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Effects of Provenances, Storage Temperature and Duration on Seed Germination of *Bombax costatum* Pellegr & Vuillet

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Abstract

Seed morphology, physiology and environmental conditions have influence on germination of any tropical seeds and their appropriate handling, processing and handling enhances seeds emergent. This study therefore investigated effects of storage durations under different temperatures on germination of *Bombax costatum* seeds from different provenances. Fresh 25 seeds of *B. costatum* in four replicates were collected from four different provenances (Aponmu, Oluwa, Ibadan and Oyo). They were surface sterilized, thoroughly washed in distilled water and stored at four different temperature regimes: 28°C, 21°C, 5°C and -17°C . Samples were taken every 2 months for germination test for 18 months. Germination assessment was carried out daily and recorded. The experimental design was 4×4×10 factorial in 4 replicates. Data were subjected to percentages and analysis of variance (ANOVA). Results showed that there were significant differences among storage temperatures, storage durations and provenances on germination percentage. Freshly collected seeds from Aponmu and Oyo had the highest mean germination of 100% followed by seeds from Ibadan (89.3%). Seeds from Oyo provenance stored 5°C had germination of 94.0%, 88.70% and 78.7% at 2nd, 4th and 6th month respectively. Seeds from Ibadan and Oyo stored at 28°C, 21°C recorded 0.0% starting from 8 months of storage. Germination of *B. costatum* seeds from the four provenances decreased with increase in storage duration at different temperatures.

Key Words: viability, emergent, temperature, storage, moisture

Introduction

Seeds are grouped according to their physiological storage potential as recalcitrant and orthodox (Bonner 1994). Recalcitrant seeds are moisture loving seeds and reducing moisture does not predictably increase longevity of viability (Adelani et al. 2020b). Orthodox seeds are tolerant of relatively extreme desiccation and will survive storage in the dehydrated state for periods that are predictable depending on the storage conditions (Lynch 1990). For efficient running of tree production programme on a large scale, the importance of an efficient and regular supply of viable seeds cannot be overemphasized. The seeds must be viable and available at the appropriate sowing season.

According to Wang et al. (1990), viable seeds are required for maximum seedling production, high rates and uniformity of germination is essential for an efficient modern afforestation programme. The main purpose of traditional seed storage therefore is to secure the supply of good quality seeds for a planting programme whenever needed. Hence seed storage serves as a buffer between demand and production. Seeds are stored during periods of abundance

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availability and taken to nurseries or other recipients when required (Schmidt 2000).

Holmes et al. (1987), defined seed storage as the preservation of viable seeds from the time of collection until they are required for sowing. The period for which seed can remain viable without germinating is greatly affected by its quality at the time of collection, its treatment at collection, storage and condition under which it is stored. Schmidt (2000) stated that it is equally wasteful to spend money on seed collection, extraction and cleaning if storage conditions are so inadequate that the seeds are 90 percent dead before they reach nursery.

Roos (1988) stated that seed storage is very crucial for germplasm preservation. It is very important to breeders, silviculturists and industries interested in seed processing and commercial plantation establishment. Seed of many species, however, lose viability after short periods of storage, making them prone to extinction and causing extensive losses. Loss of viability in storage in addition to reducing the number of plants that can be produced by a given seedlot may also result in a shift in the genetic constitution of the seeds being stored. This is particularly important in forest trees, which are predominantly out breeding (Adelani et al. 2020a).

Many factors influence seed production. They are genetical, climatic, pests, diseases, animals, human interference, forest fire etc. Also, seeding season for many hardwood species in the tropics is concentrated within few weeks. Within the short periods available, a maximum of seeds must be collected in the face of competition from game, cattle, humans, insects and generally with few resources available. Consideration must therefore be given to processing and appropriate storage for such seeds (Walters-Vertucci et al. 1996).

Cold storage has proved to prolong the viability of seeds of a number of tropical forest tree species. Gyimah (1999) also showed that cold storage is better than ambient or room temperature storage for some tropical tree species. Storage on the shelf resulted in a serious drop in seed viability. Bahuguna et al. (1987) found that storage temperature of 5°C was the best among 15°C, 20°C, 25°C and 30°C for *Terminalia myriocarpa*. Poor storage of seed in many cases contributed to a total loss of seed viability. This shows that it is essential for seeds to receive proper storage conditions in order to maintain their viability. Likewise, germination testing is often the most reliable way of assessing viability (Davies et al. 2015; Baba et al. 2021).

B. costatum is a very good species for pulp and paper production. It is the red flowered deciduous kapok tree of the forest regions found in the lowland rainforest zone, woody savannas, woodlands, secondary forest and in outliers (Arbonnier 2004). The tree grows up to 40 m high and 4 cm girth. In Ghana it is the most preferred species for food crop association and it is also considered for religious activities (Owusu-Sekyere 1999).

Influence of environmental factors and seed handling have been observed on seed germination of *B. costatum* (Ojo 2019). Among factors affecting seed germination are temperature, water, oxygen and light. Seed sources, physiology and morphology also play significant role on the germination of the species (Hartmann et al. 2002; Ojo 2019). However, there is dearth of information on the particular temperature and duration for which the species can be stored. This study therefore investigated effects of storage durations under different temperatures on germination of *B. costatum* seeds from different provenances in Southwest Nigeria.

Materials and Methods

Seeds of *Bombax costatum* were procured from four provenances in South Western Nigeria, Aponmu (latitude 7° 23' and 7° 19'N and longitude 5° 10' and 5° 05'E), Oluwa (latitude 7° 44' and 7° 48'N and longitude 3° 91' and 3° 63'E) in Ondo State, Ibadan (latitude 7° 18' and 7° 25'N and longitude 3° 42' and 3° 29'E), and Oyo (latitude 3° 55' N and 4° 42' N and longitude 3° 53' and 3° 47'E) in Oyo State.

Twenty-five trees were sampled from each provenance. The trees were numbered serially with red paint. All the selected trees were growing naturally and the tree species were within 100 m apart. Seeds were collected from the base and middle portion of the crown.

Germination tests

Freshly collected seeds from each provenance were divided into 4 and stored differently room temperature at 28°C, Air conditioned room at temperature 21°C, cold room at 5°C and in the freezer at -17°C. Twenty five seeds in 4 replicates from each provenance were freshly sown as control treatment. The same number of seedlots was subsequently taken from each storage condition/medium for germination trial at every 2 months for 18 months; that is 0 (control/freshly sown), 2, 4, 6, 8, 10, 12, 14, 16, 18 months. At each time of sowing, seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for one minute and thoroughly washed in distilled water (International Seed Testing Association 1996). The experimental design was 4×4×10 factorial in 4 replicates. The seeds were then laid on Whatman's No. 9 filter paper and placed on transparent glass sheets at Seed Store Laboratory of Forestry Research Institute of Nigeria (FRIN). The filter papers were moistened with 10 ml of distilled water and kept sufficiently moist at all times to supply the necessary moisture to the seeds. The glass sheets were placed inside Copenhagen tanks at 28°C. Germination assessment was carried out daily and recorded. Observations continued for a month after which un-germinated seeds were discarded. Germination percentage of seeds from each provenance at different temperature and storage duration was determined according to Davies et al. (2015) as follows:

$$\text{Germination Percentage (\%)} = \frac{\textit{total seeds germinated}}{\textit{Total ssed sown}} \times 100$$

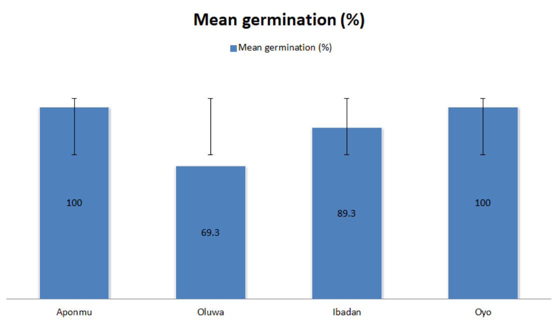

Fig. 1. Germination of fresh seeds of *Bombax costatum* from the different provenances.

Results

Germination of B. costatum seeds from different provenances as influenced by different temperature

Fresh seeds from Oyo and Aponmu provenances recorded the highest mean germination percentage of 100%. This was followed by fresh seeds from Ibadan with a germination percentage of 89.3% (Fig. 1). Fresh seeds from Oluwa provenance recorded the lowest mean germination percentage of 69.3%. Significant differences were observed in seed germination percentage from the four provenances at 5% probability level. Seedlot stored at 5°C recorded the highest mean germination percentage of 40.4%. This was followed by seeds stored at -17°C (35.3%). Seedlots stored at 21°C had mean 20.6% germination. The lowest mean germination was 19.7% observed at seedlot stored at 28°C (Table 1). Significant differences were observed in the effect of seed storage conditions on seed germination at 5%

Table 1. Mean values for effects of storage temperature on seed germination of *B. costatum* from different provenances as tested using ANOVA followed by LSD post hoc test

Storage temperature	Mean germination (%)
Room temperature (28°C)	19.7[a]
Laboratory with Air Condition (21°C)	20.6[b]
Freezer (-17°C)	35.3[c]
Cold room (5°C)	40.4[d]
LSD	1.73

Means with the same letter were not significantly different between the temperature treatment (p > 0.05).

Table 2. Mean values for effects of storage duration on seed germination of *B. costatum* from different provenances as tested using ANOVA followed by LSD post hoc test

Storage duration (months)	Mean germination (%)
0	89.7[j]
2	70.8[i]
4	53.7[h]
6	30.6[g]
8	20.4[f]
10	13.3[e]
12	7.0[d]
14	2.7[c]
16	1.3[b]
18	0.4[a]
LSD	2.74

Means with the same letter were not significantly different between the temperature treatment (p > 0.05).

probability level.

Germination of B. costatum seeds from different provenances under different storage durations

The highest mean germination (89.7%) was recorded for fresh seeds of *B. costatum*. Seeds stored for 2 months had mean germination of 70.8%. At 4 months of storage, mean germination 53.7% was obtained. Mean germination of 30.6% was obtained at 6 months of storage, while at the 12 month of storage mean germination was 7%. The lowest mean germination 0.4% was observed at 18 months of storage. Significant relationships were recorded in the effect of duration of storage on seed germination at 5% probability level (Table 2).

The highest mean germination 58.8% was recorded under seedlots from Oyo stored at 5°C. This was followed by seedlots from the same provenance stored at -17°C (48.8%). Seeds from Ibadan provenance stored at 5°C attained 39.5% followed by seeds from Aponmu provenance under the same storage condition (37.8%). The lowest mean germination 15.5% was observed for seeds from Oluwa provenance stored at 28°C. Significant difference were obtained in seed germination from the provenance at 5% probability level (Table 3).

At 6 months of storage, seedlots from all the provenances had less than 40% mean germination. There was a significant difference in the interaction effect of provenance and duration of storage on seed germination at 5% probability level (Table 4). Highest mean germination of 89.7% was observed under the four storage conditions for fresh seeds. The lowest mean germination of 0% was observed for seeds stored at 21°C and 28°C at 12th month of storage. Likewise, seeds stored at -17°C recorded the lowest mean value of 0% at 16 months of storage. There were significant interactions between storage duration and storage temperature on germination percentage at 5% probability level (Table 5).

Fresh seeds from Aponmu and Oyo provenances attained the highest mean germination of 100%. The lowest mean germination of 0% was obtained for seeds from

Table 3. Mean values for interaction effect of provenance and storage temperature on seed germination of *B. costatum* as tested using ANOVA followed by LSD post hoc test

Provenance	Storage temperature			
	R. Temp 28°C	Lab with 21°C	Freezer -17°C	Cold room 5°C
Oluwa	15.5^{a}	19.1	21.9^{cde}	25.3^{e}
Ibadan	18.3^{ab}	18.8^{abc}	35.1^{f}	39.5^{g}
Aponmu	24.2^{de}	24.7^{c}	35.3^{f}	37.8^{fg}
Oyo	20.8^{bcd}	19.9^{bc}	48.8^{h}	58.8^{i}
LSD	3.47			

Means with the same letter were not significantly different between the temperature treatment (p > 0.05).

Table 4. Mean values for interaction effect of provenance and duration of storage on seed germination of *B. costatum* as tested using ANOVA followed by LSD post hoc test

Provenance	Storage duration (months)									
	0	2	4	6	8	10	12	14	16	18
Ibadan	89.3^{q}	72.8^{o}	47.6^{n}	28.8^{ij}	21.1^{gh}	13.1^{ef}	5.8^{bcd}	0.7^{ab}	0.0^{a}	0.0^{a}
Aponmu	100.0^{r}	73.7^{o}	58.3^{n}	35.3^{kl}	20.8^{gh}	13.3^{ef}	3.8^{abc}	0.0^{a}	0.0^{a}	0.0^{a}
Oluwa	69.3	57.5^{o}	45.8^{m}	18.8^{gh}	8.5^{cde}	3.7^{abc}	0.9^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
Oyo	100.0^{r}	79.3^{p}	62.9^{n}	39.6^{l}	31.1^{jk}	23.3^{hi}	17.6^{fg}	10.2^{de}	5.2^{abcd}	1.5^{ab}
LSD	5.48									

Means with the same letter were not significantly different between the temperature treatment (p > 0.05).

Table 5. Mean values for interaction effect of storage temperature and storage duration on seed germination of *B. costatum* from different provenances as tested using ANOVA followed by LSD post hoc test

Storage duration (months)	Storage temperature (°C)			
	Lab A/C 21°C	R. Temp 28°C (%)	Cold room 5°C	Freezer -17°C (%)
0	89.7^{n}	89.7^{n}	89.7^{n}	89.7^{n}
2	63.8^{kl}	60.1^{jk}	80.7^{m}	78.7^{m}
4	40.2^{gh}	36.3^{fg}	69.31	68.9^{l}
6	10.7^{d}	6.9^{bcd}	54.6^{ij}	50.3^{i}
8	1.3^{a}	2.8^{abc}	43.5^{h}	33.7^{af}
10	0.5^{a}	1.2^{a}	31.0^{f}	20.7^{e}
12	0.0^{a}	0.0^{a}	20.0^{e}	8.0
14	0.0^{a}	0.0^{a}	8.3^{d}	2.5
16	0.0^{a}	0.0^{a}	5.2^{abcd}	0.0^{a}
18	0.0^{a}	0.0^{a}	1.5^{ab}	0.0^{a}
LSD		5.48		

Means with the same letter were not significantly different between the temperature treatment ($p > 0.05$).

Table 6. Mean values for interaction effect of provenance, storage temperature and storage duration on seed germination of *B. costatum* as tested using ANOVA followed by LSD post hoc test

Provenance storage temp	Storage duration (months)/germination (%)									
	0	2	4	6	8	10	12	14	16	18
Ibadan×21°C	89.30^{p}	60.7^{k}	32.0^{ef}	6.0^{b}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Ibadan×28°C	89.30^{p}	67.3^{kl}	24.0^{cd}	2.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Ibadan×5°C	89.30^{p}	84.70	68.0^{kl}	52.0^{i}	46.0^{h}	33.0^{ef}	19.7^{cd}	2.7^{a}	0.0^{a}	0.0^{a}
Ibadan×-17°C	89.3^{p}	78.7^{m}	66.0^{kl}	55.3^{ij}	38.3^{g}	19.3^{cd}	3.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Aponmu×21°C	100.0^{q}	69.3^{kl}	53.3^{ij}	24.3^{d}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Aponmu×28°C	100.0^{q}	65.3^{kl}	47.7^{h}	15.7^{c}	8.7^{b}	4.7^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Aponmu×5°C	100.0^{q}	79.0^{m}	64.3^{kl}	52.7^{i}	41.7^{g}	28.0^{e}	12.3^{bc}	0.0^{a}	0.0^{a}	0.0^{a}
Aponmu×-17°C	100.0^{q}	81.0^{mn}	68.0^{kl}	48.3^{h}	32.7^{ef}	20.3^{cd}	2.7^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oluwa×21°C	69.31^{kl}	58.3^{ij}	45.0^{h}	11.0^{bc}	5.0^{b}	2.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oluwa×28°C	69.31^{kl}	45.7^{h}	29.0^{e}	8.7^{b}	2.3	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oluwa×5°C	69.31^{kl}	65.0^{kl}	56.0^{i}	34.0^{ef}	16.7^{c}	8.7^{b}	3.7^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oluwa×-17°C	69.31^{kl}	61.0^{k}	53.3^{l}	21.7^{c}	10.0^{bc}	4.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oyo×21°C	100.0^{q}	67.0^{kl}	30.0^{ef}	1.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oyo×28°C	100.0^{q}	62.0^{k}	44.7^{h}	1.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Oyo×5°C	100.0^{q}	94.0^{p}	88.7^{op}	78.7^{mm}	69.7^{kl}	54.3^{c}	44.3^{g}	30.7^{ef}	20.7^{cd}	0.0^{a}
Oyo×-17°C	100.0^{q}	94.0^{p}	88.0^{op}	76.0^{m}	54.7^{ij}	39.0^{f}	26.0^{d}	10.0^{b}	0.0^{a}	0.0^{a}
LSD					5.48					

Means with the same letter were not significantly different between the temperature treatment ($p > 0.05$).

Aponmu at 8 months (21°C), 12 months (28°C) and at 14 months (5°C and -17°C). Seeds from Oyo provenance stored at 21°C and 28°C attained the lowest mean value (0%) at 8 months of storage, at 5°C (18 months) and at -17°C (16 months). Seeds from Ibadan provenance recorded the lowest mean germination of 0% under 21°C and 28°C at 8 months, while at 16 months, seeds stored at 5°C attained 0%. Seeds from Oluwa provenance also obtained the lowest mean germination of 0% at 21°C and -17°C (12 months), 28°C (10 months), 5°C (14 months). Significant differences were observed in the interaction effect of provenance, storage temperature and storage duration on seed germination at 5% probability level (Table 6).

Discussion

Storage duration on seed germination of B. costatum

Most indigenous tree species like many other trees flower and fruit once a year. In certain years, some trees do not flower at all or they produce low number of flower and fruit (Poulsen et al. 1998). This makes seed storage very vital. The maintenance of seed viability depends on the seeds inherent factors and environmental conditions under which it is stored (Poulsen et al. 1998; Akintola et al. 2021). The length of time seeds can stay viable in the natural environment depends on the type of seed themselves and the conditions around them. Some seed types do not have the ability to stay alive for a long time. These recalcitrant seeds have short physiological storability, which can only be slightly extended by storing them under controlled environment. Orthodox seeds are seeds that can be dried to low moisture content and can be stored at low temperature (Schmidt 2000).

The germination potential is inversely proportional to the storage period. This is observed in the study in which the rate of germination of *B. costatum* seeds is decreasing with increasing storage period. This is in agreement with the observation of Genes and Nyomora (2018), in assessment of storage time and temperature on germination ability of *Escoecaria bussei*. They emphasized that the seeds that did not germinate indicate that they had lost viability due to long period of being stored; that is the increase of storage period resulted in decline in ability of seeds to germinate. Moreover, Walter et al. (2005) reported that the length of storage time is strongly influenced by environmental and genetic factors such as storage temperature and seed moisture content. Rajjou and Debeaujon (2008) reported that when seeds deteriorate during storage, they lose vigor, become more sensitive to stress during germination and ultimately become unable to germinate.

Storage duration and temperature on seed germination of B. costatum

The effect of storage condition and period of storage on germination of *B. costatum* seeds were significant. Seeds stored in cold room (5°C) had the highest mean germination percentage for all the provenances. In all, cold storage had been found to favour the viability of *B. costatum* as observed in this study. This is in line with the observation of Hong et al. (1995) for *Dilplachine fusca*. *Eucalyptus delugpta* and *Eucalyptus microtheca* were also stored at low temperature to maintain viability (Boland et al. 1980). Likewise, Šimić et al. (2005) also observed that low storage temperature favoured high germination percentage of maize, soybean and sunflower oil seeds. Nasreen (1999) also observed decline in seed germination potential of wheat with increase in temperature as a result of heat stress. Roberts (1972) reported that longevity of seeds is mainly controlled by their moisture content and the storage temperature. High storage temperature accelerates seed deterioration, causing seed quality losses and therefore lower germination percentage of seed (Genes and Nyomora 2018).

Germination of B. costatum seed from different provenances as affected by storage temperatures and period of storage

Species from different provenances typically show inherited storage behavior, which may be either orthodox or recalcitrant (Agarwal and Sinclair 1997). The variability observed in the storability of the seeds from different sources could be due to their genetic and physiological makeup. Emmanuel and Dharmaswamy (1991) found large variation in storability of the seeds of Indian Teak. Significant clonal variation in storability was found between six clones of *Picea sitchensis* exposed to varying lengths of accelerated ageing (Chaisurisri et al. 1993). Oni (1993) observed that seeds of *Terminalia ivorensis* from different sources respond differently to different storage temperatures and period of storage. Storage potential is heritable.

The least germination percentage which was observed for seeds stored in room temperature (28°C) for Oluwa provenance is an indication that high temperature does not favour the storage of *B. costatum* seeds. This is contrary to the findings of Velt and Van Auken (1993) for Fullugia paradoxa and Shomade (2000) for *Mansonnia altissima*. Omran et al. (1989) observed that seeds of *Casuarina cunninghamiana* could be stored for eight month in room temperature without significant loss of viability. The physiological cause of reduce storability may be ascribed to failure of accomplishing essential stages of late maturation events, such as incomplete embryo development, inadequate protection from desiccation or inadequate formation of storage protein necessary for storability (Wiersum 1997).

Ageing can cause loss of viability during storage and the storage environment influences it, while potential storage period is prolonged by cold storage as observed in the study. Keys et al. (1995) recommended cold storage above 0°C for long-term storage of *Araucaria cuninghamii* in Queensland. Low temperature reduces microbial growth and delay physiological deterioration. Prevention of germination by low temperature storage (0 to 5°C) is possible for species that are not chilling sensitive.

Conclusion

This study had established the inverse proportional relationships that exist between the germination potential of *B. costatum* seeds and storage period and storage temperature. That is the germination potential of *B. costatum* seeds is decreasing with increasing storage period and temperature. This shows that fresh *B. costatum* seeds have the highest mean germination. Likewise, *B. costatum* seeds are best stored at 5°C for highest mean germination percentage and in order to avoid heat stress that can aggravate moisture loss.

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