relative humidities to construct water sorption isotherm²⁰.

Analysis of data: The data collected was subjected to one way analysis of variance (ANOVA) using the Genstat (11th edition) statistical package to determine significance. Mean separation was done after ANOVA, using standard error of the difference.

RESULTS

Initial moisture content of fruit, seed and seed components:

Moisture content was highest in mesocarp of seed followed by whole fruit, seed coat and then seed at the time of harvest of fruits of *G. afzelii* (Table 2). The eRH and moisture content of seeds obtained after soaking fruits in water followed by depulping measured at 20 °C when they were received fresh at the laboratory were 93.3 and 38.2%, respectively. Seeds kept in moistened vermiculite as control for 11 days had eRH and moisture content higher than the eRH and moisture content recorded for seeds dried in silica gel for the same period of time (Table 3).

Seed drying curve experiments: At a temperature of 28-31 °C, *G. afzelii* seeds with initial moisture content of 38.1% quickly lost about half of its moisture content within 96 h of drying. Seed moisture then slowly reduced as seeds were dried further and reached an equilibrium moisture content of 6.7% after 366 h of drying (Fig. 1). Similarly, at a temperature of 28-31 °C, seed samples of the species weighing 25 g quickly lost about a quarter of its fresh weight within 48 h of drying and slowly dried to an equilibrium weight of 14 g after 366 h (Fig. 2).

Seed drying effect on germination percentages: Drying of *G. afzelii* seeds to lower moisture contents resulted in reduction in seed germination percentages. Seeds desiccated in silica gel declined in viability gradually with decreasing moisture content. Below a moisture content of 25.0% (i.e., 23.3%) seed viability drastically and significantly ($p < 0.05$) declined to 43% (Fig. 3a) Similarly, seeds dried under shade resulted in a drastic and significant ($p < 0.05$) loss of viability to 43% germination below a moisture content of 25.3% (i.e., at 23.6% seed moisture) and a complete loss of viability at 11.0% seed moisture content (Fig. 3b).

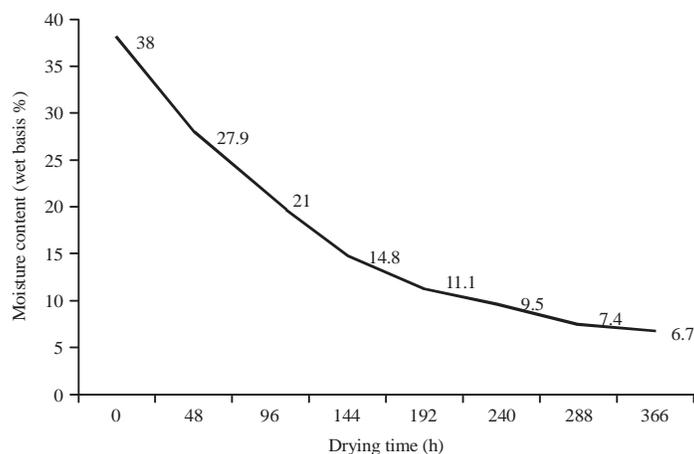


Fig. 1: Drying curve (moisture changes) of *G. afzelii* seed dried over silica gel in a ratio of 1:2 at an ambient temperature of 28-31 °C

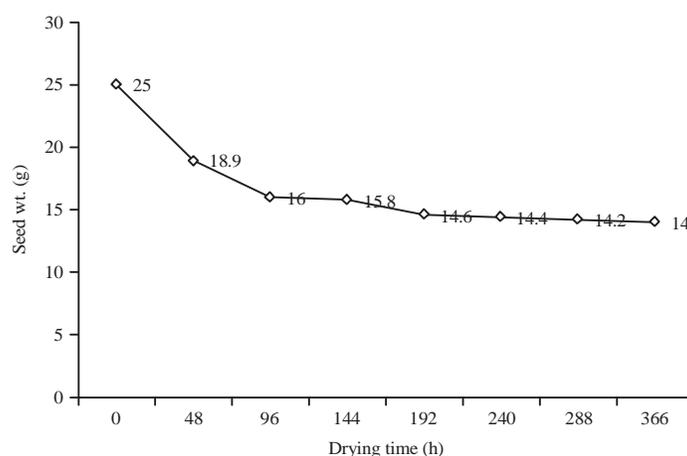


Fig. 2: Drying curve (weight changes) of *G. afzelii* seed dried over silica gel in a ratio of 1:1 at an ambient temperature of 28-31 °C

Table 2: Initial moisture content of fruit, seed and seed parts of *G. afzelii*

Species	Initial moisture content (%) of fruit, seed and seed components			
	Fruit	Seed	Mesocarp	Seed coat
<i>Garcinia afzelii</i>	65.0±2.7	35.2±1.1	73.8±2.3	61.2±2.5

The data are mean values (±SD)

Table 3: Equilibrium relative humidity (eRH) and moisture content of *G. afzelii* seeds after drying in silica gel or being kept in moistened vermiculite

Seeds	Period of desiccation in silica gel (days)	eRH of seed dried in silica gel (%)	Moisture content of seed dried in silica gel (%)	Period at which seeds were held in moistened vermiculite (control) (days)	eRH of seeds held in moistened vermiculite (Control) (%)	Moisture content of seeds held in moistened vermiculite (Control) (%)
<i>Garcinia afzelii</i>	11	28.0	13.5		91.3	33.1
		*93.3	*38.2	11	*93.3	*38.2

Figures with asterisks are initial eRH and moisture contents, respectively

Effect of drying of seed on seedling dry weight, seed vigour index and seed leachate electrical conductivity: The effect of drying on seed vigour of *G. afzelii* as indicated by seed germination speed, seedling dry weight, seed vigour index

and electrical conductivity of seed leachates are presented in Table 4. Seed germination speed at higher moisture contents was significantly higher ($p < 0.05$) than germination speed recorded at lower moisture contents. Seedling dry

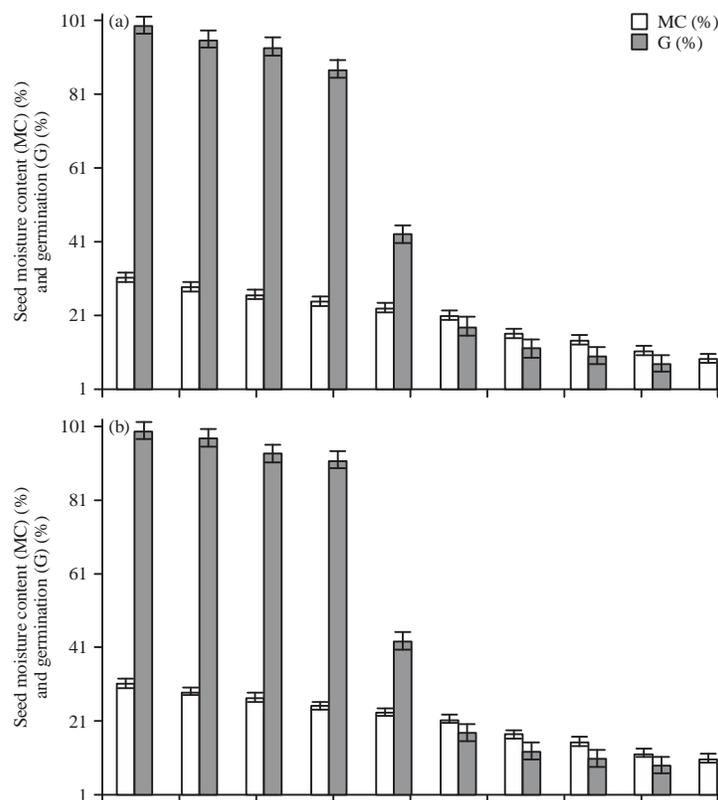


Fig.3(a-b): Moisture content effect on germination percentage of (a) *G. afzelii* seeds dried over silica gel at 28-31 °C and (b) *G. afzelii* seeds dried under shade at 28-31 °C

Error bars represent two standard error of the difference in both situations)

Table 4: Effect of drying on seed moisture content, germination speed, seedling dry weight and seed vigour index of *G. afzelii* seeds

Drying period (days)	Seed moisture content (%)	Seed germination speed	Seedling dry weight (g)	Seed vigour index	Electrical conductivity of seed leachate (μS)
0	31.5	0.1625	0.2178	1485	3.121
2	29.2	0.1625	0.2302	1513	3.101
4	27.6	0.1475	0.2408	1490	3.124
6	25.3	0.1375	0.2253	1214	5.431
8	23.6	0.0725	0.2163	559	14.374
10	21.7	0.035	0.214	208	18.343
12	17.5	0.0275	0.2053	142	25.458
14	15.5	0.02	0.1855	113	33.221
16	12.4	0.015	0.1713	84	41.035
18	11.0	0	0	0	48.843
	Replication	4	4	4	4
	df	30	30	30	30
	S.E.D	0.0585	0.004439	42.76	1.601
	Probability	<0.05	<0.05	<0.05	<0.05

weight and seed vigour index initially increased as seed moisture was reduced before it started decreasing significantly ($p < 0.05$) as seeds were dried to lower moisture contents. Electrolyte leakage from seeds measured by electrical conductivity consistently increased significantly ($p < 0.05$) as seeds were dried below 25.3% moisture content.

Water sorption isotherm for *G. afzelii* seeds: Generally seeds placed at all relative humidities from 5-98% lost fresh weight during the experimental period of 20 days (Table 5). The sorption isotherm curve generated for the species showed that seed moisture content increased with increasing relative humidity. The sorption isotherm curve has three regions and it displays a reverse sigmoidal shape (Fig. 4).

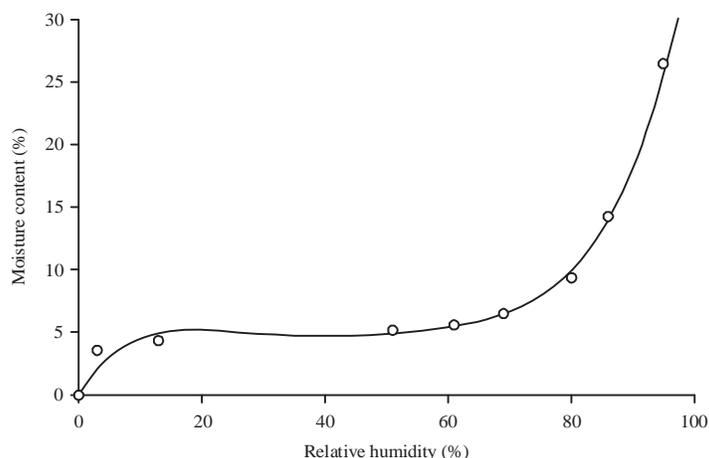


Fig. 4: Water sorption isotherm of *Garcinia afzelii* seeds showing the relationship between the equilibrium moisture and relative humidity at room temperature (28-31 °C)

Table 5: Weight changes of *G. afzelii* seed samples recorded during the first 20 days of drying over silica gel and Lithium chloride solutions posing various relative humidity conditions at room temperature (28-31 °C)

Drying period (days)	Relative humidity (%)									
	98	95	86	80	69	61	51	47	13	5
Seed sample weight (g)										
0	10.24	9.78	10.25	9.58	10.45	9.57	9.72	10.36	9.84	9.45
2	9.85	9.29	9.11	8.16	8.35	7.36	7.22	7.11	7.26	6.89
4	9.71	8.98	8.34	7.28	7.35	6.45	6.17	6.66	6.34	6.06
6	9.51	8.71	7.76	6.71	6.82	6.07	5.88	6.35	6.07	5.81
8	9.32	8.28	7.48	6.33	6.51	5.93	5.79	6.21	5.95	5.73
10	9.12	8.12	7.19	6.19	6.44	5.89	5.72	6.16	5.93	5.71
12	8.83	7.87	6.94	6.06	6.39	5.85	5.68	6.13	5.87	5.69
14	8.67	7.74	6.81	6.02	6.37	5.83	5.66	6.13	5.82	5.67
16	8.52	7.57	6.72	5.99	6.35	5.81	5.65	6.12	5.81	5.66
18	8.41	7.53	6.68	5.98	6.35	5.81	5.65	6.12	5.80	5.66
20	8.33	7.47	6.65	5.98	6.34	5.80	5.65	6.12	5.80	5.66

DISCUSSION

In the seed industry, knowing seed eRH at harvest can inform post-harvest handling¹⁹. Equilibrium relative humidity (eRH) is the value of relative humidity into which a hygroscopic product can be placed where no net exchange of moisture between the product and the surrounding environment can take place. The eRH and moisture content values of seeds measured at 20 °C when they were received fresh at the laboratory were 93.3 and 38.2%, respectively. Even seed samples exclusively extracted from fruits not soaked in water for the purpose of establishing the exact moisture content at which seed and seed components were shed had a moisture content of 35.2%. These high values are indicative of the fact that the seeds were shed at the time when moisture content was high. It was reported by Chin *et al.*²¹ that desiccation sensitive seeds do not undergo maturation drying and even after shedding from the mother plant they have high moisture content, ranging²² from 30-70%, leading to their

“wetness” and high eRH values MSBP²³. This is an important characteristic of the Genus *Garcinia* (family Clusiaceae) as reported by Morton²⁴. Seeds maintained in moistened vermiculite for 11 days still had high eRH and moisture content values close to freshly harvested seeds indicating that the medium had the competence to keep the seeds in the fresh state for at least that length of time. In carrying out a seed desiccation study, it is important to know whole seed moisture as well as individual seed components moisture content. This helps in determining the variation in moisture content within the seed components¹¹. The initial test indicated that mesocarp of *G. afzelii* seed had higher moisture content compared to the whole fruit, whole seed and seed coat. In view of the fact that the fruits of the species are fleshy^{25,26} their mesocarps were expected to contain a lot of water. Gyimah²⁷ reported that the seed coat of *G. afzelii*, although, was thin and leather-like had higher moisture content than the whole seed.

The shape of the drying curves indicated that seed moisture content and seed weight declined steeply during the initial stages of drying in silica gel. As seed moisture content reduced the rate of seed moisture lost during drying also reduced. This observation could be explained by what Vertucci and Leopold²⁸ described as types of water in seed tissues. The moisture present in the seeds at the start of drying is what is termed the type 3 or freezable water which is easily lost, hence, the faster rate of moisture loss from the seeds at the start of drying. As drying progressed, the water present in the seeds entered the type 2. This water is tightly associated with macromolecular surfaces of the cells, its mobility is reduced and it constitutes the "unfreezable water" which is difficult to remove by drying. When the seeds were dried further, the water status entered the type 1. This water is very difficult to remove by further drying and that could be the reason for the slow rates of drying when the seed moisture content were approaching very low levels²⁸.

The relationship between seed moisture content and germination capacity revealed that fresh seeds with 31.5% seed moisture germinated 100%. Desiccation of the seed either in silica gel or under shade at ambient temperature resulted in the reduction of seed viability which followed a decreasing trend with decreasing seed moisture content. Below seed moisture content of 25% (silica gel drying) and 25.3% (shade drying), seed germination drastically reduced to 43%. *G. afzelii* can therefore, be described as sensitive to desiccation. The critical moisture content (CMC) of the species was found to be 25% in this study. It was suggested by King and Roberts²⁹ that seed death resulting from desiccation occurs at or below critical moisture and is caused by membrane related physiological damages or an accumulation of by-products of biochemical enzymatic breakdown. Different drying rates of seeds (relatively faster in silica gel) and a slower rate under shade did not significantly affect the critical moisture content of the seed as it has been achieved with the excised embryos of some recalcitrant seeds³⁰. The reason is that it is difficult to dry large seeds fast²⁵ compared to the excised embryos of the seed⁹.

There is a connection between seed moisture content, seed viability and vigour in recalcitrant seeds³⁰. In the present study, it was observed that reduction in speed of germination, seedling dry weight, vigour index and electrical conductivity of leachate from seeds was associated with seed desiccation. Bhattacharyya and Basu³¹ and Raja *et al.*³⁰ observed decreased viability and seedling vigour during the desiccation of recalcitrant avocado (*Persea* species) and jackfruit (*Artocarpus heterophyllus*) seeds. Asomaning *et al.*³² also reported that seeds of *Garcinia kola* dehydrated to lower moisture contents released more electrolytes into solutions

shown by increased electrical conductivity, an indication of decrease vigour. The study has further shown that seedling dry weight and vigour index of seeds of *G. afzelii* increased with desiccation up to a certain level and then decreased. This trend was also reported in lychee (*Litchi chinensis*) and longan (*Dimocarpus longan*)³³ in horse chestnut (*Aesculus hippocastanum*)³⁴.

Moisture sorption isotherm curves are developed for seeds in order to study the behaviour of water in their tissues³⁵. The net loss of moisture (desorption) from *G. afzelii* seeds samples placed in all relative humidity chambers from 5-98% indicated by loss in fresh weight can be explained by the fact that the seeds had high moisture contents. All seeds are hygroscopic and automatically absorb or desorb moisture by diffusion along a water potential gradient between the seed and the surrounding air. If the water potential of the seed is greater than the surrounding air, the seed will lose water and become drier³⁶.

According to Vertucci and Leopold²⁸, under desiccation intolerant condition, isotherms are more hyperbolic indicating that water binding differs between plant tissues that are intolerant as compared to those that are tolerant to desiccation. Isotherms of a hyperbolic form (monotonic isotherms) have been reported for some species of desiccation sensitive seeds including cocoa (*Theobroma cacao*)³⁷. According to the Langmuir sorption theory, these monotonic isotherms indicate that there is little or no contribution to the water absorption characteristics by the strong binding sites³⁸. *Garcinia afzelii* has been classified as a desiccation sensitive species with 'critical moisture content' of 25% in this study. However, the shape of the sorption isotherm developed for the species has the sigmoidal character with three regions, reflective of water-binding strength with inflection points. However, Asomaning *et al.*³⁹ indicated that moisture sorption isotherm of recalcitrant *Garcinia kola* also showed the typical sigmoid shape curve. Kapseu *et al.*⁴⁰ observed that dried shea nut kernels (*Vitellaria paradoxa*) which has also been described as desiccation sensitive, losing viability below 20% moisture content, as found by Gamene *et al.*⁴¹ showed the typical sigmoid shape similar to what has been developed for *G. afzelii* in this study.

CONCLUSION

Seeds of *G. afzelii* attain maturity at high moisture content of above 35%. The species is desiccation sensitive with "Critical moisture content" of about 25%. Seed germination percentage decreased gradually with reduction in seed moisture content until after 25% moisture content

when germination percentage reduced drastically to 43% (at 23% moisture content). Seedling dry weight, seed vigour index and speed of germination also decreased as seeds were dried. Electrical conductivity of leachate from seeds increased with desiccation of seeds. Water sorption isotherm curve for the species showed the typical sigmoid shape curve.

SIGNIFICANCE STATEMENT

This study discovers that *Garcinia afzelii* seed is shed from the mother plant at a high moisture content above 35%. The study further discovers that the critical moisture content of the seed which is desiccation sensitive is 25%. This implies that fresh seed must not be dried below 25% moisture content value so as not to cause radical reduction in viability and vigour of seed. The findings from this study will greatly help researchers and tree nurserymen to handle the fresh seed properly so as to prevent excessive drying which resulted in seed viability losses as reported by handlers of the seed in their quest to raise seedlings for conservation planting and other purposes.

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