



SOAKING AND STORAGE DURATION EFFECT ON GERMINATION AND GROWTH OF WILD SOURSOP (*Annona senegalensis* Pers): PROSPECT FOR CONSERVATION

Amadi, J. O., *Alaje, V. I., Oyedeji O.F., Geply, O.A., Appah, O.R., and Alaje, M. A,
Forestry Research Institute of Nigeria, P.M.B. 5054, Ibadan, Oyo State, Nigeria

*Correspondence Address: alajeveronica@gmail.com +2348035267527

Abstract

Rapid genetic erosion of wild species from deforestation coupled with the immense benefits of wild species calls for urgent massive conservation efforts. This study was therefore carried out to assess the effect of soaking and storage duration on the germination and growth of wild soursop with the aim of conserving it. The two factors nursery experiment comprised of storage time (0, 2, 4, and 6 weeks) and soaking time (0.8, 16 and 24 hours) arranged in completely randomized design in five replications. Data collected on germination: number of leaves, plant height, collar diameter and biomass, were subjected to descriptive as well as analysis of variance using SAS and significant means were separated using Fishers least significant difference at 5% level of probability. Results showed that seed stored at 0 weeks soaked for 8 hours had the highest germination percentage of 92% while the least was observed in 6 weeks with 0 hours soaking. The result also showed that soaking duration had significant effect $P \leq 0.05$ on number of leaves, plant height and biomass. The effect of storage was also significant on number of leaves and biomass. The interaction between soaking and storage duration had significant effect $P \leq 0.05$ on collar diameter and biomass. The study demonstrated that soaking seeds of wild soursop enhances germination resulting in uniform seedlings growth as well as improved growth parameter of this wild species.

The study recommends that seeds collected for conservation purposes, should not be stored for more than two weeks at room temperature.

Key words: wild soursop, germination, storage, soaking, growth

Introduction

Annona senegalensis, a wild fruit tree commonly known as wild soursop or wild custard apple belonging to the family Annonaceae has immense benefits to man, animal and industries (NRC, 2008). The fleshy pulp is eaten by man and the leaves are used as fodder for livestock. The leaves are also used for treating pneumonia and as a tonic to promote general well being. The bark is used as an insecticide and also used for treating guinea worms and other worms, diarrhoea, gastroenteritis, snakebite, toothache and respiratory infections. The gum from the bark is used in sealing cuts and wounds (Orwa *et al.*, 2009). The roots are used for stomachache and venereal diseases (Orwa *et al.*, 2009). With these enormous benefits of this wild species to man and livestock, it is expedient to conserve and improve upon the traits to meet the demand of man and other users.

The use of wild plants or crop wild relatives in breeding cannot be overemphasized. They are useful genetic resources used in breeding improved varieties that are high yielding, nutritious, pest and disease resistant, stress tolerant and resilient. These benefits derived from wild species may be hampered by their inadequate representation in gene banks and the loss of wild varieties to deforestation, unsustainable harvesting and usage, climate change and other impacts (Castañeda-Álvarez *et al.* 2016). There is therefore the need to prevent the loss of these useful genetic resources and to maximize their availability. This demand an urgent attention in ensuring their appropriate conservation and sustainable use.

The immense benefits of this wild species and the threat posed by mass genetic erosion calls for massive germplasm collection and conservation efforts. Through germplasm collection, the genetic diversity essential to any tree improvement programme could be safe guarded, (Asaah *et al.*, 2006). Seed storage which is an *ex situ* germplasm conservation is an essential step for the long-term conservation of plant genetic resources. Seed storage is employed in maintaining seed viability for longer period and this is very crucial in preserving the genetic integrity in stored seeds (Pradhan and Badola, 2012). Seed storage mostly in genebanks is the most practical *ex situ* conservation technique for many plant species (Maxted *et al.*, 2013)

Seed is the reproductive unit which also houses the genetic material of any plant. Viable seeds are essential for continuity of any species. Seed storage plays an important role in making large volume of viable seeds available for use (Merritt and Dixon, 2011). Different types of seed respond different to storage duration. Orthodox seeds could store successfully for long durations at low temperature and low moisture content (Fennessy, 2002) while recalcitrant seeds do not store because as the storage period increases, moisture content further reduces and respiration rate declines (Warren and Adams, 2001).

Seed germination is an important aspect of plant conservation. The key to success in seed propagation is proper timing and high germination percentage. Plants that possess hard coat poses serious problem to seed germination by hindering water permeability and germination thereby resulting in seeds taking too long to germinate or have random germination when no pre-germination treatment is applied (Azad *et al.* 2010). Pre-germination treatment technique such as soaking enhances germination in hard coated

seed. Seed soaking before sowing is employed to shorten the lag phase in germination thereby resulting in faster seedling establishment (Azad et al. 2010).

Since storage is an important factor in preserving viable seeds from time of collection until they are required for sowing (Ojo, 2008), it is thus important to identify how long seed stored will maintain viability. The study also aimed at identifying the most effective soaking period for germination, growth and development of wild soursop. This will add to the information necessary for the conservation and utilization of this wild species as the use of crop wild relatives is expected to increase with better information about the species (Castañeda-Álvarez et al. 2016). This study was, therefore conducted to determine the effect of storage and soaking duration on the seed germination and seedling growth of wild soursop for conservation purpose.

Materials and methods

Matured fruits of *A. senegalensis* were sourced and allowed to ferment for easy extraction of seeds. Seeds were extracted from the softened fruit manually with the use of hands. The extracted seeds were tested for their viability by the floatation method. Only the unfloated seeds were considered viable and suitable for the experiment. The floated seeds are mainly the immature seeds and were therefore discarded. The seeds were stored at room temperature in the laboratory for further usage.

Experimental site

The experiments were carried out at the Physiology Nursery of Forestry Research Institute of Nigeria (FRIN) Headquarters, Jericho, Ibadan. FRIN is located on the longitude 07023'18"N to 07023'43"N and latitude 03051'20"E to 03051'43"E. The climate of the study area is the West African monsoon with dry and wet seasons. The dry season is usually from November through March and is characterized by dry cold wind of harmattan. The wet season usually starts from April to October with occasional strong winds and thunderstorms. Mean annual rainfall is about 1548.9 mm, falling within approximately 90 days. The mean maximum temperature is 31.90C, minimum 24.20C while the mean daily relative humidity is about 71.9% (FRIN, 2015).

Experimental procedure

A total of 800 seeds was used for the entire experiment which was divided according to the treatment combinations below: the treatment comprised of two factors at four levels each. Factor one is storage time (0, 2, 4 and 6 weeks) while factor 2 is soaking time (0.8, 16 and 24 hours). These were combined in the following ways:

Table 1: Treatment combination for the experiment

Treatment combinations		
	Storage time (weeks)	Soaking time (hours)
T1	0	0
T2	0	8
T3	0	16
T4	0	24
T5	2	0
T6	2	8
T7	2	16
T8	2	24
T9	4	0
T10	4	8
T11	4	16
T12	4	24
T13	6	0
T14	6	8
T15	6	16
T16	6	24

Each of the treatments combinations comprised of 10 seeds replicated five times making a total of 50 seeds for each of the treatment combination.

The seeds were broadcasted on a germination sieve filled with top soil. These are arranged in a completely randomized design in a nursery shed. Watering was done daily.

After germination, the seedlings were potted in a 6”X 8” polythene pots and nursed for 6 weeks. At 6 weeks, 80 uniformly growing seedlings were selected from all the treatment combinations for assessments. The assessment was done at two weeks interval for six months.

Data collection and analysis

Data was collected on the following germination parameters

- Number of seeds germinated: the number of seeds that germinated was counted on a daily basis from the onset of seed germination until no further germination occurred. *Annona senegalensis* exhibit epigeal form of germination, germination was therefore said to occur when the plumule rises above the soil.

- Germination percentage (GP): It is computed as follows:

$$GP = \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

- Number of leaves : Physical counting of the leaves
- Collar diameter (mm): By the use of digital vernier caliper placed at the marked collar
- Plant height: By the use of meter rule. This was placed at the base of the plant and stretched to the tip of the plant. The distance between the base to the tip of the plant is the plant height
- Biomass: this was destructively measured at the beginning and end of the experiment. The seedlings were dipped into a bowl of water to loosen the soil off the root. The young plants are then dried in an oven until a constant weight is obtained. This serves as dried biomass weight.

The collected data was subjected to analysis of variance (ANOVA) using SAS (2000) and means that are significant were separated using Fishers least significant difference at 5% level of probability. Results are presented in graph and tables.

Results

The results revealed that higher percentage of 50% and above were recorded for the seeds stored from 0 to 2 weeks irrespective of the soaking duration while seeds stored for 6 weeks irrespective of the soaking duration recorded very low germination percentage of less than 20%. However, the result further revealed that seeds sown immediately after extraction with 8 hours soaking had the overall highest germination of 92%. This was closely followed by freshly extracted seeds sown after zero hours of soaking and seeds stored for one week and eight hours of soaking. They both produced the same germination percentage of 88%. The least of all was recorded in seeds sown after 6 weeks storage without soaking.

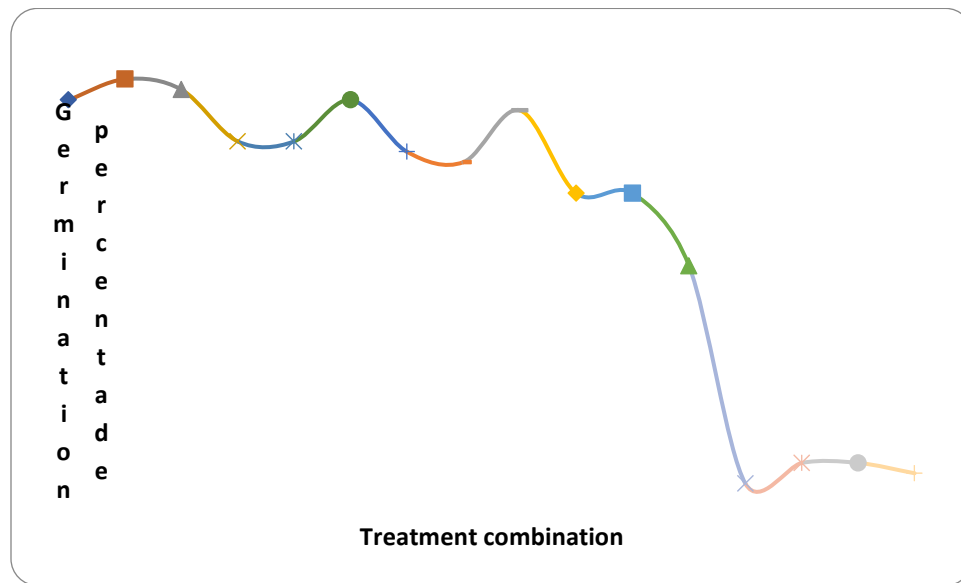


Figure 1: Germination percentage (%) of wild soursop as affected by soaking and storage duration

The mean square analysis for the effect of soaking and storage duration on the growth traits of wild soursop showed that soaking duration had significant effect $P \leq 0.05$ on number of leaves, plant height and biomass (Table 2). The effect of storage was also significant on number of leaves as well as on biomass. The two way interaction between soaking and storage duration had significant effect $P \leq 0.05$ on collar diameter and biomass.

Table 2: Mean square effect of storage and soaking duration on the growth traits of *A. senegalensis*

Sources of variation	DF	NOL	Height	CD	Biomass
Soaking	3	125.381*	132.482*	16.179*	14.508*
Storage	3	215.547*	70.48ns	1.200ns	24.584*
soaking*storage	9	69.825ns	51.501ns	4.381*	32.028*
Error	64	41.706	31.949	1.436	1.84
total	79				

*significant ($p < 0.05$), ns- not significant ($p > 0.05$)

Seeds soaked for 16 hours produced the highest number of leaves followed by seeds soaked for 24 hours (Table 3). The least number of leaves was observed in seedlings produced from seeds soaked for 8 hours though not significantly different from each other (Table 2).

Seeds soaked for 24 hours produced the tallest plant of 17.375cm followed by seeds soaked for 16 hours and the shortest plant was produced from seeds soaked for 8 hours.

The seedlings produced from seeds soaked for 24 hours performed better than all the other soaking durations in terms of collar diameter as it recorded a mean collar diameter of 4.432mm and the least was observed among the seedlings produced from 0 hours soaking with a mean collar diameter of 2.478mm.

The effect of soaking duration on biomass was significant effect. The highest biomass of 6.741g was observed in 16 hours soaking, next to this is 8 hours and 24 hours soaking which produced the same effect.

Table 3: Effect of soaking time on growth traits of wild soursop

soaking time (hours)	NOL	Height (cm)	CD (mm)	Biomass (g)
0	13.69a	12.32c	2.478c	4.934b
8	13.3a	11.73c	2.623bc	6.558ab
16	18.65a	14.715b	2.902b	6.741a
24	14.05a	17.375a	4.432a	6.592ab
Sig	0.037	0.100	0.00	0.00

The storage duration had significant effect $p \leq 0.05$ on number of leaves and biomass (Table 2). The mean effect revealed that 0 weeks storage had the highest number of leaves of 18.94 followed by 2 week storage which produced number of leaves of 15.90 (Table 4). The least number of leaves was observed in 6 weeks storage.

The highest biomass of 7.362g was produced from 0 weeks storage followed by 2 week storage and the least biomass of 5.131g was observed among seedlings raised from seeds stored for 6 weeks.

Table 4: Effect of storage time on the growth traits of wild sour sop

Storage duration (weeks)	Number of leaves	Height (cm)	Collar diameter (mm)	Biomass (g)
0	18.94a	16.085a	3.428a	7.362a
2	15.90b	12.37a	3.099a	6.937b
4	13.60c	15.17a	3.075a	5.393c
6	11.25d	12.51a	2.832a	5.131c
Sig	0.003	0.096	0.479	0.000

Table 5: Interactive effect of soaking and storage duration on the growth traits of wild soursop

The two way interaction between soaking and storage duration on wild soursop had no significant effect on number of leaves produced. Nonetheless, the highest number of leaves of 23.00 was observed among the seedlings raised from seeds soaked for 16 hours after 0 week of storage, next to this were seeds soaked for 24 hours at 0 weeks storage with a biomass of 21.76g. Higher

biomass of 19.40 was recorded for 8 hours soaking and 0 weeks storage as well as 20.00g for 24 hours at 2 weeks of storage. The least of all was observed among seedlings raised from seeds stored for 6 weeks at 0 hour soaking.

Soaking duration (hours)	Storage (weeks)	Number of leaves	Height (cm)	Collar diameter (mm)	Biomass (g)
0	0	14.60a	13.12a	2.596c	9.85a
0	2	11.20a	13.54a	2.138d	7.028b
0	4	10.60a	17.72a	2.152d	3.484e
0	6	7.80a	14.48a	4.722ab	6.600c
8	0	19.40a	11.44a	2.93c	4.634d
8	2	10.20a	9.60a	3.38b	2.988e
8	4	8.60a	14.20a	2.49c	5.284cd
8	6	15.00a	11.68a	1.692e	6.830c
16	0	23.00a	17.24a	2.674c	9.186a
16	2	20.00a	11.98a	2.144d	9.652a
16	4	15.80a	14.10a	2.702c	5.408cd
16	6	15.80a	5.96a	2.394cd	1.986f
24	0	21.76a	22.54a	3.128b	5.78cd
24	2	20.00a	14.38a	4.736ab	8.08b
24	4	10.00a	14.66a	4.958a	7.398b
24	6	13.00a	17.92a	4.906a	5.108d
Sig		0.114	.131	.004	0.000

Discussion

Rapid genetic erosion of wild species from unsustainable tropical deforestation coupled with the immense benefits of wild species calls for urgent massive conservation efforts. Seed storage is a critical step in conservation as this will greatly influence the lifespan of seeds because seeds need to be stored from time of collection until they are required for sowing (Ojo (2008). Hard-coated tree species poses serious problem in germination which hinders these species for conservation, therefore finding alternative to this problem is necessary.

Higher percentage of 80% and above recorded for seeds stored for 0 weeks irrespective of the soaking duration and subsequent decline to about 50% after 4 weeks of storage and to less than 20% seed germination for seeds stored for 6 weeks is an indication that the seed loses viability as storage time increases. This finding agrees with the findings of Kandari (2008) who observed a steady decrease in viability of tropical tree species when stored beyond 2weeks.

On the soaking effect, seed soaking enhances germination of wild soursop. This is in line with the thought of Copeland (1976) who observed that most seeds when swollen in water and sown germinate faster than unsoaked seeds. This also is in line with many authors who reported that soaking seeds before sowing increase seed germination (Alamgir and Hossain, 2005, Azad *et al.*, 2010, Olatunji *et al.*, 2013 and Billah *et al.*, 2015; Odoi *et al.*, 2019)

The result also showed that soaking duration had significant effect $P \leq 0.05$ on number of leaves, plant height and biomass (Table 2). Seeds soaked for 16 hours produced the highest number of leaves followed by seeds soaked for 24 hours. Seeds soaked for 24 hours produced the tallest plant of 17.375cm followed by seeds soaked for 16 hours. The highest biomass of 6.741g was observed in 16 hours soaking. The high performance in 16 and 24 hour soaking treatment suggest the seed of *A. senegalensis* require an optimal level of moisture through soaking to activate the embryo to commence the process of cell division, differentiation and multiplication to grow into a seedling. This according to Sabongari and Aliero, 2004 is due to hydrolysis of complex into simple sugars that are readily utilized in the synthesis of auxins and proteins. The auxins produced help to soften cell walls to facilitate growth and the proteins readily utilized in the production of new tissues (Sabongari and Aliero, 2004).

The storage duration had significant effect $p \leq 0.05$ on number of leaves and biomass (Table 2). The seed stored for 0 weeks had the highest number of leaves of 18.94 followed by 2 weeks storage which produced 15.90. The highest biomass of 7.362g was produced from 0 weeks storage followed by 2 week storage. The least of these two were produced by the 6 weeks storage. This is a pointer

that as seed loses moisture during storage, even though germination eventually takes place, the vigour of the seedling produced will be adversely affected and these seedlings may not be able to survive further thus hampering the conservation of the species. This result is also a clear indication that sowing seeds immediately after collection not only produced the highest germination percentage but also enhanced the growth of this wild species. Thus, suggesting the inadequacy of ambient temperature in storing seeds.

Conclusions and recommendations

The study demonstrated that soaking seeds of wild soursop enhances germination resulting in high germination percentage as well as uniform seedlings growth. Soaking of seed also improved the collar diameter, height and biomass accumulation of this wild species.

The result of this study also demonstrated seed storage duration affect seeds germination as sowing immediately without storing the seeds of wild soursop also resulted in the production of seedlings with good vigour in terms of number of leaves and biomass accumulation.

It concludes convincingly that there is prospect for the conservation of this wild species by giving due consideration to the storage as well as soaking time because storage duration affects the seed germination percentage in wild soursop which declines with increasing storage duration irrespective of soaking duration as well as affected the vigour of seedling produced.

The study recommended that for producing seedlings with good vigour for conservation of this wild species, seeds collected should not be stored for more than 2 weeks at room temperature for best result. Research on storage conditions necessary for long time storage for the conservation of this species is advocated.

References

- Alamgir, M. and Hossain, M.K. (2005). Effect of pre-sowing treatments on germination and initial seedling development of *Albizia saman* in the nursery. *Journal of Forestry Research* 16(3): 200-204.
- Asaah, E., Alain, R., Atangana, A.R. and Slain, T., (2006). Germplasm collection, production and Distribution in Zac Tchoundjeu, Edit. Regional coordinator. Concepts and principles of Tree domestication. Pp. 26 – 33.
- Azad, M.S., Musa Z.A. and Matin A. (2010). Effect of pre-sowing treatments on seed germination of *Melia azedarach*. *Journal of Forestry Research* 21(2):193- 196.
- Billah M.A.S., Kawsar M.H., Titu A.P., Pavel M.A.A., Masum K.M. (2015). Effect of Pre-Sowing Treatments on Seed Germination of *Tectona grandis*. *International Journal of Bioinformatics and Biomedical Engineering*. Vol. 1, No. 1, pp. 37-42
- Bharat K. Pradhan and Hemant K. Badola (2012). Effect of storage conditions and storage periods on seed germination in eleven populations of *Swertia chirayita* : A critically endangered medicinal herb in himalaya. *The ScientificWorld Journal*.(2012) pp 1-9
- Copeland LO (1976). Principles of seed science and technology, Burges Pub. Co. U.S.A. pp.55-200.
- Fennessy, J. (2002): The collection storage treatment and handling of broadleaved tree seeds. Coford Ireland. 155Pp
- FRIN, (2015): Forestry Research Institute of Nigeria, Annual Meteorological Report.
- Maxted, N., Magos Brehm, J. and Kell, S. (2013). Resource book for preparation of national conservation plans for crop wild relatives and landraces. Section Context 1.5.
- Merritt, D. J. Dixon KW (2011). Restoration seed banks—a matter of scale. *Science* 332: 424–425.
- National Research Council (2008): Custard Apples, Lost crops of Africa. National Academies Press Vol 3: ISBN 978-0-309-10596-5
- Ojo, M.O. (2008). Effect of seed storage condition on the germination of the seeds of *Bombax costatum* Pellergr and Vuillet from four provenances within Nigeria. Unpublished PhD thesis. University of Ibadan, Nigeria. Pp 226.
- Olatunji D, Maku J O and Odumefun O P. (2013). The effect of pre-treatments on the germination and early seedlings growth of *Acacia auriculiformis* Cunn. Ex. Benth. *African Journal of Plant Science* Vol. 7(8), pp. 325-330
- Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., and Anthony, S. (2009): Agroforestry Database: a tree reference and selection guide version 4.0 (<http://www.worldagroforestry.org/sites/treedatabases.asp>)
- Kandari, L. S., Roak, S., Maikhuri, R. K. and Chauhan, K. (2008): Effect of pre-sowing, temperature acclimatization to light. *Plant cell and Environment* 24(6) 597 – 609.
- Sabongari, S and Aliero, B. L. (2004). Effects of soaking duration on germination and seedling growth of tomato (*Lycopersicum esculentum* Mill) *African Journal of Biotechnology* Vol. 3 (1), pp. 47-51.
- Odoi, J. B., Mugeni, D., Kiiza, R., Apolot, B. and Gwali S(2019). Effect of Soaking Treatment on Germination of Hard Coated Tropical Forest Tree Seeds. *Uganda Journal of Agricultural Sciences* Volume 19 Number 2 (2019) pp. 1 – 9
- Waren, C. R. and Adams, M. A. (2001). Distribution of nitrogen and Photosynthesis in *Pinus pinaster* and acclimatization to light. *Plant cell and Environment* 24(6) 597 – 609.