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PROVENANCE AND STORAGE DURATION EFFECT ON GERMINATION AND GROWTH OF WILD SOURSOP (*Annona senegalensis* Pers)

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ABSTRACT

Annona senegalensis is a wild species that has enormous benefits that has not be fully utilized and improved on. Upon this, the effect of provenance and storage time on seed quality traits was assessed to determine the source of germplasms collection as well as appropriate storage time. Seeds were sourced from Abeokuta in Ogun state, Ibadan in Oyo state, Ikire in Osun state and Ore in Ondo state and subjected to 0,2,4 and 6 weeks storage at room temperature (25^0C). These were arranged in completely randomized design with five replications. Data collected on germination percentage, number of leaves, seedling height, collar diameter and biomass accumulation were subjected to analysis of variance and significant means were separated using Duncan multiple range test at 5% level of probability. Results revealed that the effect of provenance on almost all the seed quality traits measured were significant ($P<0.05$) except number of leaves. The effect of storage were significant ($P<0.05$) on seed germination as well as biomass accumulation. The two way interactive effect of provenance and storage time were significant ($P<0.05$) for all the traits except number of leaves. Seeds from Ore had the best germination percentage (75.88%), seedling height of 20.84cm, collar diameter of 7.07mm and biomass of (7.07g). Seeds sown immediately after air dry had the highest germination capacity of 70.36% followed by seeds stored for 2 weeks (65.60%) and the least was observed when seeds were sown after six weeks (50.91%) of extraction. Seeds from Ore could therefore serve as superior provenance and seeds should be sown between 0-2weeks after extraction.

Keywords: *Annona senegalensis*, wild relatives, improvement, conservation, provenance

Introduction

Increasing human population has led to increase in human food consumption and agricultural land, unsustainable use of natural resources and urbanization which has resulted in displacement wild species of plants. These are threats to the world's rich and highly adapted plant genetic resources. The wild relative of plants especially those growing in their natural environment are known to be genetically diverse, locally adapted and represent a potential source of genes and alleles for adapting crops to changing environmental conditions and human needs (FAO, 2017). They are paramount to continuous human existence because most of them have high nutritional values such as protein, carbohydrate, vitamins, fat, minerals and fibre, while others are appreciated for their medicinal values, cultural symbols and fuel (Moss, 1995). *Annona senegalensis* like other wild species with diverse usage and benefits to man are mostly found in the wild

To prevent further loss of plant wild relatives and to maximize their availability for human use, there is an urgent need to ensure appropriate in *situ* conservation strategies that will ensure active management and

monitoring of plant populations within their natural habitats as well as ex situ strategies which will involves management of plant outside their natural environment (FAO, 2017).

A range of *ex situ* conservation techniques are available, but seed storage mostly in genebanks predominates as the most practical *ex situ* conservation technique for many plant species (Maxted *et al.*, 2013) and it is expected that seed storage will play an increasingly important role in making sufficient viable seeds available for use or for producing larger volumes of seeds (Merritt and Dixon, 2011).

Seed is the common propagation material for most tropical tree species (Bowes, 1999; Adebisi *et al.*, 2011). Propagation from seed is inexpensive and usually effective, and is therefore a viable method for their ex-situ conservation (Abirami *et al.*, 2010). An understanding of seed physiology could expectedly contribute to the effort of seed-based in-situ conservation as well as ex-situ cultivation (Sharma and Sharma, 2006). The proper handling of seed through the processes of collection from good source, processing, storage and germination is required for success in producing seedlings and subsequently, plant establishment. Often, seed collection regime does not correspond with the time of seedling production, some species do not produce seeds all year round and some do not produce at some years. Seed storage is therefore vital to secure the supply of good quality and quantity seeds for planting (Onyekwelu and Fayose, 2007; Siddique and Wright, 2003).

Annona senegalensis is a wild fruit trees commonly known as wild soursop or wild custard apple belonging to the family Annonaceae. The species occurs along riverbanks, fallow land, swamp forests and at the coast (Orwa *et al.*, 2009). The pulp is edible and the leaves are used as fodder for livestock. The bark is used as an insecticide and also for treating guinea worms and other worms, diarrhoea, gastroenteritis, snakebite, toothache and respiratory infections. Gum from the bark is used in sealing cuts and wounds. The leaves are used for treating pneumonia and as a tonic to promote general well being. The roots are used for stomach-ache 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 to meet the demand of man. To achieve this, seeds need to be sourced from superior source and appropriate storage duration documented. Thus it will be necessary to determine which seed source will yield good viable seeds and subsequently, plant development as well as determine when the seeds will lose viability at storage. Therefore this study was carried out to evaluate the possible effect of provenance and storage time on germination and seed quality traits performances *of A. senegalensis* seeds with the aim of broadening genetic base for conservation and improvement of this useful wild species.

Materials and Method

Sources and extraction of experimental seeds

The experimental seeds were sourced from fruits from four southwest states viz: Abeokuta in Ogun State, Ibadan in Oyo State, Ikire in Osun State and Ore in Ondo State. The collected fruits were allowed to ferment naturally to aid seed extraction. The viability of the extracted seed was tested

using the water floatation method. The floated seeds were considered not viable and thus discarded while the sank seeds were taken as the viable ones. The seeds were air dried in an open chamber for two days and then stored in the laboratory under room temperature for subsequent usage in germination trial according to storage duration.

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 and design

The factorial experiment was laid down in a Completely Randomized Design (CRD) with five replications. There were two factors: provenance (4) and storage time (4) as follows.

Provenance

The four seed sources include: Abeokuta, Ibadan, Ikire and Ore

Storage time (weeks)

The extracted and air dried seeds from each provenance were stored at two weeks interval between 0 and 6 weeks viz.

1. Week 0 (immediately after extracting and air drying)
2. Week 2 (after extracting and air drying)
3. Week 4 (after extracting and air drying) and
4. Week 6 (after extracting and air drying)

Experimental procedure and maintenance of seedlings

Eighty propagating sieves of 18cm diameter were filled with washed and sterilized river sand to about two-third full. Eight hundred seeds were broadcasted over it according to treatment (50 seeds per treatment) and then covered with enough river sand to avoid exposure of the seeds when watering. These were transferred into a midst propagator chamber and watering were done as and when due. Daily observations were done to determine when germination occurs. *Annona senegalensis* exhibit epigeal form of germination, germination is therefore said to occur when the plumule rises above the soil. The seedlings were transplanted into nursery pots containing top soil and these were later transferred to the nursery shed for further actions. Eighty uniformly growing seedlings (four leaves stage) were selected for growth Watering was done daily and weeding was carried out as and when due.

Seed Quality Assessment

The following seeds quality traits were assessed for period of nine months:

1. Germination percentage: germination count was carried out daily at the emergent of plumules. Germination percentages were derived from the germination count as:

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$$\frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown} x100$$

2. Number of leaves: Physical count of leaves were done fortnightly
3. Seedling height (cm): meter rule was used to measure from the base of the plant to the apex. This was done fortnightly for.
4. Seedling collar diameter (mm): This was done with the aid of digital venier caliper fortnightly.
5. Biomass accumulation (g): Total initial and final biomass was carried out. The seedlings were dipped in a bowl of water to loosen the soil off the roots. The plants were gradually uprooted, washed and separated into roots, stems and leaves. Fresh weights were determined using sensitive weighing balance. The separated plant parts were placed in oven at constant temperature of 70^{o}C until constant weights were obtained after 72 hours.

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using SPSS and significant treatment means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

Results

Result in Table 1 shows the summary of Analysis of Variance (ANOVA) for the effects of provenance and storage time on seed quality traits of *A. senegalensis* seeds. The effects of provenance on almost all the seed quality traits measured were significant (P<0.05) except number of leaves. The effect of storage were significant (P<0.05) on seed germination as well as biomass accumulation but a non significant effect on number of leaves, seedling height as well as collar diameter. The two way interactive effect of provenance and storage time were significant (P<0.05) for all the traits except number of leaves.

Table: 1 Summary of Analysis of variance (ANOVA) showing the effects of provenance and storage time on seed quality traits of *A. senegalensis* seeds.

Source of variation	Df	Seed germination	Number of leaves	Seedling height	Collar diameter	Biomass
Provenance (P)	3	3055.935**	49.917ns	565.528**	18.603**	35.647**
Storage (S)	3	1370.373**	67.55ns	13.489ns	1.924ns	101.017**
P*S	9	224.019**	103.106ns	90.685**	3.385**	1.847**
Error	64	8.715	50.025	39.157	1.293	1.499

** significant @ p<0.05 ns not significant

The mean effects of provenance as well as storage time on seed quality parameters evaluated in *A. senegalensis* seeds as presented in Table 2 indicated that seed sourced from Ore gave the best germination percentage (75.88%) which was significantly different from other sources. This was followed by Ibadan (69.83%) while seeds sourced from Abeokuta gave the poorest germination percentage of 51.30%. The effect of provenance on number of leaves was not pronounced as all the sources had the same effect. Nonetheless, Seedlings from Abeokuta had the highest number of leaves

(14.05) while seedlings from Ore had the least (10.75). The effect of provenance on seedling height were significant (P<0.05). Ore had the highest seedling height of 20.84cm closely followed by Ibadan (16.34) while Abeokuta recorded the shortest height of 8.01cm. Seeds sourced from Ore in Ondo State had the widest collar diameter of 7.07mm which was significantly different (P<0.05) from the values recorded by other sources. Seedlings from Ibadan had 3.92mm, Ikire 3,25mm and the least was from Abeokuta. The effect of provenance on the four seed sources was significant. Ore (7.07g) and Ibadan (6.74g) had the same higher effect which was different from other sources. Ikire recorded the least weight of biomass of 4.10g.

The mean effect of storage on seed quality of wild soursop (Table 2) indicated a significant different on seed germination percentage and biomass accumulation. It was observed that there was a gradual reduction in seed germination over the storage time as seeds sown immediately after air dry had the highest germination capacity of 70.36%. This was followed by seeds stored for 2 weeks (65.60%) and ranking third in the performance was seed sown after 4 weeks (62.64%) of extraction. The least germination percentage was observed when seeds were sown after six weeks (50.91%) of extraction.

The effect of storage on number of leaves, seedling height and collar diameter was not pronounced as storing the seeds of *A. senegalensis* for 0, 2, 4 and 6 weeks under room temperature had the same effect on the three traits evaluated. However, the effect of storage were pronounced in biomass accumulation as seeds sown immediately after extraction had the highest biomass (8.37g) which was significantly different from other storage time. Storing seed for 2 and 4 weeks had the same effect on biomass while seeds stored for 6 weeks had the least biomass of 2.98g.

Table 2: Mean effects of provenance and storage time on seed quality parameters evaluated in *A. senegalensis* seeds.

	Treatment	Germination (%)	Number of leaves	Seedling height (cm)	Collar diameter (mm)	Biomass (g)
Provenance						
	Abeokuta	51.306c	14.05a	8.015c	2.902c	5.708b
	Ibadan	69.832b	12.15a	16.34ab	3.924b	6.741a
	Ikire	52.493c	13.95a	14.715b	3.255bc	4.105c
	Ore	75.888a	10.75a	20.845a	5.093a	7.076a
Storage (weeks)						
	0	70.362a	13.90a	15.92a	3.807a	8.369a
	2	65.601b	13.72a	15.255a	3.524a	6.597b
	4	62.64c	10.00a	14.77a	3.619a	5.681b

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6	50.917d	13.25a	13.97a	4.224a	2.981c

Means followed by the same alphabet under each column are not significantly different from each other by the Duncan's Multiple Range Test at 5% level of probability

The interactive effect of provenance and storage time on seed germination (%) observed in wild soursop is presented in Table 3. It revealed that seeds sown between 0 and 2 weeks after extraction produced good number of germination percentage across the four seed sources with gradual reduction from the 4th week after extraction. Seeds sown after 6 weeks of extraction gave the poorest germination percentage across the four sources. However, seeds sourced from Ore (86.81%) and Ibadan (84.54%) sown after 2 weeks of extraction gave the best germination effect, this was closely followed by seeds sourced from Ore (82.02%) sown immediately after extraction. It was observed that seeds sourced from Abeokuta sown after 6 weeks of extraction gave the poorest germination percentage of 42.36%.

Table 3: Interactive effect of provenance and storage time (weeks) on seed germination (%) observed in wild soursop

	Storage (weeks)			
Provenance	**0**	**2**	**4**	**6**
Abeokuta	54.807def	58.807cde	49.249f	42.362g
Ibadan	77.798b	84.584a	64.584c	52.362ef
Ikire	53.333def	55.473def	51.251f	49.915f
Ore	82.022ab	86.807a	75.696b	59.029cd

Means followed by the same alphabet under each column are not significantly different from each other by the Duncan's Multiple Range Test at 5% level of probability

The interactive effect of provenance and storage on number of leaves evaluated in seed quality traits of wild sour sop is presented in Table 4. The interactive effect has no significant different on the number of leaves evaluated. Nonetheless, seeds sourced from Ikire that was sown immediately after extraction had the highest number of leaves closely followed by seed sourced from Abeokuta sown after 2 weeks of extraction. The least number of leaves were observed from seeds sourced from Ikire sown after 6 weeks of extraction.

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Table 4: Interactive effect of provenance and storage on number of leaves evaluated in seed quality traits of wild soursop

	Storage (weeks)			
Provenance	**0**	**2**	**4**	**6**
Abeokuta	14.6a	19.80a	11.20a	10.60a
Ibadan	12.20a	12.60a	13.80a	10.00a
Ikire	20.8a	18.0a	9.04a	7.6a
Ore	11.20a	15.80a	8.00a	8.00a

Means followed by the same alphabet under each column are not significantly different from each other by the Duncan's Multiple Range Test at 5% level of probability

The interactive effect of provenance and storage on seedling height (cm) evaluated in seed quality traits of wild soursop as presented in Table 5 revealed a significant different (P=0.05). The tallest seedling (27.86cm) was observed in seeds sourced from Ore sown immediately after extraction closely followed by the same source after two weeks of extraction. Seeds sown after six weeks of extraction across the four seed sources recorded the shortest plant, however, seeds from Abeokuta sown after six weeks of extraction had the least plant height of 6.66cm

Table 5: Interactive effect of provenance and storage on seedling height (cm) evaluated in seed quality traits of wild soursop

	Storage (weeks)			
Provenance	**0**	**2**	**4**	**6**
Abeokuta	10.36bcd	8.5cd	7.54cd	6.66d
Ibadan	16.54abcd	21.2abc	14.2abcd	13.42bcd
Ikire	17.32abcd	17.72abcd	14.5abcd	13.12bcd
Ore	27.86a	23.7ab	13.54bcd	14.48abcd

Means followed by the same alphabet under each column are not significantly different from each other by the Duncan's Multiple Range Test at 5% level of probability

Table 6 shows the interactive effect of provenance and storage on seedling collar diameter (mm) evaluated in wild soursop. There was significant difference between the four seed sources as well as storage time on collar diameter. All the sources recorded their highest collar diameter when seeds were sown immediately after extraction. Overall, seeds

sources from Ore produced the superior collar diameter (5.59mm) closely followed by seeds sourced from Ibadan (5.30mm) sown immediately after extraction. Next to this is Ibadan sown after 4 weeks of extraction and Abeokuta provenance (4.72mm) sown immediately after extraction. The least collar diameter of 2.14mm was observed from seeds sources from Abeokuta sown after six weeks of seed extraction.

Table 6: Interactive effect of provenance and storage on seedling collar diameter (mm) evaluated in seed quality traits of wild soursop

	Storage (weeks)			
Provenance	**0**	**2**	**4**	**6**
Abeokuta	4.722ab	2.596abc	2.152c	2.138c
Ibadan	5.302a	4.232abc	4.754ab	4.728ab
Ikire	4.14abc	3.68abc	2.56bc	2.64bc
Ore	5.588a	4.176abc	3.55abc	3.738abc

Means followed by the same alphabet under each column are not significantly different from each other by the Duncan's Multiple Range Test at 5% level of probability

The interactive effect of provenance and storage on biomass accumulation (g) evaluated in seed quality traits of wild soursop is presented in Table 7. The interactive effect revealed a significant effect (P=0.05) on biomass accumulated by the plant under investigation. Seeds sourced from Ore sown immediately after seed extraction produced the highest biomass (9.85g) which was different from others whereas the least biomass (1.41g) was observed from seed collected from Ikire sown after six weeks of extraction.

Table 7: Interactive effect of provenance and storage on biomass accumulation (g) evaluated in seed quality traits of wild sour sop

	Storage (weeks)			
Provenance	**0**	**2**	**4**	**6**
Abeokuta	8.118abc	7.028bcd	6.60bcd	3.484ef
Ibadan	8.656ab	7.684abc	4.912de	7.05bcd
Ikire	6.854bcd	3.262ef	4.896de	1.408f
Ore	9.85a	6.78bcd	2.12f	5.812cde

Means followed by the same alphabet under each column are not significantly different from each other by the Duncan's Multiple Range Test at 5% level of probability

Discussion

Germplasms collection from good source is very crucial in ensuring the genetic diversity as well as plant growth and development which is essential to any tree improvement programme (Anegbeh *et. al.*, 2003; Atangana

et. al., 2002). The threat to mass genetic erosion of wild species from rapid tropical deforestation calls for urgent massive germplasms collection and conservation efforts. In addition, seed storage is also very important as this will greatly influence their lifespan since seeds need to be stored from time of collection until they are required for sowing Ojo (2008).

The result from this study revealed that there were significant differences among provenance for seed germination percentage, seedling height, collar diameter and biomass accumulation. Significant differences (P=.05) were also observed among storage time for seed germination as well as biomass accumulation. The two way interaction between provenance and storage time had significant effect on seed germination percentage, seedling height, collar diameter and biomass accumulation. This implies that seed quality traits of *A. senegalensis* were affected by provenance as well as storage time. Consequently, conservation and improvement in these traits could be achieved by giving due consideration to source of seeds as well as post seed extraction factors such as storage duration.

The differences in performances of the provenance could be attributed to differences in environmental factors, genetic component as well as the genotype by environment interactions of the plant population used in this study. This is in line with the works of various authors who investigated the effect of different provenance on growth and development of plant species such as *Cordia africana, Dacryodes edulis, Prosopis africana and Parkia biglobosa,* as affected by environmental factors (Singh *et al.,* 2010; Gutterman, 2000; Loha *et al*, 2006; Oyun, 2003), genetic factors of source population/plant (Shu *et al*., 2012, Asinwa *et al.,* 2008) and the impact of plant genotype by environmental interaction (Alaje *et al*., 2018). Gutterman (2000) stated that environmental factors such as day length, temperature, light quality, water availability and altitude affect plant growth and development. Asinwa *et al*. (2008), maintained that seedlings of the same family grown under the same climatic and edaphic condition may differ in their growth development due to the differences in genetic composition. Alaje *et al.* (2018) further added that plant growth and development for increased yield is affected by genotype by environment interaction and this poses great difficulties in predicting performance in selection programme.

The study also revealed that the effect of storage was pronounced as seeds sown between 0-2 weeks after seed extraction irrespective of the provenance had good germination percentage. The high germination obtained from seeds sown between 0-2 weeks and progressive decline in germination capacity with increase in storage time indicated that seeds of *A.senegalensis* lose viability gradually during storage. This is true as tree species loses viability when stored under room temperature (Kandari, 2008). This finding agrees with the findings of Kandari (2008) who reported a steady decrease in viability of tropical tree species when stored beyond 2wks.

Conclusion and Recommendations

The findings from this study indicated that significant differences were observed in seed germination percentage, plant height, and collar diameter and biomass accumulation due to differences in provenance. However, provenance does not have effect on fruit colour.

Seed obtained from Ore provenance had the highest seed germination percentage, seedling height, collar diameter and biomass accumulation while seed from Abeokuta provenance had the highest number of leaves. The differences in traits observed as effect of provenance offers potential for selection among the provenance for trait improvement in *A. senegalensis*

Significant differences were also observed in seed germination capacity as well as biomass accumulation due to differences in the storage time as seeds sown between 0-2weeks after extraction gave the best germination percentage as well as biomass accumulation. Storage time had no effect on number of leaves, seedling height and collar diameter as both sowing immediately and sowing after 6 weeks had the same effects..

It is therefore recommended that seeds of *A. senegalensis* should be sourced from good provenance such as Ore as this provenance could serve as gene pool for this for the improvement of this multipurpose species. The seeds of wild soursop though with gradual reduction in germination capacity still germinate even after six weeks of extraction thus the storage time should also be extended beyond six weeks of extraction to validate when *A. senegalensis* looses viability on storage.

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