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Effect of Temperature and Duration of Storage on Viability of *Plukenetia Conophora* (Walnut) Mull Arg Seeds using Tetrazolium Test

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Abstract **– In this study the effect of temperature and duration of storage on viability of *Plukenetia conophora* seeds using Tetrazolium (T_2C) test was investigated. The seeds were subjected to five different storage temperature including a control, viz: room temperature (28°C), freezer (-5°C), refrigerator (7°C), plastic container filled with dry soil (29°C). The seeds were stored in these treatments for six months. For the control, seeds were tested immediately after extraction. Viability test of embryo from 20 seeds per treatment was carried out fortnightly for the six months by soaking in T2C for 24 hours, after which seeds were examined for colour change. Viable seeds changed to red colour. There was significant difference between viability of fresh seeds (100%) and seeds stored at different temperatures at $p<0.05$. Seeds stored at 7°C in refrigerator had the highest viability (40%) after 30 weeks of storage.**

Keywords **– Viability, Storage Temperature, Tetrazolium Test, *Plukenetia Conophora,* Seeds, Recalcitrant.**

I. Introduction

The success of any afforestation or reforestation project depends largely on regular availability of good quality seeds in adequate quantity for seedling production, thus the importance of seeds in forestry cannot be over emphasized [1]. Seeds are grouped according to their physiological storage potential as recalcitrant and orthodox [2]. Recalcitrant seeds are those that lose their viability easily, their storage life are usually a few days. On the other hand, the orthodox seeds are those that can conveniently be stored by different means until when required for propagation. Most climax tropical rainforest tree species produce recalcitrant seeds and thus must be stored as growing seedlings rather than seeds [3]. *Plukenetia conophora* (Mull ARG) commonly known as walnut is a tropical rainforest liana with much economic potential. It produces recalcitrant seeds that deteriorates and lose viability easily making propagation difficult after the fruiting season. Seed deterioration during storage is an inevitable and irreversible process which depends on external factors (relative humidity and temperature) and internal factors (genetics and seed quality) [4]. To circumvent the problem of unavailability of viable seeds immediately after the fruiting season of walnut, there is need to identify most suitable temperature of storing the seeds for a longer viability. Hence, this study was designed to determine ideal temperature for better storage of *P. conophora* seeds for regeneration.

II. Materials and Methods

The experiment was carried out in the seed storage unit of the National Centre for Genetic Research and Biotechnology (NACGRAB) Ibadan, Nigeria, where a regulated standard facility with varying storage temperatures and constant power supply was used. Four thousand (4000) freshly extracted seeds of *P. conophora* procured from a farmer in Ibadan were used in this experiment. The seeds were subjected to five different storage temperatures including a control viz:

- Stored at room temperature 28°C
- Stored in a freezer at -5°C
- Refrigerator condition 7°C
- Buried in a plastic container filled with dry soil 29°C
- Control (tested immediately after extraction).

A total of 240 seeds were randomly allocated to each treatment. Each seed lot was stored in sealed polythene bag before storage at the various treatments. Assessment of viable seeds was carried out fortnightly with 20 seeds per treatment using one percent (1%) Tetrazolium salt test (T_2C). Seeds were cracked using a small pestle and mortar in the laboratory to expose the endosperm which was opened with a knife to release the embryo. The embryo were placed in each petri dish and tested by covering them with the T_2C for 24 hours. After which they were examined for colour change. Red colour indicates viability while non viable seeds remained unchanged. At the end of the study period of 30 weeks, the data collected were subjected to Analysis of variance. Where significant difference occurred, LSD test was used to separate the means [5].

Assessment was done by simple count of embryo that changed to red colour, which is an indication of formazan

II. Results and Discussions

Effect of Storage Condition on Viability

The control, (seeds extracted and tested immediately) recorded the highest viability percentage of 100%. The 20 embryo tested with Tetrazolium salt (T_2C) overnight changed to colour red.

Seeds Stored at Room Temperature (28°C)

Seeds stored at room temperature (28^0c) had their highest mean viability percentage of 85% at the second week of storage, this gradually decreased until the 18 week of storage when only 10% of the seeds were viable,

after then all the remaining seeds could no longer show signs of viability when tested. (Fig. 1)

Seeds Stored in Refrigerator (7°C)

These seed lot had the highest viability percentage of 100% from the 2nd week through 4th week. At the sixth week the viability percentage reduced to 95% and gradually decreased to 40% at the 30th week of storage, after which no signs of viability were observed (Fig. 2).

Seeds Stored Under Freezer Condition (-5°c)

Had a viability percentage of 80% at the 2nd week of storage, this gradually reduced to 25% at the 10th week of storage, after which no more signs of viability were observed. (Fig. 3)

Buried in a Plastic Container Filled with Dry Soil 29°C

Seeds stored in a plastic container filled with dry soil at 29°C had a viability percentage of 70% at the 2nd week of storage which gradually reduced to 15% at the 10th week of storage after which the seeds lost viability (Fig. 4).

Interaction Effect of Storage Medium and Viability

Analysis of variance showed that the storage medium used had significant effect on the number of seeds that changed to red (viable) at <0.001 (Table 2). Table of means also indicated that the storage temperature used had significant effect on the number of seeds that changed to red (Table 3).

Interaction effect of storage medium used and viability showed that there was a significant difference between the medium used and percentage viability at <0.001 (Table 1)

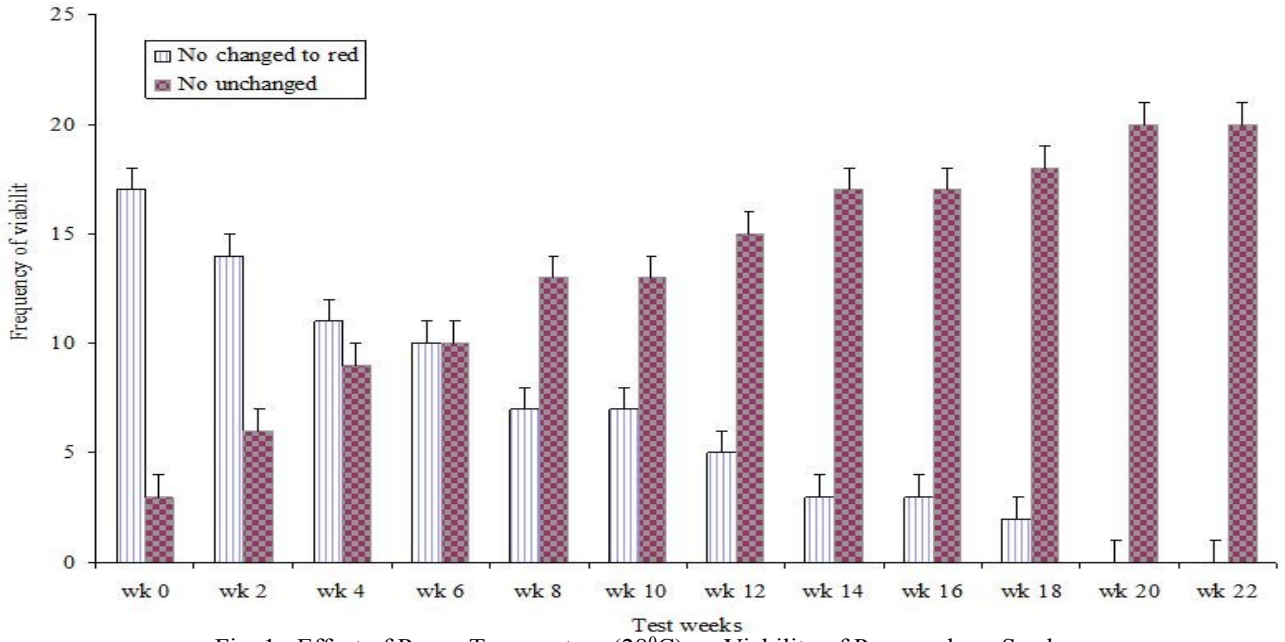

Fig. 1. Effect of Room Temperature (28°C) on Viability of P. conophora Seeds

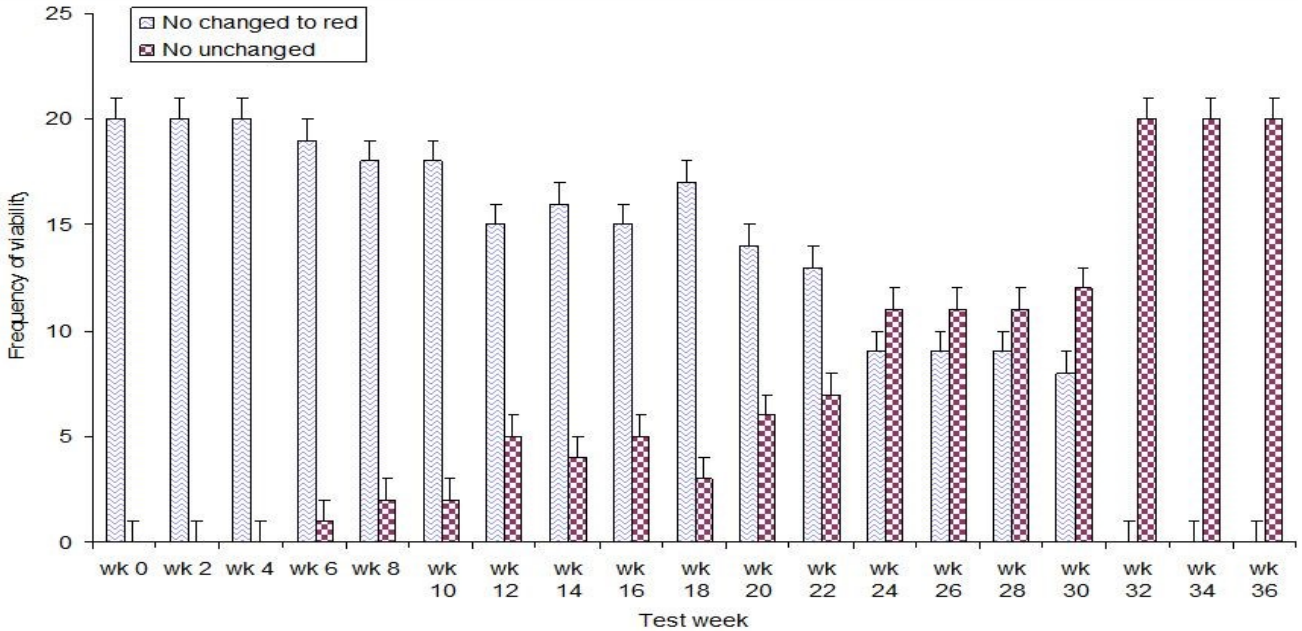

Fig. 2. Effect of Refrigerator Storage (7°c) on Viability of P. conophora Seeds.

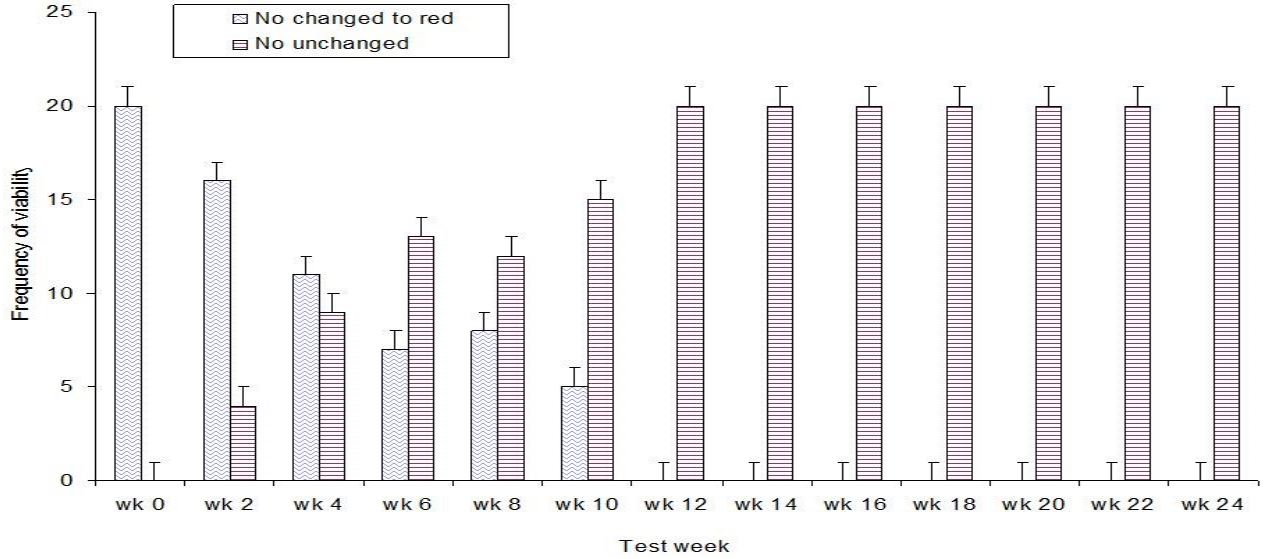

Fig. 3. Effect of Storage in freezer (-5^{O}C) on Viability of P. conophora Seeds.

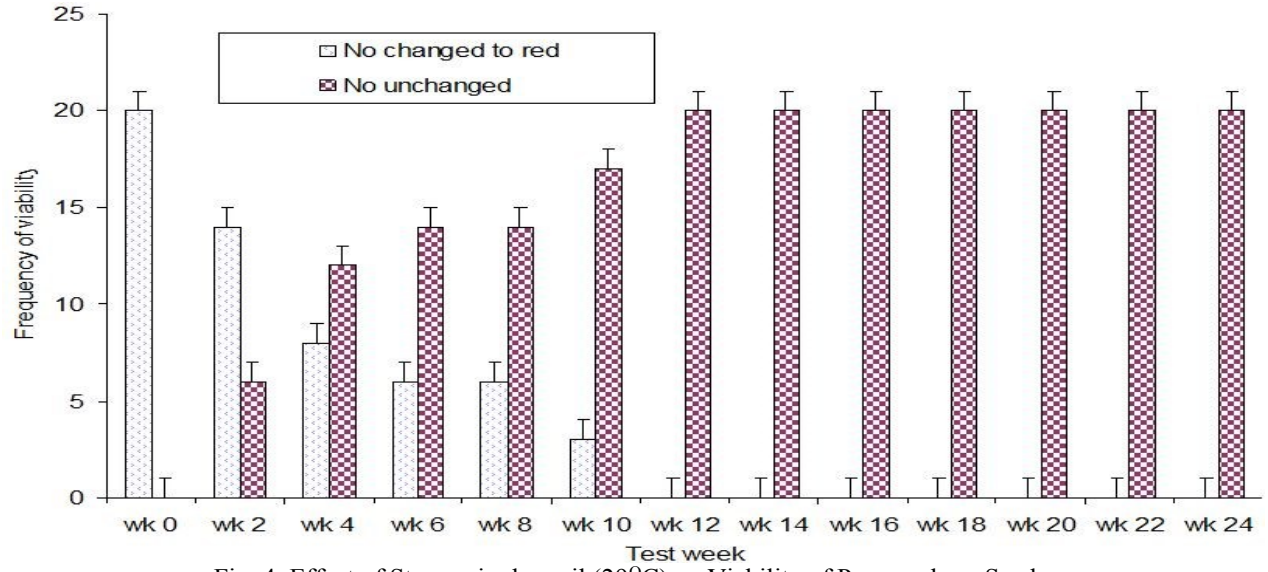

Fig. 4. Effect of Storage in dry soil (29^{O}C) on Viability of P. conophora Seeds.

Table 1. ANOVA for the effect of storage medium on Number of seeds that change to red colour

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F	F.pr
Temp.	3	1407.30	469.10	14.71	<0.001
Residual	60	1909.31	31.82		
Total	63	3316.61			

*Significant at P< 0.001

Table 2 ANOVA for the effect of storage on Seed Viability

Source of variation	Degree of Freedom	Sum of Square	Mean Square	F	F.pr
Temp.	3	35406.4	11802.1	14.62	<.001
Residual	59	47619.4	807.1		
Total	62	82915.1			

*Significant at P< 0.001

Table 3. Means for the Effect of Storage Temperature on viability of Plukenetia conophora

TREATMENT TEMP	PARAMETER MEASURED CHANGED RED	UNCHANGED	% VIABLE
5^{0}C	4.19	10.81	20.9
7^{0}C	15.00	6.25	75.0
28^{0}C	4.94	8.07	24.0
29^{0}C	3.56	12.69	17.8
LSD	3.989	NS	20.10

III. Discussion

Germplasm is the reproductive unit (genetic material) of any plant, it could be seed, pollen, vegetative propagules or other materials, though normally seed. Through germplasm collection, the genetic diversity essential to any tree improvement programme could be safe guarded [6]. In recognition of the threat to mass genetic erosion from accelerated tropical deforestation, massive

germplasm collection and conservation efforts are inevitable. The physiological state and physical storage conditions of seeds greatly influence their lifespan. Since storage is an important factor in preserving viable seeds from time of collection until they are required for sowing [7], poor storage may greatly affect seed viability, and since most tropical tree species fruit seasonally, it is thus important to identify the best storage condition to maintain seed viability. Seeds are either orthodox (seeds that maintain their viability for long periods if processed (low moisture content) or they are recalcitrant, (seeds that lose viability very fast, even under conditions that are normally conducive to seed longevity). *Plukenetia conophora* is a recalcitrant species. The effect of different storage temperature and duration on the viability of its seed were highly significant (<0.001) Seeds stored at refrigerator condition (7°c) had the highest viability percentage of 45% at 24 weeks and longest storage period of 30 weeks with 40% of seeds still viable. This is in agreement with Ojo [7], who observed that cold storage at 5^0c favored the viability of *Bombax costatum*. However, Fennessy [8], explained that normal (orthodox) seeds could store successfully for long durations at low temperature conditions and at low moisture content, Warren and Adam [9], explained further that recalcitrant seeds do not store because as the storage period increases, moisture content further reduces, respiration rate declines and oil seeds will thus lose viability. He concluded that starchy seeds tend to last longer in storage than oily seeds. In this study, cold storage of *Plukenetia conophora* at 7^0c favoured the viability of the species till the 7^{th} month, which is in agreement with Pierizzi *et al.* [10], who worked on preservation of germination capacity of *Araucaria angustifolia* using refrigerated storage at 0^0c, 4^0c and 10^0c and concluded that germination capacities were best at 0^0c for 12 months. This study is also in agreement with Pasquini *et al.* [11], who investigated different storage conditions in recalcitrant seeds of *holm oak* and concluded that low temperature and drying tolerance storage under different temperature can increase viability. Storage in ambient temperature decreased the viability of *P. conophora*; this was observed in seeds stored in room temperature (28^0c), that lost their viability in less than 20 weeks while those buried in dry soil in plastic containers (29^0c) also lost their viability at 10 weeks. However extremely low temperature does not favour the viability of this recalcitrant species as seeds kept in freezer condition -5^0c caked, and lost their viability at 10 weeks.

IV. Conclusion and Recommendation

Through the study, it could be suggested that *P. conophora* could best be stored at 7°C in a refrigerator for about 6 months after harvesting for onward propagation. However, it is recommended that further research should be carried out on cheaper storage methods other than these that could prolong viability of the species for regeneration.

References

[1] Oni, O. (1995): Management and maintenance of stock plants for effective vegetative propagation of forest tree species, paper presented at the training Workshop on the training Workshop on the vegetative propagation of Forest Trees, organized by Extension Research and Liaison section (ERLS) FRIN 7 – 11 Nov. 1995. Iwo, Osun State. 15pp.

[2] Bonner, F. T, Vozzo, J. A, Eloiam W. W and Land, S. B. (1994): Tree seed technology training course instructors manual, Southern forest experimental station. U.S. Department of Agriculture. General Technical report pp. 50 – 106.

[3] Michael Allaby (2004): A Dictionary of Ecology 2004.

[4] Research Interest (2010):Seed Lab Wageningen. www.wageningeninfo.com.

[5] Gomez, K. A. and Gomez, A. A. (1984): Statistical procedure for agricultural research. 2nd edition John Wiley and Sons 680 Pp.

[6] Ebenezar Asaah, Alain Rene, Atangana, A.R. and Slain Tsobeng (2006): Germplasm collection production and Distribution in Zac Tchoundjeu, Edit. Regional coordinator. Concepts and principles of Tree domestication. Pp. 26 – 33.

[7] Ojo, M.O. (2008): Effect of seed storage conditions on the germination of the seeds of Bombax costatum Pellergr. & Vuillet from four provenances within Nigeria. Unpublished Ph.D thesis. University of Ibadan, Nigeria. Pp 226.

[8] Fennessy J. (2002): The collection storage treatment and handling of broadleaved tree seeds Coford Ireland.

[9] Waren, C. R. and Adams M. A. (2001): Distribution of nitrogen, Rubisco and Photosynthesis in Pinus pinaster and acclimation to light. Plant cell and environment 24(6) 597 – 609.

[10] Pieruzzi Fernanda P., Leanardo L.C. Dias, Tiago S. Balbuena, Claulete Santa – Catarina Andre W. dos santos and Enyl S. Floh (2011): Polyamines, IAA and ABA during germination in two recalcitrant seeds Araucaria angustifolia (Gymnosperm) and Ocotea odorifera (Angiosperm). Ann. Bot. (2011) doi 10. 1093/acb/mcr 33. June 17, 2011.

[11] Pasquini, S, Braidot, E., Petrussa, E. Vianellot, A. (2011): Effect of different storage conditions in recalcitrant seeds of Holm oak (Quercus ilex L) during germination. Seed science and Technology. Vol. 39 No. 1. April 2011. Pp 165 – 177 (13).