

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

Amaranth (*Amaranthus cruentus* L.) is a very important plant cultivated for its leaves that are very rich in vitamins and minerals however, availability of quality seeds for sustainable production to meet the high demand in Nigeria becomes a big challenge thus, the conservation of amaranth seeds is important in order to guarantee seed supply. 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 and 16 months in storage and evaluated for germination. Results of analysis of variance (ANOVA) on germination percentage revealed that the 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*. However, there was non-significant difference between germination of amaranth seeds stored under medium term (70.85%) and short term (70.46%) storage conditions suggesting that efforts should be made to optimise storage conditions in these environments. Moreover, the significant combined effect of storage environment and duration on seed germination of *A. cruentus* suggests that in assessing germination potential of *A. cruentus* seeds, storage environment and duration should be given prime consideration in order to avoid wrong conclusion on germination potential of amaranth seedlot.

Keywords: Germination, storage, environments, duration, amaranth

1. INTRODUCTION

Amaranth (*Amaranthus cruentus* L.) is a very important plant cultivated from the tropics to the temperate zones for its leaves that are very rich in vitamins and minerals especially Vitamins A and C, iron and calcium [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]. Temperature also plays an important role in the viability of seeds. Within the range of environmental parameters for the normal biological activity of seeds, the activities of insects and microbial moulds usually increase as temperature increases. High moisture and temperature increased the infection of *Aspergillus flavus* and decreased the germination of soybean seed [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.

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 [7] 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* (NGB 01259 and NGB 01276) were sourced from the gene banks of NACGRAB, Ibadan. The seeds were produced during the late growing season of 2013 at the experimental field of NACGRAB. The laboratory experiment was conducted at the seed testing laboratory of 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 29.5°C while that of the short term storage environment ranged from 15.07 to 20.30°C. The lowest temperature value was observed under medium term storage environment which ranged from -8.18 to 3.10°C. Relative humidity in short and medium term storage environments ranged from 26.88 to 50.67% and 42.72 to 72.10% respectively (Table 1).

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

Storage environment	Temperature (°C)	Relative humidity (%)
Ambient	24 ⁰ C to 29.5 ⁰ C	---
Short term	15.07 ⁰ C to 20.30 ⁰ C	26.88 to 50.67
Medium	-8.18 ⁰ C to 3.10 ⁰ C	42.72 to 72.10

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 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°C. The seeds were kept moist every day for seven days thereafter germination percentages were determined [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 [9]. 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 Analysis of Variance

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 [10]. However, the results further revealed that the overall performance of amaranth seedlot depended on the combined effect of amaranth accession, storage duration and environments.

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 (%)
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Replication	2	51.36 ns
Accessions (ACC)	1	117.36 ns
Storage environment (ENV)	2	4518.88**
Storage period (STR)	7	2437.77**
ACC x ENV	2	562.97**
ACC×STP	7	167.81**
ENV×STP	14	843.58**
ACC×ENV×STP	14	250.74**
Error	94	57.32
Total	143	344.97
CV		11.66
R ²		0.89
Mean		64.93

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

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116 3.2 Germination Performance of *A. cruentus* as influenced by Accession, Storage 117 Environment and Storage duration

118 The result of mean comparison showed that seeds stored under ambient (ENV1) conditions had
119 lowest mean germination percentage (51.71%) whereas the mean germination percentages for
120 amaranth seeds stored under medium (ENV3) and short (ENV2) term storage conditions were

121 70.85% and 70.46% respectively (Table3). The over 70% germination percentages observed on
 122 materials stored under short and medium term storage conditions is a clear indication that the lower
 123 the temperature of the storage environment, the longer the storability of the seed stored in the environment.
 124 The results corroborated with the findings of Chauhan and Nautical [11] who reported lesser seed
 125 viability under room temperature than under low temperature storage for *Nardostachys jatamansi*.
 126 However, in this present study, a non-significant difference was observed between the germination
 127 percentages of amaranth seeds stored in the short and medium term storage chambers could be
 128 attributed to the fluctuation in electricity supply, which could have masked the anticipated
 129 differences between the two cold rooms used in this study.

130 There was variation in germination of amaranth seed due to storage period. The mean germination
 131 at eighth month (78.56%) in storage was significantly higher than mean germination observed at
 132 twelfth month (64.72%) in storage. However, the mean germination at fourth (57.22%) and
 133 sixteenth (56.89%) month in storage were not significantly different (Table 3). The significantly
 134 lower mean germination at fourth month (first storage period) in storage suggests that amaranth
 135 seeds exhibited after-harvest dormancy which might be overcome after some periods during
 136 storage. This results supported the findings of Cristaudo *et al.* [12] who reported that germination of
 137 amaranth seeds was strongly influenced by after-harvest periods in nine Amaranth species. They
 138 further stated that primary dormancy in *Amaranthus* plays a fundamental role in extending
 139 germination over a longer period so that the probability of seedling survival is maximised.
 140 However, the germination significantly increased at eighth month in storage (second storage period).
 141 This is in line with findings of some authors that postharvest duration of dry seed storage (after
 142 ripening) is an important factor in dormancy release equation of seeds and is a common requirement
 143 for loss of primary dormancy and promoting seed germination [13, 14,15]. Moreover, germination
 144 of amaranth seeds decreased significantly after storage at twelfth and sixteenth month.

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

Factors		Seed germination (%)
A.	Variety	
	NGB 01259 (ACC1)	65.11a
	NGB 01276 (ACC2)	63.58a
	LSD	2.13
B.	Storage Environment	
	Ambient (ENV1)	51.71b
	Short term (ENV2)	70.46a
	Medium (ENV3)	70.85a
	LSD	2.61
C.	Storage Period	
	Fourth month (STP1)	57.22c
	Eight month (STP2)	78.56a
	Twelfth month (STP3)	64.72b
	Sixteenth month (STP4)	56.89c
	LSD	3.01

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

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149 These findings agreed with those obtained by Jantana, *et al.* [16] who reported that, as seeds
 150 deteriorate during storage, their performance potential and vigor decline before any loss in viability.

151 **3.3 Germination Performance of *A. cruentus* as influenced by the interactive effect of**
 152 **Accession, Storage Environment and Duration**

153 The interactive effect of accession by storage environment by storage period (ACC x ENV x STP)
 154 was highly significant ($P < 0.01$) on germination of amaranth seed (Table 4). This implies that in
 155 assessing germination of amaranth seeds, among other factors, genotype, storage environment and
 156 storage duration should be given prime consideration in order to avoid wrong conclusion on
 157 germination potential of amaranth seedlots. Amaranth accession, NGB 01259 (ACC1) for instance
 158 had highest germination (83.00%) observed when the seeds were stored for eight months (STP2) in
 159 the medium term storage chamber whereas germination value of 72.67%, 69.33% and 52.67% were
 160 observed at sixteenth (STP4), twelfth (STP3) and fourth month in the same storage environment.
 161 Germination response of accession NGB 01276 (ACC2) stored in the medium term were 82.33%,
 162 80.67%, 65.67% and 60.67% at sixteenth, eighth, twelfth and fourth period respectively. Under the
 163 short term storage conditions, the germination values of 79.33%, 77.33%, 61.33 and 57.00% were
 164 observed for NGB 01259 (ACC1) at eighth, sixteenth, twelfth and fourth month respectively while
 165 NGB 01276 (ACC2) the germination values of 80.33%, 80.00%, 67.67% and 60.67% at sixteenth,
 166 eighth, twelfth and fourth month respectively (Table 4). Germination performance under ambient
 167 conditions (control) revealed that accession NGB 01259 (ACC1) had germination percentages of
 168 80.67%, 71.67%, 52.67 and 23.67% at eighth, twelfth, sixteenth and fourth month in storage
 169 respectively while NGB 01276 (ACC2) the germination values of 67.67%, 59.67%, 52.67% and
 170 5.00% at eighth, fourth, twelfth and sixteenth month in storage respectively (Table 4). From this
 171 findings, it clearly showed that accession NGB 01259 (ACC1) can be stored safely for up to sixteen
 172 weeks with over 70% viability value under short and medium term storage conditions. Similarly,
 173 accession NGB 01276 (ACC2) can be stored safely for up to sixteen weeks with over 80% viability
 174 value under short and medium term storage conditions. However, the materials must be stored in
 175 containers that are air-tight such as aluminium cans used in this study so as to maintain seed
 176 moisture content. The results generally support the findings of Hartmann *et al.* [17] who reported
 177 that amaranth seeds can be stored safely for up to three years at a temperature below 8°C and at
 178 10% RH in a tightly closed moisture resistant container. They further stated that containers that are

179 air-tight such as a sealed glass jars, metal cans or foil envelopes are ideal for storing amaranth seeds
 180 as they maintain seed moisture content best. In addition, they recommended that seeds in containers
 181 should be stored in a cool, shady and dry

182 place to extend seed shelf life [17].

Accessions	Storage environments	Storage periods	Germination Percentage (%)
ACC1	ENV3	STP2	83.00a
ACC2	ENV3	STP4	82.33a
ACC2	ENV3	STP2	80.67a
ACC1	ENV1	STP2	80.67a

4. CONCLUSION

184 This study led to the conclusion that there
 185 was differential response of germination
 186 of amaranth seed in the different
 187 storage environments, with

188 lowest germination percentage observed for seed stored under ambient conditions. Also there was
 189 differential response of germination of amaranth seed to duration in storage. However, there was no
 190 significant difference between the germination of amaranth seeds stored under medium and those
 191 in short term storage despite the different storage conditions. This suggests that duration power
 192 supply to the genebanks should be given priority in order to obtain anticipated differences from the
 193 cold environments. Furthermore, it can be concluded that amaranth seed can be stored safely for up
 194 to sixteen months with over 70% viability at a temperature range of 15.07 to 20.30⁰C and relative
 195 humidity of 26.88 to 50.67% or -8.18⁰C to 3.10⁰C and relative humidity of 42.72 to 72.10% with at
 196 least twelve hours power supply to the storage environments. In addition, the materials must be
 197 stored in containers that are air-tight such as aluminium cans to maintain seed moisture content.

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

200	ACC2	ENV2	STP4	80.33a
	ACC2	ENV2	STP2	80.00ab
201	ACC1	ENV2	STP2	79.33ab
	ACC1	ENV2	STP4	77.33abc
	ACC1	ENV3	STP4	72.67bdc
202	ACC1	ENV1	STP3	71.67dc
	ACC1	ENV3	STP3	69.33d
203	ACC2	ENV2	STP3	67.67ed
	ACC2	ENV1	STP2	67.67ed
	ACC2	ENV3	STP3	65.67edf
204	ACC1	ENV2	STP3	61.33egf
	ACC2	ENV2	STP1	60.67egf
205	ACC2	ENV3	STP1	60.67egf
	ACC2	ENV1	STP1	59.67hgf
	ACC1	ENV2	STP1	57.00hg
206	ACC1	ENV3	STP1	52.67h
	ACC1	ENV1	STP1	52.67h
207	ACC2	ENV1	STP3	52.67h
	ACC1	ENV1	STP4	23.67i
	ACC2	ENV1	STP4	5.00j
208	LSD			7.38

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

211 ENV1=Ambient conditions, ENV2= Short term conditions, ENV3 =Medium term conditions

212 STP1=First storage period, STP2= Second storage period, STP3= Third storage period,

213 STP4=Fourth storage period,

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