

Original Research Article

Effects of seed source, moisture content and duration of storage on the viability of *Vitellaria paradoxa* C.F. Gaertn

ABSTRACT

Viability of tropical seeds is crucial in plantation establishment. Temperature – moisture duration relationship of the seed is of great significance in seed viability. 3000 ripe fruits of *V. paradoxa* were collected from each of three sources where it is endemic: Eruwa, Saki and New-Bussa. The fruits were depulped; a sample of 100 seeds was drawn from each of the three sources and sown in washed and sterilized river sand to monitor the germination percentage of the seeds. The seeds were dried under dehumidifier to attain two moisture contents (43% and 32%). Thereafter, each seed lot from the three sources was divided into two batches (A and B) of 1200 seeds each, divided into four and stored at four different temperature regimes: -20°C, 5°C, 22°C and 28± 2°C. Samples were taken monthly for germination test for six months. Data were subjected to percentages and analysis of variance (ANOVA). Results showed that freshly collected seeds of *V. paradoxa* had 98% germination when sown within 48 hours of fallen and collection which eventually dropped to 4.4% after the six months storage. Storage temperature had significant effect ($P > 0.05$) on germination of the seeds. Seeds stored at 28±2°C had the highest mean germination (72.2%), those stored at 22°C had 70.2% while seeds stored at -20°C and 5°C failed to germinate. The seeds of *V. paradoxa* readily lost viability with time, hence fallen fruits should be collected during fruiting season processed and sown immediately or within 7 days of collection.

Keywords: *Vitellaria paradoxa*, germination, moisture content, storage temperature, duration of storage, seed source.

INTRODUCTION

Quality tree plantings with outputs marching the expectations of the tree planters require good quality seed with good germination potential and good genetic properties (Davidson *et al.*, 1996). In order to ensure successful stand establishment, the need for effective and regular supply of seeds of high genetic and physiological qualities should not be overlooked (Toon *et al.*, 1990). This is because seed constitutes unique genetic composition from combined parental genetic materials. Seeds must be viable and available at the appropriate sowing season for production of seedlings for plantation establishment. Most indigenous tree species like many other trees flower and fruit once a year while some do every other year. Therefore, availability of viable seeds is very vital. Seed storage is an important factor in the preservation of viable seeds from the time of collection until they are required for sowing (Toon *et al.*, 1990; Davidson *et al.*, 1996).

Seed production, quality and quantity in most species are not stable from year to year and are influenced by several factors including climate, pest and diseases, animal, human interference and forest fires. In view of this, consideration must be given to appropriate storage to maximize the seeds collected (Vertucci *et al.*, 1996). Lady (2000) stated that the two most crucial necessities for storing seeds are constant temperatures and low humidity; although, seeds from different sources respond differently to storage temperatures and durations.

Vitellaria paradoxa seed is recalcitrant hence require adequate silvicultural attention for viability. It is a dicotyledonous plant in the family Sapotaceae. It is commonly known as butter tree, shea tree, shea butter tree or shea nut tree (Hall *et al.*, 1996). The seed has high oil content, oil rancidity during storage, oil freezing in cold condition and inaccessibility of the embryo for the supply of the stored nutrient (Lamien *et al.*, 2007). Recalcitrant seeds have low tolerance to drying and cooling. Many recalcitrant species contain about 40% moisture at maturity and do not tolerate drying to less than 20-30%. Similarly, *V. paradoxa* had various nut fresh weights ranging from 8-10g containing 43-68% moisture by weight

(Bonkougou, 2005). In contrast, most orthodox seeds can be dried below 5% moisture content without losing viability. Therefore, this study was carried out to highlight the germination response of *Vitellaria paradoxa* seed from different sources to different moisture contents, storage temperature and duration of storage with a view to recommending best storage option(s) for the species.

MATERIALS AND METHODS

The study was carried out in three locations namely Eruwa, Saki and New-Bussa in Nigeria. Ripe fruits of *V. paradoxa* were collected from three sites along its natural range: Eruwa (latitudes $7^{\circ} 47' N$ and $7^{\circ} 42' N$; longitudes $3^{\circ} 51' E$ and $3^{\circ} 47' E$); Saki (latitudes $8^{\circ} 40' N$ and $8^{\circ} 36' N$; longitudes $3^{\circ} 19' E$ and $3^{\circ} 16' E$) and New-Bussa (latitudes $9^{\circ} 35' N$ and $9^{\circ} 33' N$; longitudes $3^{\circ} 33' E$ and $3^{\circ} 29' E$) in the derived, southern guinea and northern guinea savanna ecological zones respectively. The forest floor around the selected trees were cleared of fruits, leaves, twigs and fallen fruits before the fallen ripe fruits were daily for a period of 7 days. A total of 1500 fruits were collected from each source and packed in jute bags to the laboratory where depulping was carried out to extract the seeds and thereafter separated from dirt. A sample of 100 clean seeds was drawn from each of the three sources and immediately sown in washed and sterilized river sand under a non-mist propagator to monitor the initial germination percentage of the seed. Each seed lot (2400) from the three sources was divided into two batches of 1200 seeds each (A and B) and dried to two moisture contents (43% and 32%) under a dehumidifier at the Tree Improvement Nursery, Forestry Research Institute of Nigeria (FRIN), Ibadan Nigeria. The moisture content was determined by weighing seeds on a sensitive weighing balance and there after oven drying at $60^{\circ}C$ for 17 hours until they attained constant dry weight.

Seedlots from the three sources grouped into A and B were each divided into four (300 seeds/pack) and packed into transparent re-sealable plastic containers before storage at four different temperature regimes: ($-20^{\circ}C$), ($5^{\circ}C$), ($22^{\circ}C$) and ($28 \pm 2^{\circ}C$). The seeds were stored in the Gene bank at National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan. Samples of *V. paradoxa* seeds stored under the four temperatures were taken from each batch fortnightly and sown in washed and sterilized river sand under a non-mist propagator. Germination percentage was monitored fortnightly for 6 months. Twelve sampling periods were observed and germination was monitored for two weeks and percentage germination within two weeks of sowing determined. The experimental design used was $3 \times 6 \times 4 \times 2$ factorial experiments in Completely Randomized Design where the first factor was the three seed sources, the second factor was six storage duration, the third factor was four storage temperature and the fourth factor was two moisture contents, the treatment combinations were replicated four times. The data collected were subjected to Analysis of variance (ANOVA) and where significant differences were obtained, least significant difference (LSD) test was used to separate the means.

RESULTS AND DISCUSSION

Results

Effect of seed source and moisture content on percentage germination of the different seedlots

The germination of the seeds of *V. paradoxa* varied from 94 to 98%. Seeds from Saki had the highest initial germination of 98%, followed by the seeds from New-Bussa with 97% while the lowest initial percentage germination of 94% was observed among the seeds collected from Eruwa (Table 1). However, sources of seeds and moisture contents did not show any significant effect on the percentage germination of the seeds (Table 2)

Effect of temperature on percentage germination of stored seeds

Storage temperature and duration of storage had significant effect ($p \leq 0.05$) on germination of the seeds from the sources. The mean percentage germination of seeds stored at $28 \pm 2^{\circ}C$ and $22^{\circ}C$ were not significantly different from each other but differed significantly from the seeds stored at $-20^{\circ}C$ and $5^{\circ}C$. However, the seeds stored at $28 \pm 2^{\circ}C$ had higher mean germination (72.2%) than those stored at $22^{\circ}C$ with 70.2% while seeds stored at $-20^{\circ}C$ and $5^{\circ}C$ had zero germination each (Table 3).

Effect of duration of storage on percentage germination of stored seeds

Duration of storage substantially influenced the percentage germination of the seeds from the three sources under the four temperature regimes throughout the period of assessment. The variation pattern showed a systematic decline in percentage germination in all the three seedlots as well as in seeds stored at $28 \pm 2^{\circ}C$ and $22^{\circ}C$ over the storage periods. The seedlots from all the sources stored at $28 \pm 2^{\circ}C$ and $22^{\circ}C$ temperature regimes showed consistent decrease in percentage germination ranging from 88.4 to 5.6% while the seeds stored at $-20^{\circ}C$ and $5^{\circ}C$ exhibited sudden germination failure. (Table 3).

Effect of temperature and duration of storage on germination of stored seeds

Interaction of storage period and temperature had significant effect on the percentage germination of different seedlots. The percentage germination of seedlots stored at $-20^{\circ}C$ and $5^{\circ}C$ were not significantly different from one another; but were significantly different from those kept in $-20^{\circ}C$ and $5^{\circ}C$ only in the 1st month (Table 2).

90 **Table 1: Germination percentage of *V. paradoxa* seeds from three sources.**

GPS Coordinates			
Seed Source	Latitude	Longitude	Germination %
Saki	8° 40'N and 8° 36'N	3° 19'E and 3° 16'E	98
New Bussa	9°35'N and 9°33'N	3°33'E and 3°29'E	97
Eruwa	7° 47' N and 7° 42'N	3°51'E and 3°47'E	94

91 **Table 2: Analysis of variance for the percentage germination of *V. paradoxa***
 92 **seeds subjected to viability variables**

Parameter	df	ms	f-value	P-level
Source (S)	2	0.04	0.10ns	0.902
Storage duration (SD)	5	42.04	98.69*	0.000*
Storage temperature (ST)	3	114.41	268.61*	0.000*
Moisture content (MC)	1	0.52	1.22ns	0.270
S X SD	10	0.16	0.37ns	0.959
S X ST	6	0.36	0.82ns	0.998
SD X ST	15	14.24	33.44*	0.000*
S X MC	2	0.13	0.31ns	0.734
SD X MC	5	0.51	1.20ns	0.311
ST X MC	3	0.25	0.59ns	0.625
S X SD X ST	30	0.12	0.27ns	0.999
S X SD X MC	10	0.16	0.38ns	0.954
S X ST X MC	6	0.55	0.38ns	0.954
SD X ST X MC	15	0.25	0.58ns	0.892
S X SD X ST X MC	30	0.17	0.40ns	0.998
Error	288	0.43		

112 Note: *Significant at 5% Probability level, ns = not significant

113 **Table 3: Mean values of percentage germination of *V. paradoxa* seeds subjected to different**
 114 **temperature and duration of storage**

Parameter	Mean Germination %
Storage temperature	
-20°C	0.00a
5°C	0.00a
22±1°C	70.4b
28±2°C	72.4b
Duration of Storage (Months)	
6	5.6a
5	11.2a
4	22.4b
3	36.8c
2	50.0d
1	88.4e

130 Note: Means with the same letters are not significantly different from each other at $p \leq 0.05$

131 **DISCUSSION**

132 Germination of viable seeds is dependent on varying temperature and moisture contents and other environmental
 133 conditions which seeds must be adapted to (Gamaéné *et al.*, 1999). Each plant species has a specific optimum and a
 134

135 range within which germination will occur (Shaban, 2013). The closer the temperature is to optimum temperature required
136 by seed, the quicker the germination will occur (Beyranvand *et al.*, 2013; Baskin and Baskin, 2004).

137 Seeds of *V. paradoxa* irrespective of source responded to varied storage temperatures as well as storage durations.
138 Germination which was highest when freshly collected seeds were sown and declined in a geometrical progression when
139 stored at $22\pm 1^{\circ}\text{C}$ and $28\pm 2^{\circ}\text{C}$ affirm the recalcitrant nature of the species. According to Hall *et al.*, (1996), *V. paradoxa* is a
140 recalcitrant species which are remarkably short-lived and cannot be dried to moisture content below 20-30% without injury
141 and are unable to tolerate freezing. They are also termed as desiccation sensitive seeds, difficult to be successfully stored
142 and their ex-situ conservation is problematic (Vertucii *et al.*, 1996). This implies that the species should be sown
143 immediately after collection as a substantial level of germination would be achieved. This finding conforms with Gamene
144 *et al.* (1999) who reported that *V. paradoxa* is recalcitrant in nature and thus has short physiological storability and viability
145 status. Roberts, (1988) reported that seed quality maintenance in storage from the time of production until the seed is
146 planted is imperative to assure its planting value (Roberts, 1988). This implies that decrease in seed viability with storage
147 duration in *V. paradoxa* seeds could be ascribed to influence of storage conditions in terms of temperature. The findings
148 of this study where 32 and 43% moisture contents had no significant effect on the viability of the seeds corroborates the
149 result of Pritchard *et al.* (1996), on storage trials of *V. paradoxa* in Burkina Faso and Senegal with regulated storage
150 temperature and moisture contents over a period of 6 months where the drying to moisture content above 37 % did not
151 affect germination percentage of the seeds of *V. paradoxa*. This is also in line with findings of Daws *et al.*, (2004) that
152 mortality of the seeds of *V. paradoxa* increased at moisture contents that ranged from 20 – 26%.

153 The significance of the effects of storage duration and temperature on germination percentage of *V. paradoxa* seeds
154 recorded in his study is in line with Yameogo *et al.*, (2000) who recorded highest germination in the 1st month at $20\text{-}25^{\circ}\text{C}$
155 and progressive decline in germination as the duration of storage increased confirming the seed to be recalcitrant and
156 losing viability very rapidly. On the other hand, lower germinations were recorded in low temperature storage. This is
157 probably because of the recalcitrant nature of the seed which requires high moisture for optimum germination (Lars,
158 2000).

159 Seed moisture content is a function of environmental conditions which is chiefly influenced by humidity and temperature
160 of the environment. Invariably, the main aim of storing seeds is to maintain the critical moisture content required for the
161 optimum germination or recondition it to attain the optimum moisture level either through drying or soaking (Bradford,
162 1995; Covell *et al.*, 1986). The decrease in germination potentials with increase in storage period and decreased
163 temperature confirms the recalcitrant nature of the seed which result in impairment of certain physiological activities at
164 temperatures below the optimum required. This implies that moisture and temperature are basic requirements for
165 germination, they are essential for enzyme activation, breakdown, translocation, and use of reserve storage material. In
166 their resting state, seeds are characteristically low in moisture and relatively inactive metabolically. That is, they are in a
167 state of quiescence (Bradford, 1995). Thus, quiescent seeds are able to maintain a minimum level of metabolic activity
168 that ensures their long-term survival in the soil and during storage. Moisture availability is described in various ways. Field
169 capacity moisture is about optimum for germination in soil; however, germination varies among species and may occur at
170 soil moistures near the permanent wilting point (Beyranvand *et al.*, 2013). Most seeds have critical moisture content for
171 germination to occur. For example, Orthodox seeds which are long-lived seeds can be successfully dried to moisture con-
172 tents as low as 5% without injury while species such as *Chrysophyllum albidum* and *Caesalpinia bonduc* which are
173 recalcitrant seeds cannot germinate readily at moisture content below 20% (Kazeem-Ibrahim *et al.*, 2016). Once that
174 critical seed moisture content is attained in the seed, sufficient water is present to initiate germination (Ellis *et al.*, 1986).
175 On the other hand, if internal moisture content decreases below the critical moisture content, seeds will essentially decay
176 in the soil (Bradford, 1995). Seed germination is a complex process involving many individual reactions and phases, each
177 of which is affected by temperature (Covell *et al.*, 1986). The characteristics exhibited by *V. paradoxa* may be an
178 indication of ecological adaptation of the species since its natural range is within the savanna eco region where
179 temperature is relatively high all the year round. This implies that low temperatures do not favour the viability of the seeds
180
181

CONCLUSION

It could be inferred from this finding that seeds of *V. paradoxa* could be collected from any of the sources within the distribution range of the species most especially for germination purpose. There is obvious evidence that the seeds should be sown fresh since seeds from all the sources recorded impressive initial germination percentage. Nursery managers and silviculturists who want to raise the seedlings don't have to bother about provision of cold storage. This information is crucial in ensuring successful germplasm conservation programme so that seedlots that lose their viability quickly may be kept in storage for a short period and sown out before they lose their viability. However, further studies are needed on improving the longevity in storage of *V. paradoxa* seed.

REFERENCES

- Baskin JM, Baskin CC. A classification system for seed dormancy. *Seed Sci. Res.* 2004;14:1–16.
- Bewley JD, Bradford K, Hilhorst H.. *Seeds: Physiology of development, germination and dormancy*. Springer Science and Business Media, 2012; p: 392.
- Beyranvand H, Farnia A, Nakhjavan SH, Shaban M. Response of yield and yield components of maize (*Zea mayz* L.) to different bio fertilizers. *International Journal of Advanced Biological and Biomedical Research*. 2013;Volume 1, Issue 9: 1068-1077.
- Bonkougou EG. The Shea Tree (*Vitellaria paradoxa*) and African Shea Parklands, *Proceedings of the International Workshop on Processing and Marketing of Shea Products in Africa, Sénégal*, 4-6 March 2002. 2005;CFC Technical paper No 21, Food and Agriculture Organization. Rome.
- Bradbeer JW. *Seed dormancy and germination*. Springer Science and Business Media, 2013;P: 146.
- Bradford KJ. Water relations in seed germination. Pages 351–396 in J. Kigel and G. Galili, eds. *Seed Development and Germination*. 1995;New York, Marcel Dekker, Inc.
- Cibele CM, Bovi ML, Nakagawa J. Desiccation effects on germination and vigor of King palm seeds. *Hort Brasileira* 2003; 21: 88-92.
- Covell S, Ellis RH, Roberts EH, Summerfield R.J. The influence of temperature on seed germination rate in grain legumes. I. A comparison of chickpea, lentil, soybean, and cowpea at constant temperatures. *Journal. of Botanical Science*. 1986;37: 705–715.
- Daws, M. L. Gamene, C. S. Glidewell, S.M. Pritchard, H. W. Seed mass variation potentially masks a single critical water content in recalcitrant seeds. *Seed Science Research* 2004;14(02):185 – 195 DOI: 10.1079/SSR2004168
- Davidson RH, Edwards DG, W., Sziklai, O. and El-Kassaby, YA. Variation in germination parameters among Pacific silver fir populations. *Silvae Genet.*, 1996.;45: 165-171.
- Ellis RH, Covell S, Roberts EH, Summerfield RJ. The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *Journal. Exp. Botany* 1986; 37:1503-1515.
- Gamaéné SH, Pritchard C, Harris W. *Vitellaria paradoxa*. The project on handling and storage of recalcitrant and intermediate tropical forest tree seeds, Newsletter 1999; 5: 15-16. Danida Forest Seed Centre, Humlebæk, Denmark.
- Hall JB, Aebischer DP, Tomlinson HF, Osei-Amaning A, Hindle JR.. *Vitellaria paradoxa*, A Monograph. School of Agricultural and Forest Sciences, University of Bangor. UK. 1996; pp. 105.
- Kazeem-Ibrahim F, Akinyele OA, Asinwa Ol.. Influence of different storage temperatures and periods on the germination of *Ceasalpinia bonduc* (L.) Roxb seeds. *Journal of Forestry Research and Management*. 2016; Vol. 13, 71-82; 2016, www.frin.gov.ng/frin1/journals.html Publication of Forestry Research Institute of Nigeria
- Lady B J .<http://www.wildflower.org/2002>. Guidelines for seed collection.
- Lars S. *Guide to handling of tropical and subtropical forest seed* (eds) Kersten,O. Danida Forest Seed Centre. 2000;511pp.
- Lamien N, Tigabu M, Guinko S, Oden PC. Variations in dendrometric and fruiting characters of *Vitellaria paradoxa* populations and multivariate models for estimation of fruit yield. *Agroforestry Systems* 2007;69: 1–11.
- Pritchard HW, Yameogo CS, Daws MI. Desiccation, storage and germination of *Butyrospermum paradoxum* (*Vitellaria paradoxa*) seed from Burkina Faso. *International Plant Genetic Resources Institute Annual Report*1996;.51.
- Roberts EH. Temperature and seed germination. in S. P. Long and F. I.Woodward, eds. *Plants and Temperature*. Cambridge, U.K: 1988 Society for Experimental ;Pages 109–132 Biology.
- Shaban M. Application of seed equilibrium moisture curves in agro physics. *International Journal of Advanced Biological and Biomedical Research*. 2013;Volume 1, Issue 9: 885-898.
- Toon PG, Haines RJ Dieters MJ. Relationship between seed weight, germination and seedling-height growth in *Pinus caribae*. Morele. var. *hondurensis* barre and Golfri. *Seed Science Technology*. 1990, 19: 389-402.
- Vertucii CW, Crane J, Vane NC. Physiological aspects of *Taxus brevifolia*. *Physiologia planetarium* 1996;98:1-12.