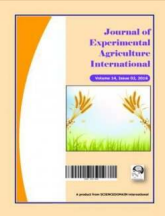

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The Effect of Storage Environments and Duration on Seed Germination of Amaranth (*Amaranthus cruentus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author O. Adam designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors O. Anthony, OO, AO and AAM managed the analyses of the study, literature searches and over all planning and supervision of the experiment. All authors read and approved the final manuscript.

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ABSTRACT

Amaranth (*Amaranthus cruentus* L.) is a very important leafy vegetable especially in Nigeria however, availability of quality seeds for sustainable production to meet the high demand has become a big challenge. This study was carried out to investigate the influence of storage environments and duration on the germination of amaranth seeds. Seeds of two accessions of amaranth (NGB 01259 and NGB 01276) produced during the late growing season of 2013 were used for the study. The laboratory experiment was conducted at Seed Testing Laboratory of The National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria between 2014 and 2015. Ten grams of each accession were partitioned to three equal parts. Samples from each accession were kept separately in three different storage environments using aluminum cans as packaging materials. The seed samples were drawn at regular intervals of 4, 8, 12 and16

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months in storage and evaluated for germination. Results of analysis of variance (ANOVA) on germination percentage revealed that the effect of storage environment and duration were highly significant (P = .01) on seed germination of *A. cruentus*. Also, the combined effect of storage environment and duration differed with seed germination of *A. cruentus* indicating that germination of amaranth seeds observed at each storage period varied with storage environment. However, germination percentage observed under short and medium term storage conditions at the end of sixteen months in storage were not significantly different in spite of different storage conditions with respective values of 78.5 and 77.5%. In conclusion, amaranth seed can be stored safely for up to sixteen months with over 70% viability at a temperature range of 15.1 to 20.3ºC and relative humidity of 26.9 to 50.7% or -8.2ºC to 3.1ºC and relative humidity of 42.7 to 72.1% with at least twelve hours electricity supply to the storage environments.

Keywords: Germination; storage; environments; duration; amaranth.

1. INTRODUCTION

Amaranthus cruentus L. (red amaranth) is a species in the genus Amaranthus which contains approximately 112 to 193 species and belongs to the family of the Amaranthaceae. It is cultivated for both its seeds which are used as a grain and its leaves which are used as a vegetable. Both leaves and seeds contain protein of an unusually high quality. The leaves of both the grain and vegetable types may be eaten raw or cooked [1].The demand for this crop as vegetable has increased especially in the urban centres where people are not usually involved in primary production of food crops [2]. This has made the vegetable to become an important commodity in most markets. Due to the high demand for the leafy vegetable for food, availability of quality seeds for sustainable production becomes a big challenge thus, the conservation of amaranth seeds is important in order to guarantee seed supply. The longevity of seed during storage is controlled by several factors among which are, nature of the seeds, temperature of the storage environment, seed moisture content, storage duration and relative humidity [3]. Of all these, the seed moisture content is probably the most important factor influencing seed viability during storage [4]. High seed moisture content may reduce seed germination by promoting fungal growth and insect infestation [5]. Moisture content and temperature are the two key environmental factors that influence growth of molds and fungi [6]. Storage potential of seeds is also influenced by the relative humidity of the surrounding atmosphere. Seeds are hygroscopic in nature which means they pick up and release moisture from and to the surrounding air until the vapor pressures of seed moisture and air reach equilibrium, a situation that can lead to rapid seed deterioration especially where humid conditions prevail. According to Samuel et al. [7], even after drying, maize grain harvested in tropical countries retained a certain amount of moisture, and when exposed to air, exchanges of moisture between the maize grains and surrounding occur until the equilibrium is reached.

Seed germination, which is the resumption of active growth of the embryo that results in the rupture of the seed coat and the emergence of the young plant [8] forms a crucial aspect of seed quality. Germination tests therefore are used worldwide to determine the maximum germination potential of a seed batch under optimum conditions.

In Nigeria, one of the conservation strategies of genetic resources was the establishment of The National Centre for Genetic Resources and Biotechnology (NACGRAB) located in Ibadan. NACGRAB was involved in the distribution of amaranth seedlots from her working collections to meet the requirements of researchers in the national agricultural research system and farmers while duplicate samples in the medium term collections were reserved for safety purpose. However, conservation of germplasm in the genebanks of NACGRAB is of recent being confronted with some challenges such as interruption in power supply which often results to fluctuation in temperature and relative humidity in her short and medium term storage chambers. Information on duration of amaranth seed under these storage conditions is therefore necessary to ensure proper conservation strategy of amaranth seeds. This would also furnish additional information for amaranth seed producers. The objective of this study therefore was to investigate the influence of three different storage environments namely, ambient (control), short and medium term storage conditions at different storage duration on germination of amaranth seeds.

2. MATERIALS AND METHODS

2.1 Genetic Materials and Location of the Experiment

Seeds of two accessions of *Amaranthus cruentus* (NGB01259 and NGB01276) were sourced from the gene banks of NACGRAB, Ibadan. The materials were randomly selected among the accessions harvested and processed during the late season of 2013 and stored in one of the genebanks of NACGRAB. The laboratory experiment was conducted at NACGRAB between April 2014 and June 2015.

2.2 Seed Storage and Measurement Temperature and Relative Humidity

Five grams of processed seeds of each accession were drawn and further partitioned into three parts. Samples from each accession were kept separately under ambient (control), short term and medium term conditions using aluminum cans as packaging materials. Temperature and relative humidity of both short and medium term storage environments were taken daily however only temperature of ambient environment was also taken daily. Electricity supply of at least ten hours was ensured in both short and medium term. Mean temperature under ambient conditions ranged from 24 to 29.5ºC while that of the short term storage environment ranged from 15.1 to 20.3ºC. The lowest temperature value was observed under medium term storage environment which ranged from -8.2 to 3.1ºC. Relative humidity in short and medium term storage environments ranged from 26.9 to 50.7% and 42.7 to 72.1% respectively (Table 1).

2.3 Experimental Design

The stored seed samples were drawn at regular intervals of 4, 8, 12, and 16 months and evaluated for germination percentage. The experiment was arranged in 2 x 3 x 4 factorial using completely randomized design (CRD) in three replication.

2.4 Standard Germination Test

Standard germination test was conducted at the seed testing laboratory of NACGRAB between April 2014 and June 2015. The test was assayed by placing 100 seeds from each sample in petri plates lined with double layer filter paper moistened with 5ml of distilled water. The petri plates were placed inside a germinating chamber at 25℃. The seeds were kept moist every day for seven days thereafter germination percentages were determined at seven days after planting according to International Seed Testing Association (ISTA) rules [9].

2.5 Data Analysis

Data on germination percentage were log transformed to ensure conformity to normality and subjected to analysis of variance (ANOVA) using Statistical Analysis Software, SAS Version 9.1 [10]. However, since ANOVA did not detect any significant difference between transformed and untransformed values, untransformed values were hereby presented. Pertinent means were thereafter separated by the use of the least significant difference (LSD) at 0.05 level of probability using SAS software.

3. RESULTS AND DISCUSSION

3.1 Germination Performance of Amaranth Seeds

Analysis of variance (ANOVA) revealed that the influence of storage environment (ENV), storage duration (STP) and interaction of accession by storage environment by storage duration (ACC x ENV x STP) were highly significant (*P* = .01) on germination of amaranth seeds (Table 2). This findings emphasise the fact that storability of seeds is affected by a wide number of factors such as nature of the seed, duration in storage and storage environment [11]. However, the results further revealed that the overall performance of amaranth seed depended on the combined effect of amaranth accession, storage duration and environments.

Table 1. Mean temperature (ºC) and relative humidity (%) ranges in short and medium term storage chambers and mean temperature range under ambient conditions

Storage environment	Temperature (ºC)	Relative humidity (%)
Ambient	24ºC to 29.5ºC	---
Short term	15.1ºC to 20.3ºC	26.9 to 50.7
Medium	-8.2ºC to 3.1ºC	42.7 to 72.1

Table 2. Mean squares from analysis of variance for germination test conducted on accessions of amaranth (*Amaranthus cruentus*) seeds at NACGRAB, Ibadan

Source of variation	DF	Germination (%)
Replication	2	51.4 ns
Accessions (ACC)	1	117.4 ns
Storage environment (ENV)	2	4518.9**
Storage period (STR)	7	2437.8**
ACC x ENV	2	563.0**
ACC×STP	7	167.8**
ENV×STP	14	843.6**
ACC×ENV×STP	14	250.7**
Error	94	57.3
Total	143	345.0
CV		11.7
R^2		0.9
Mean	64.9	

**, **, Significant at probability level of .05 and .01, respectively; ns = not significant*

3.2 Germination Performance of *A. cruentus* as Influenced by Accession, Storage Environment and Storage Duration

The result of mean comparison showed that seeds stored under ambient (ENV1) conditions had lowest mean germination percentage (51.7%) whereas the mean germination percentages for amaranth seeds stored under medium (ENV3) and short (ENV2) term storage conditions were 70.9% and 70.5% respectively (Table3). The over 70% germination percentages observed on materials stored under short and medium term storage conditions is a clear indication that the lower the temperature of the storage environment, the longer the storability of the seed stored in the environment. The results corroborated with the findings of Chauhan and Nautical [12] who reported lesser seed viability under room temperature than under low temperature storage for *Nardostachys jatamansi.* However, in this present study, a non-significant difference was observed between the germination percentages of amaranth seeds stored in the short and medium term storage chambers could be attributed to the fluctuation in electricity supply, which could have masked the anticipated differences between the two cold rooms used in this study. Yakubu, 2009 [13] gave similar report that fluctuation of temperature and relative humidity in tropical countries accelerates rapid multiplication of molds and insects, which facilitate further spoilage of grain.

There was variation in germination of amaranth seed due to storage period. The mean germination at eighth month (78.6%) in storage was significantly higher than mean germination observed at twelfth month (64.7%) in storage. However, the mean germination at fourth (57.2%) and sixteenth (56.9%) month in storage were not significantly different (Table 3).The significantly lower mean germination at fourth month (first storage period) in storage suggests that amaranth seeds exhibited after-harvest dormancy which might be overcome after some periods during storage. This results supported the findings of Cristaudo et al. [14] who reported that germination of amaranth seeds was strongly influenced by after-harvest periods in nine Amaranth species. They further stated that primary dormancy in Amaranthus plays a fundermantal role in extending germination over a longer period so that the probability of seedling survival is maximised. However, the germination signifcantly increased at eighth month in storage (second storage period). This is in line with findings of some authors that postharvest duration of dry seed storage (after ripening) is an important factor in dormancy release equation of seeds and is a common requirement for loss of primary dormancy and promoting seed germination [15,16,17]. Moreover, germination of amaranth seeds decreased significantly after storage at twelfth and sixteenth month.

These findings agreed with those obtained by Jantana, et al. [18] who reported that, as seeds deteriorate during storage, their performance potential and vigor decline before any loss in viability.

3.3 Germination Performance of *A. cruentus* as Influenced by the Interactive Effect of Accession, Storage Environment and Duration

The main objective of this study was to investigate the influence of storage environments and storage duration on germination of amaranth seeds. The significant interactive effect of storage environment by storage duration (ENV x STP) on germination of amaranth seeds indicates that germination of amaranth seeds observed at each storage period varied with storage environment. However, germination percentage observed under short (ENV2) and medium (ENV3) term storage conditions were

not significantly different in spite of different storage conditions with respective values of 78.5 and 77.5% (Table 4). This could be attributed to the fluctuation in electricity supply, which could have masked the anticipated differences.

Table 3. Germination percentages of *A. cruentus* seed as influenced by accession, storage environment and period at NACGRAB, Ibadan

Factors	Seed germination (%)
A. Variety	
NGB 01259 (ACC1)	65.1a
NGB 01276 (ACC2)	63.6a
LSD	2.1
B. Storage environment	
Ambient (ENV1)	51.7b
Short term (ENV2)	70.5a
Medium (ENV3)	70.9a
LSD	2.6
C. Storage period	
Fourth month (STP1)	57.2c
Eight month (STP2)	78.5a
Twelfth month (STP3)	64.7b
Sixteenth month (STP4)	56.9c
LSD	3.0

Means with different letters within the column of the same factor are significantly different at P=.05

Moreover, the interactive effect of accession by storage environment by storage period (ACC x ENV x STP) was highly significant (P<0.01) on germination of amaranth seed (Table 5).This implies that in assessing germination of amaranth seeds, among other factors, genotype, storage environment and storage duration should be given prime consideration in order to avoid wrong conclusion on germianation potential of amaranth seedlots. Amaranth accession, NGB01259 (ACC1) for instance had highest germination (83.00%) observed when the seeds were stored for eight months (STP2) in the medium term storage chamber whereas germination value of 72.67%, 69.33% and 52.67% were observed at sixteenth (STP4), twelfth (STP3) and fourth month in the same storage environment. Germination response of accession NGB 01276 (ACC2) stored in the medium term were 82.33%, 80.67%, 65.67% and 60.67% at sixteenth, eighth, twelfth and fourth period respectively. Under the short term storage conditions, the germination values of 79.33%, 77.33%, 61.33 and 57.00% were observed for NGB 01259 (ACC1) at eighth, sixteenth, twelfth and fourth month respectively while NGB 01276 (ACC2) the germination values of 80.33%, 80.00%, 67.67% and 60.67%at sixteenth, eighth, twelfth and fourth month respectively (Table 5). Germination performance under ambient conditions (control) revealed that accession NGB 01259 (ACC1) had germination percentages of 80.67%, 71.67%, 52.67 and 23.67% at eighth, twelfth, sixteenth and fourth month in storage respectively while NGB 01276 (ACC2) the germination values of 67.67%, 59.67%, 52.67% and 5.00% at eighth, fourth, twelfth and sixteenth month in storage respectively (Table 5).

Table 4. Germination percentages of *A. cruentus* seed as influenced by the interaction between storage environment and period at NACGRAB

Storage environments	Storage periods	Germination percentage (%)
ENV1	STP1	56.2
ENV2	STP1	58.8
ENV3	STP1	56.7
ENV1	STP2	74.2
ENV2	STP2	79.7
ENV3	STP2	81.8
ENV1	STP3	62.2
ENV2	STP3	64.5
ENV3	STP3	67.5
ENV1	STP4	14.3
ENV2	STP4	78.8
ENV3	STP4	77.5
LSD		7.38

ENV1=Ambient conditions, ENV2= Short term conditions, ENV3 =Medium term conditions STP1=First storage period, STP2= Second storage period, STP3= Third storage period, STP4=Fourth storage period,

From this findings, it clearly showed that accession NGB 01259 (ACC1) can be stored safely for up to sixteen weeks with over 70% viability value under short and medium term storage conditions. Similarly, accession NGB 01276 (ACC2) can be stored safely for up to sixteen weeks with over 80% viability value under short and medium term storage conditions. However, the materials must be stored in containers that are air-tight such as aluminium cans used in this study so as to maintain seed moisture content. The results generally support the findings of Hartmann et al. [19] who reported that amaranth seeds can be stored safely for up to three years at a temperature below 8ºC and at 10% RH in a tightly closed moisture resistant container. They further stated that containers that are air-tight such as a sealed glass jars, metal cans or foil envelopes are ideal for storing amaranth seeds as they maintain seed moisture

Table 5. Germination percentages of *A. cruentus* seed as influenced by the interaction between accession, storage environment and period at NACGRAB

Accessions	Storage environments	Storage periods	Germination percentage (%)
ACC1	ENV3	STP2	83.00
ACC2	ENV3	STP4	82.33
ACC2	ENV3	STP2	80.67
ACC1	ENV1	STP2	80.67
ACC2	ENV2	STP4	80.33
ACC2	ENV2	STP2	80.00
ACC1	ENV2	STP2	79.33
ACC1	ENV2	STP4	77.33
ACC1	ENV3	STP4	72.67
ACC1	ENV1	STP3	71.67
ACC1	ENV3	STP3	69.33
ACC2	ENV2	STP3	67.67
ACC2	ENV1	STP2	67.67
ACC2	ENV3	STP3	65.67
ACC1	ENV2	STP3	61.33
ACC2	ENV2	STP1	60.67
ACC2	ENV3	STP1	60.67
ACC2	ENV1	STP1	59.67
ACC1	ENV2	STP1	57.00
ACC1	ENV3	STP1	52.67
ACC1	ENV1	STP1	52.67
ACC2	ENV1	STP3	52.67
ACC1	ENV1	STP4	23.67
ACC2	ENV1	STP4	5.00
LSD			7.38

ENV1=Ambient conditions, ENV2= Short term conditions, ENV3 =Medium term conditions STP1=First storage period, STP2= Second storage period, STP3= Third storage period, STP4=Fourth storage period.

content best. In addition, they recommended that seeds in containers should be stored in a cool, shady and dry place to extend seed shelf life.

4. CONCLUSION

This study led to the conclusion that there was differential response of germination of amaranth seed in the different storage environments, with lowest germination percentage observed for seed stored under ambient conditions. Also there was differential response of germination of amaranth seed to duration in storage. However, there was no significant difference between the germination of amaranth seeds stored under medium and those in short term storage despite the different storage conditions. This suggests that duration power supply to the genebanks should be given priority in order to obtain anticipated differences from the cold environments. Furthermore, it can be concluded that amaranth seed can be stored safely for up to sixteen months with over 70% viability at a temperature range of 15.1 to 20.3ºC and relative humidity of 26.9 to 50.7% or -8.2ºC to 3.1ºC and relative humidity of 42.7 to 72.1% with at least twelve hours power supply to the storage environments. In addition, the materials must be stored in containers that are air-tight such as aluminium cans to maintain seed moisture content. Furthermore, the significantly lower mean germination at fourth month in storage, suggests that amaranth seeds exhibited after-harvest dormancy that may overcome after some periods during storage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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