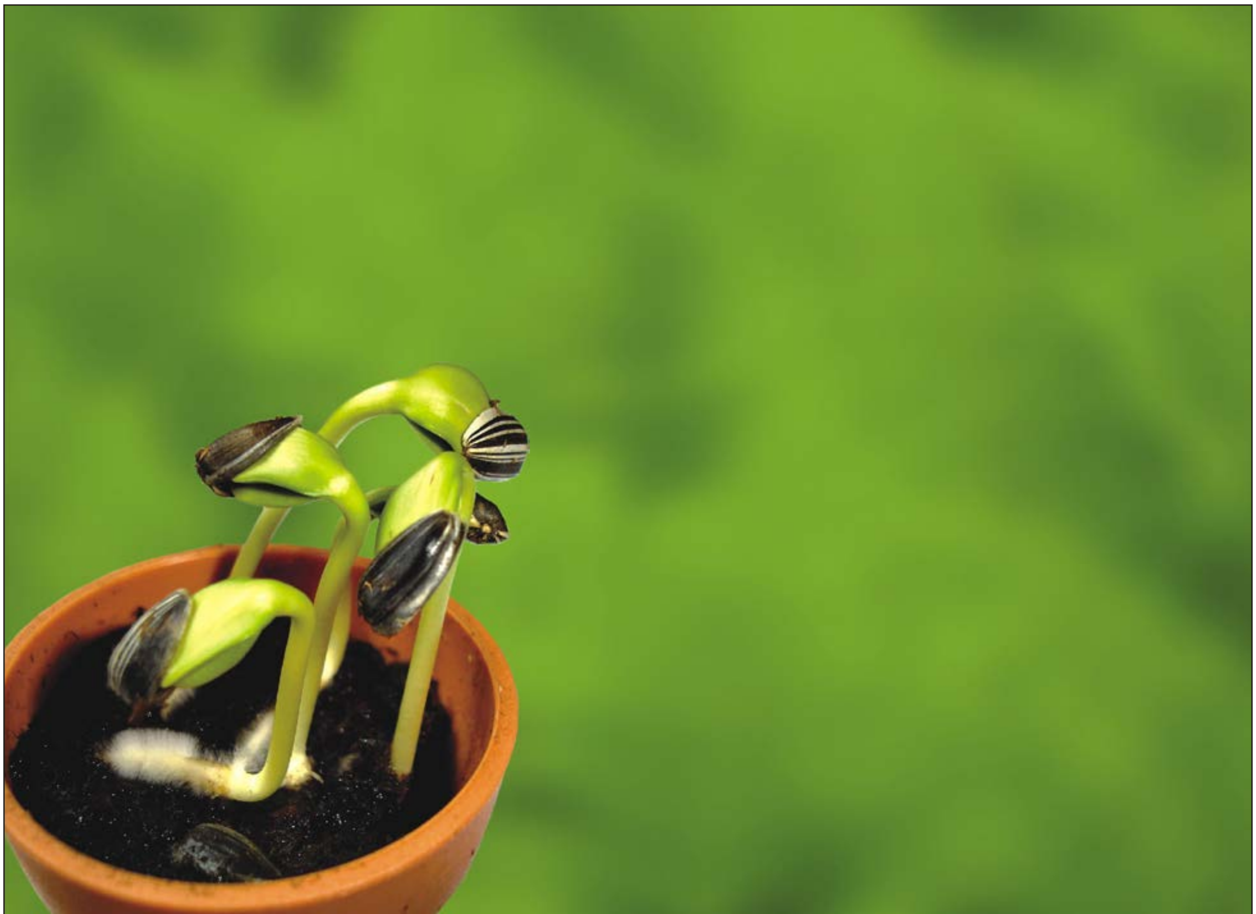

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Physiological and Genetic Integrity of Amaranth (*Amaranthus* spp.) Seeds During Storage

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ABSTRACT

The longevity of seeds in storage is a good indicator of seed quality and vigour in many crops. This study examined the physiological and genetic integrity of nineteen amaranth accessions during storage. Seeds of each accession were subjected to Artificial Ageing (AA) in an oven at 45°C and 80% RH for 3, 6, 24, 27, 48, 51 and 72 h for viability and vigour tests. Also, the seeds were placed in a cotton bag and kept in a seed store, under ambient conditions for 6 months. Stored seeds were tested for viability and vigour at 30 day interval. Furthermore, electrophoresis analysis was conducted on seeds of three accessions to investigate the total protein bands after AA and ambient storage for 6 months. The result revealed that after 3 months of storage under ambient conditions, viability and vigour reduced from 41.67-28.25% and 2.37-0.66%, respectively. Seeds progressively declined in viability and vigour after 24-27 h of ageing from 28.42-2.89% and 1.54-0.12%, respectively. Accession NG/AO/11/08/123 had the best storage potentials (P_{50} = 24 h) and (P_{50} = 150 days) under ambient storage conditions. In accessions (NG/AO/08/123 and NG/AO/09/024), intensity of profile staining was optimal at 24 h ageing maintained profile intensity for the 72 h. These periods correspond between 30 and 60% germination and vigour index of 2.3, suggesting that Amaranth seeds begin to lose genetic integrity when germination capacity is below 40%. Storage under ambient conditions should not exceed 3 months for best performance of amaranth seeds.

Key words: Electrophoresis, genetic integrity, longevity, physiological integrity, seed storage, seed viability, SDS-PAGE, ageing

INTRODUCTION

Amaranth is an herbaceous annual with upright growth habit, cultivated for both its seeds which are used as a grain and its leaves which are used as a vegetable or green. Both leaves and seeds contain protein of an unusually high quality. The grain is milled for flour or popped like popcorn. The leaves of both the grain and vegetable types may be eaten raw or cooked (Berkelaar and Alemu, 2008).

Seeds are stored for short term as required for carry-over seeds or for considerably longer term as required for germplasm accessions and high value seed stocks. The full benefits of any storage system are realized only when the seeds intended for storage have high initial quality. Therefore, maximum seed quality and vigour are of paramount importance in germplasm management.

The purpose of conservation of germplasm in gene banks in the form of seeds is to maintain the integrity of the material conserved to the highest standard over prolonged periods of time. It is necessary to set standards based on current scientific knowledge and available technologies for the proper handling and storage of seeds in gene banks that will ensure their conservation over the longest possible time, without the need for frequent costly regeneration. Standards for routine gene bank operations and quality assurance were described by Ehsan and Engles (2003).

Seed preservation is the most common method of *ex-situ* maintenance of genetic resources of about 70% of the earth's plant species that possess the orthodox seed storage characteristics, accounting for 90% of the 7.4 million accessions conserved in *ex-situ* gene banks globally (Pritchard, 2004; FAO, 2009). A variety of problems has been acknowledged to be associated with *ex-situ* conservation strategies, one of which is the problem of genetic changes in storage due to ageing and/or field rejuvenation (Roberts, 1988).

In Nigeria, one of the institutional strategies for genetic resources conservation was the establishment of National Centre for Genetic Resources and Biotechnology (NACGRAB) located at Ibadan, Nigeria. To date, many of the tropical species maintained in the gene bank at NACGRAB had not been experimented to generate appropriate individual seed banking procedures. It was therefore, thought imperative to investigate the physiological and genetic changes to understand the basis of seed deterioration in amaranth. This would help not only in identifying reasons for improving storage life of the seed but also provide information that would enable incorporation of trait for better storability in the genetic background of the high yielding varieties. Thus, this study therefore focused on *Amaranthus* vegetable species due to its incredible nutritional content and health benefits to humans.

MATERIALS AND METHODS

This study was carried out between July and December, 2010 at the Federal University of Agriculture, Abeokuta, Nigeria.

Seed materials: Seeds of nineteen (19) accessions of amaranths were sourced from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan as shown in Table 1.

Seed viability: Germination counts of normal seedlings was conducted seven days after sowing (ISTA, 1985) when the radicals have elongated beyond the length of the seed.

Seedling vigour index: This was carried out together with the germination test described above. Five normal seedlings were randomly selected from each replicate for measuring seedling length. Seedling vigour index was then calculated by multiplying seedling length by standard germination percent divided by 100 (ISTA, 1985).

Artificial ageing (AA): The seeds of each seed lot were subjected to AA in an oven at 45°C and 90% RH for 72 h following the procedure of Daniel *et al.* (2009). During the course of ageing, 3 replicates of 10 seeds of each treatment were removed from the ageing chamber at 3, 6, 24, 27, 48, 51 and 72 h for viability tests.

Seed storage at ambient tropical storage conditions: Fifty grams of each seed lot from each of the 19 accessions was weighed and put into cotton bags of 12×10 cm dimension. The experiment was presented in a completely randomized design. Seeds in cotton bags were placed under ambient tropical storage conditions for 6 months (July-December, 2010) at the seed laboratory, Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta. Seeds were then taken from each cotton bag for seed viability at 30 day interval for 6 months during the storage. Genetic test was conducted at the end of the 6 months storage while freshly harvested seeds serve as control for the genetic test.

Electrophoretic analysis: A total of 0.05 g seeds from each of the three selected accessions were used to obtain crude extracts. The seed were weighed on a sensitive balance into an Eppendorf tube and crushed in 500 µL of 0.1 M tris HCl (pH 7.6) after which centrifuging was done for 2 min at 10,000 rpm. The supernatant was then collected into clean Eppendorf tubes. The SDS-PAGE analysis was carried out using the bio-rad mini protean II (10 mL capacity) for the seed proteins with soybean-based protein molecular weight marker. The separating gel was composed of deionized water (3.35 mL), 1.5 M Tris-HCl (2.5 mL), 10% sodium dodecyl sulphate (100 µL), acrylamide/bis-acrylamide solution (4.0 mL), 10% ammonium persulphate (75 µL) and TEMED (7.5 µL). The stacking gel contained deionized water (6.1 mL), 0.5 M tris-HCl at pH of 6.8 (2.5 mL), 10% sodium dodecyl sulphate (100 µL), acrylamide/bis-acrylamide solution (1.33 µL), 10% ammonium persulphate (50 µL) and TEMED (10 µL). β-mercaptoethanol (7.5%) in sample buffer was used for the preparation of the protein samples. The supernatant and the sample buffer was added at ratio 4:1 and heated at 95°C for 5 min in a water bath. The mixture was then loaded into the walls on the gel. The separation of the protein was carried out with the aid of bio-rad electrophoresis system using the bio-rad mini protean II cell at 150 V for 50 min (Ramiro *et al.*, 1995; Maselli *et al.*, 1996).

At the completion of the electrophoresis, the gels were carefully removed under water and placed in a staining solution of 0.1% coomassie blue in 1:4 acetic acid and methanol for 45 min. The staining solution was later removed and the gels were destained with the destaining solution which contains 40% distilled water in 1:4 acetic acid and methanol for 24 h. The de-stained gels were then scanned and the bands scored visually (Lazaro and Aguinagalde, 1998).

Statistical analyses

Probit analysis: Probit analysis was used to estimate potential seed longevity for each seed lot during AA and ambient storage. Seed longevity parameters were values of Ki, slope of survival curve ($1/\sigma$), σ (standard deviation of individual life spans of seeds) and P_{50} (seed half-life) (Ellis and Roberts, 1980).

Analysis of variance: Data on seed viability, seedling length and seedling vigour index were subjected to Analysis of Variance (ANOVA). Means were compared using Duncan's Multiple Range Test (DMRT) at 5% probability level (Duncan, 1955).

Analysis of electropherograms: The percentage of seeds showing activity for the protein marker was calculated for each seed lot at the various deterioration levels.

Table 1: Estimate of probit seed longevity for 19 amaranth accessions after AA

Accessions	K_i	1/σ	σ	P_{50} (h)
NG/AA/SEP/09/104	0.79	-0.12	10.08	7.13
NGB/06/104	8.48	-5.64	0.18	1.50
NHGB/ASPP/09/100	0.19	-0.04	23.16	3.62
NGHB/ASPP/09/091	8.48	-5.64	0.18	1.50
NG/AO/AUG/09/001	3.02	-0.96	8.16	7.19
NHGB/ASPP/09/086	-0.34	-0.04	23.19	-9.70
NG/AO/APR/09/024	0.38	-0.05	22.68	7.49
NGB/06/103	0.83	-0.04	25.41	20.55
NG/AO/11/08/039	0.99	-0.05	19.33	19.05
NG/AO/11/08/040	0.93	-0.15	12.26	9.18
NG/AO/11/08/042	1.15	-0.07	14.77	17.04
NG/SA/DEC/07/043	0.61	-0.18	15.79	2.98
NG/SA/07/205	0.67	-0.05	22.08	14.57
NG/SA/JAN/09/140	1.15	-0.06	16.85	19.97
NG/MR/MAY/09/014	0.48	-5.64	0.18	1.50
NG/FO/JUN/09/008	0.88	-0.05	21.55	19.07
NGB/06/105	0.33	-0.05	20.11	6.53
NG/AO/11/08/123	1.20	-0.43	25.39	27.71
NG/TO/AUG/09/007	8.48	-5.64	0.17	1.50

K_i: Intercept, 1/σ: Slope, σ: Time taken for seed lot to lose 1 probit viability, P_{50}: Seed half life or measure of time to 50% seed viability in storage

RESULTS

Table 1 shows the estimate of potential seed longevity for seeds after AA. The probit programme gave values of intercepts, slopes and half life (P_{50}) while the ageing values were recorded in hours. NG/TO/AUG/09/007 had the highest value of K_i (8.48) with NHGB/ASPP/09/086 (-0.34) recording the lowest. The result also shows the negative values of estimates of slope of the seed viability for all the seed lots; revealing a certain degree of deterioration in the seed lots, irrespective of the ageing hour. Estimate of seed half life viability period in hours (P_{50}) were also different in all the amaranth accessions; with accession NG/AO/11/08/123 having significant highest value (27.71) of the parameter.

Estimate of probit parameters of potential seed longevity for seeds stored under ambient conditions for 6 months are shown in Table 2. The values obtained for intercept (estimates for initial probit viability and a measure of seed viability before storage) were generally higher in accession NG/MR/MAY/09/014. Also, a comparison of the rate of deterioration as indicated by magnitude of negative slope values revealed that NG/AO/11/08/123 recorded prolonged longevity while NG/MR/MAY/09/014 recorded the highest rate of deterioration. NG/AO/11/08/123 also recorded the highest P_{50} value (6.87) while NG/MR/MAY/09/014 the lowest.

Table 3 shows the mean of seed viability variables for the amaranth accessions evaluated after AA. At 3 hours of ageing, germination was significantly higher for accessions NG/AO/11/08/123 and NG/AO/11/08/042. At 6 h, accession NG/AO/11/08/123 also had the highest significant germination value. Accessions NGB/06/103 and NG/FO/JUN/09/008 had higher germination value at 24 h. At 27 h of ageing, accession NGB/06/103 had the highest value. At 48 and 51 h, accession NG/AO/11/08/123 had the highest germination value and only this accession had germination value (6.61) at 72 h while others had zero estimates.

Table 2: Estimate of probit parameters of potential seed longevity for 19 amaranth accessions during storage under ambient conditions for 6 months

Accessions	K_i	$1/\sigma$	σ	P_{50}
NG/AA/SEP/09/104	1.24	-0.64	1.66	1.97
NGB/06/104	0.92	-1.07	1.33	0.77
NHGB/ASPP/09/100	2.25	-1.12	0.93	1.99
NGHB/ASPP/09/091	5.45	-8.78	0.41	0.69
NG/AO/AUG/09/001	1.23	-0.87	1.35	1.34
NHGB/ASPP/09/086	1.34	-0.75	1.36	1.80
NG/AO/APR/09/024	2.17	-0.76	1.35	2.82
NGB/06/103	1.98	-0.51	1.96	3.84
NG/AO/11/08/039	2.46	-0.59	1.72	4.10
NG/AO/11/08/040	1.39	-0.41	2.44	3.33
NG/AO/11/08/042	3.14	-0.73	1.37	4.30
NG/SA/DEC/07/043	2.64	-1.67	0.60	1.59
NG/SA/07/205	2.41	-0.63	1.59	3.74
NG/SA/JAN/09/140	1.13	-0.28	3.69	4.10
NG/MR/MAY/09/014	8.47	-16.90	0.06	0.50
NG/FO/JUN/09/008	1.78	-0.47	2.19	3.77
NGB/06/105	1.46	-0.41	2.45	3.56
NG/AO/11/08/123	1.06	-0.16	6.57	6.87
NG/TO/AUG/09/007	7.14	-7.85	0.13	0.90

K_i: Intercept, $1/\sigma$: Slope, σ: Time taken for seed lot to lose 1 probit viability, P_{50}: Seed half life or measure of time to 50% seed viability in storage

Table 3: Mean values of seed viability of amaranth accessions after AA

	Aging duration (h)						
Accessions	3	6	24	27	48	51	72
NG/AA/SEP/09/104	50.0^{cde}	40.0^{bcd}	13.33^{efgh}	3.33^{d}	0.00^{b}	0.00^{c}	0.00^{b}
NGB/06/104	6.67^{h}	0.00^{e}	0.00^{h}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{b}
NHGB/ASPP/09/100	30.00^{fg}	43.33^{abcd}	23.33^{defgh}	6.67^{d}	10.00^{ab}	0.00^{c}	0.00^{b}
NGHB/ASPP/09/091	0.00^{h}	0.00^{e}	0.00^{h}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{b}
NG/AO/AUG/09/001	43.33^{def}	33.33^{bcd}	6.67^{gh}	6.67^{d}	6.67^{ab}	0.00^{c}	0.00^{b}
NHGB/ASPP/09/086	33.33^{ef}	23.33^{cde}	20.00^{defgh}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{b}
NG/AO/APR/09/024	13.33^{gh}	43.33^{abcd}	36.67^{bcde}	20.00^{bcd}	6.67^{ab}	0.00^{c}	0.00^{b}
NGB/06/103	56.67^{cd}	56.67^{ab}	70.00^{a}	46.67^{a}	6.67^{ab}	6.67^{b}	0.00^{b}
NG/AO/11/08/039	63.33^{c}	56.67^{ab}	53.33^{abc}	33.33^{abc}	6.67^{ab}	0.00^{c}	0.00^{b}
NG/AO/11/08/040	50.00^{cde}	40.00^{bcd}	26.67^{defg}	13.33^{bcd}	0.00^{b}	0.00^{c}	0.00^{b}
NG/AO/11/08/042	86.67^{a}	50.00^{abc}	43.33^{bcd}	23.33^{abcd}	0.00^{b}	3.33^{bc}	0.00^{b}
NG/SA/DEC/07/043	30.00^{fg}	20.00^{de}	10.00^{fgh}	10.00^{cd}	0.00^{b}	0.00^{c}	0.00^{b}
NG/SA/07/205	63.33^{c}	33.33^{bcd}	56.67^{abc}	16.67^{bcd}	6.67^{ab}	6.67^{b}	0.00^{b}
NG/SA/JAN/09/140	83.33^{ab}	50.00^{abc}	56.67^{abc}	33.33^{abc}	6.67^{ab}	3.33^{bc}	0.00^{b}
NG/MR/MAY/09/014	0.00^{h}	0.00^{e}	0.00^{h}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{b}
NG/FO/JUN/09/008	66.67^{bc}	43.33^{abcd}	70.00^{a}	36.67^{ab}	10.00^{ab}	0.00^{c}	0.00^{b}
NGB/06/105	26.67^{fg}	26.67^{cde}	33.33^{cdef}	23.33^{abcd}	0.00^{b}	0.00^{c}	0.00^{b}
NG/AO/11/08/123	86.67^{a}	70.00^{a}	60.00^{ab}	33.33^{abc}	13.33^{a}	26.67^{a}	6.67^{a}
NG/TO/AUG/09/007	0.00^{h}	0.00^{e}	0.00^{h}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{b}

Means followed by the same alphabet along the column are not significantly different at 5% probability level according to Duncan's multiple range test (DMRT)

Table 4: Mean values of seed viability of amaranth accessions under ambient storage for six months

	Storage duration (month)					
Accessions	1	2	3	4	5	6
NG/AA/SEP/09/104	60.00^{def}	36.67^{ef}	40.00^{d}	10.00^{cd}	0.00^{a}	3.30^{d}
NGB/06/104	13.33^{hi}	16.67^{gh}	6.67^{f}	0.00^{d}	6.67^{e}	0.00^{d}
NHGB/ASPP/09/100	86.67^{abc}	33.33^{efg}	26.67^{de}	0.00^{d}	0.00^{e}	0.00^{d}
NGHB/ASPP/09/091	3.33^{i}	3.33^{h}	3.33^{f}	0.00^{d}	0.00^{e}	0.00^{d}
NG/AO/AUG/09/001	46.67^{fg}	23.33^{fg}	13.33^{ef}	0.00^{d}	6.67^{e}	0.00^{d}
NHGB/ASPP/09/086	56.67^{ef}	46.67^{de}	23.33^{e}	0.00^{d}	0.00^{e}	3.33^{d}
NG/AO/APR/09/024	80.00^{abcd}	70.00^{bc}	66.67^{bc}	20.00^{c}	0.00^{e}	0.00^{d}
NGB/06/103	86.67^{abc}	73.33^{abc}	73.33^{abc}	56.67^{b}	40.00^{bc}	0.00^{d}
NG/AO/11/08/039	100.00^{a}	70.00^{bc}	80.00^{ab}	60.00^{ab}	46.67^{b}	0.00^{d}
NG/AO/11/08/040	70.00^{cde}	60.00^{cd}	60.00^{c}	53.33^{b}	36.67^{bcd}	0.00^{d}
NG/AO/11/08/042	100.00^{a}	90.00^{a}	83.33^{a}	60.00^{ab}	46.67^{b}	0.00^{d}
NG/SA/DEC/07/043	76.67^{bcde}	33.33^{efg}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{d}
NG/SA/07/205	96.67^{ab}	80.00^{ab}	63.33^{c}	50.00^{b}	36.67^{bcd}	0.00^{d}
NG/SA/JAN/09/140	66.67^{cdef}	60.00^{cd}	66.67^{bc}	53.33^{b}	43.33^{bc}	26.67^{b}
NG/MR/MAY/09/014	0.00^{i}	0.00^{h}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{d}
NG/FO/JUN/09/008	86.67^{abc}	70.00^{bc}	66.67^{bc}	50.00^{b}	33.33^{cd}	10.00^{c}
NGB/06/105	80.00^{abcd}	63.33^{bcd}	60.00^{c}	50.00^{b}	33.33^{cd}	0.00^{d}
NG/AO/11/08/123	60.00^{def}	70.00^{bc}	83.33^{a}	70.00^{a}	70.00^{a}	40.00^{a}
NG/TO/AUG/09/007	26.67^{gh}	0.00^{h}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{d}
Standard error (SE)	11.49	8.78	7.25	6.21	6.48	3.24

Means followed by the same alphabet along the column are not significantly different at 5% level of probability according to Duncan's multiple range test (DMRT)

Table 4 shows the mean of seed viability variables for the amaranth accessions evaluated at monthly intervals after ambient storage conditions. The highest viability value at 1 month after storage was recorded in accession NG/AO/11/08/039. In all the accessions, a progressive decline in viability was observed with storage period with accession NG/AO/11/08/123 recording highest germination value at month 4 and 5.

The mean of seedling vigour variables after AA are presented in Table 5. Accession NGB/06/103 recorded highest seedling vigour at 3 hours though not statistically different from the values observed in accessions NG/AO/11/08/042, NG/SA/JAN/09/140 and NG/AO/11/08/123. At 6 h, accession NGB/06/103 gave the highest value which was not significantly different from that of NG/AO/11/08/123. Also at 24 and 27 h of ageing, accession NGB/06/103 recorded the highest seedling vigour value. However, at 51 and 72 h, accession NG/AO/11/08/123 still recorded seedling vigour value which was significantly different from all other accessions.

Means of seedling vigour variables of the 19 amaranth accessions evaluated under ambient storage condition for 6 months are shown in Table 6. Accession NGB/06/103 recorded highest vigour values over the first 4 months of storage. After 5 and 6 months of storage, accession NG/AO/11/08/123 recorded the highest vigour variable which was significantly different from all other accessions.

Amaranth seed total protein: Activity of seed protein markers appeared in a range of peptides varying from 6.5 to 97 kDa for the freshly harvested control samples (Fig. 1-3). Comparing protein activity of freshly harvested seeds with ageing seed by the banding profile showed

Table 5: Mean values of seedling vigour index of amaranth accessions after AA

	Aging duration (h)						
Accessions	3	6	24	27	48	51	72
NG/AA/SEP/09/104	2.81^{de}	2.20^{bc}	0.55^{fg}	0.22^{cd}	0.00^{a}	0.00^{c}	0.00^{b}
NGB/06/104	0.34^{hi}	0.00^{d}	0.00^{g}	0.00^{d}	0.00^{a}	0.00^{c}	0.00^{b}
NHGB/ASPP/09/100	1.45^{fgh}	1.80^{bcd}	0.96^{efg}	0.30^{bcd}	0.42^{a}	0.00^{c}	0.00^{b}
NGHB/ASPP/09/091	0.00^{i}	0.00^{d}	0.00^{g}	0.00^{d}	0.00^{a}	0.00^{c}	0.00^{b}
NG/AO/AUG/09/001	2.85^{cde}	2.31^{bc}	0.38^{fg}	0.37^{bcd}	0.43^{a}	0.00^{c}	0.00^{b}
NHGB/ASPP/09/086	1.40^{gh}	0.99^{cd}	1.65^{def}	0.00^{d}	0.00^{a}	0.00^{c}	0.00^{b}
NG/AO/APR/09/024	0.41^{hi}	2.17^{bc}	1.73^{de}	0.95^{bcd}	0.28^{a}	0.00^{c}	0.00^{b}
NGB/06/103	5.12^{a}	4.93^{a}	4.42^{a}	4.04^{a}	0.24^{a}	0.34^{b}	0.00^{b}
NG/AO/11/08/039	2.61^{def}	2.82^{bc}	2.42^{cde}	1.40^{bcd}	0.33^{a}	0.00^{c}	0.00^{b}
NG/AO/11/08/040	2.51^{defg}	1.96^{bc}	1.64^{def}	1.30^{bcd}	0.00^{a}	0.00^{c}	0.00^{b}
NG/AO/11/08/042	4.30^{ab}	2.68^{bc}	2.43^{cde}	1.30^{bcd}	0.00^{a}	0.08^{bc}	0.00^{b}
NG/SA/DEC/07/043	1.49^{fgh}	1.05^{cd}	0.41^{fg}	0.47^{bcd}	0.00^{a}	0.00^{c}	0.00^{b}
NG/SA/07/205	3.22^{bcd}	1.93^{bcd}	2.92^{abcd}	1.19^{bcd}	0.32^{a}	0.33^{b}	0.00^{b}
NG/SA/JAN/09/140	3.98^{abc}	2.40^{bc}	2.77^{bcd}	1.54^{bcd}	0.20^{a}	0.08^{bc}	0.00^{b}
NG/MR/MAY/09/014	0.00^{i}	0.00^{d}	0.00^{g}	0.00^{d}	0.00^{a}	0.00^{c}	0.00^{b}
NG/FO/JUN/09/008	3.33^{bcd}	2.88^{bc}	4.27^{ab}	1.80^{bc}	0.47^{a}	0.00^{c}	0.00^{b}
NGB/06/105	1.99^{efg}	1.93^{bcd}	2.16^{cde}	2.01^{b}	0.00^{a}	0.00^{c}	0.00^{b}
NG/AO/11/08/123	4.29^{ab}	3.45^{ab}	3.32^{abc}	1.85^{bc}	0.46^{a}	1.16^{a}	0.24^{a}
NG/TO/AUG/09/007	0.00^{i}	0.00^{d}	0.00^{g}	0.00^{d}	0.00^{a}	0.00^{c}	0.00^{b}

Means followed by the same alphabet along the column are not significantly different at 5% probability level according to Duncan's Multiple Range Test (DMRT)

Table 6: Mean values of seedling vigour index of amaranth accessions under ambient storage for six months

	Storage duration (month)					
Accessions	1	2	3	4	5	6
NG/AA/SEP/09/104	4.53^{bcd}	2.64^{bcd}	2.11^{cd}	0.53^{ef}	0.00^{c}	0.01^{c}
NGB/06/104	0.77^{f}	0.9^{efg}	0.20^{fg}	0.00^{f}	0.17^{c}	0.00^{c}
NHGB/ASPP/09/100	4.54^{bcd}	1.64^{de}	1.11^{ef}	0.00^{f}	0.00^{c}	0.00^{c}
NGHB/ASPP/09/091	0.03^{f}	0.13^{fg}	0.13^{g}	0.00^{f}	0.00^{c}	0.00^{c}
NG/AO/AUG/09/001	3.04^{de}	1.10^{ef}	0.49^{efg}	0.00^{f}	0.10^{c}	0.00^{c}
NHGB/ASPP/09/086	2.96^{de}	3.70^{b}	1.20^{de}	0.00^{f}	0.00^{c}	0.01^{c}
NG/AO/APR/09/024	2.91^{e}	3.05^{bc}	3.01^{bc}	0.83^{de}	0.00^{c}	0.00^{c}
NGB/06/103	6.88^{a}	5.43^{a}	5.49^{a}	2.41^{a}	2.16^{b}	0.00^{c}
NG/AO/11/08/039	4.43^{bcde}	2.53^{cd}	4.71^{a}	0.49^{ef}	2.02^{b}	0.00^{c}
NG/AO/11/08/040	3.65^{cde}	3.51^{bc}	3.09^{b}	0.17^{f}	1.98^{b}	0.00^{c}
NG/AO/11/08/042	5.90^{ab}	5.10^{a}	5.55^{a}	0.93^{de}	2.73^{b}	0.00^{c}
NG/SA/DEC/07/043	4.32^{bcde}	1.35^{e}	0.00^{g}	0.00^{f}	0.00^{c}	0.00^{c}
NG/SA/07/205	5.30^{ab}	4.98^{a}	3.73^{b}	1.23^{cd}	1.83^{b}	0.00^{c}
NG/SA/JAN/09/140	3.33^{cde}	2.94^{bc}	3.41^{b}	0.84^{de}	2.02^{b}	0.19^{b}
NG/MR/MAY/09/014	0.00^{f}	0.00^{g}	0.00^{g}	0.00^{f}	0.00^{c}	0.00^{c}
NG/FO/JUN/09/008	4.89^{bc}	3.52^{bc}	2.91^{bc}	1.68^{bc}	2.18^{b}	0.05^{c}
NGB/06/105	6.85^{a}	5.19^{a}	4.77^{a}	0.97^{de}	0.87^{c}	0.00^{c}
NG/AO/11/08/123	3.02^{de}	3.64^{b}	4.76^{a}	2.13^{ab}	3.90^{a}	0.50^{a}
NG/TO/AUG/09/007	1.03^{f}	0.00^{g}	0.00^{g}	0.00^{f}	0.00^{c}	0.00^{c}

Means followed by the same alphabet along the column are not significantly different at 5% probability level according to Duncan's multiple range test (DMRT)

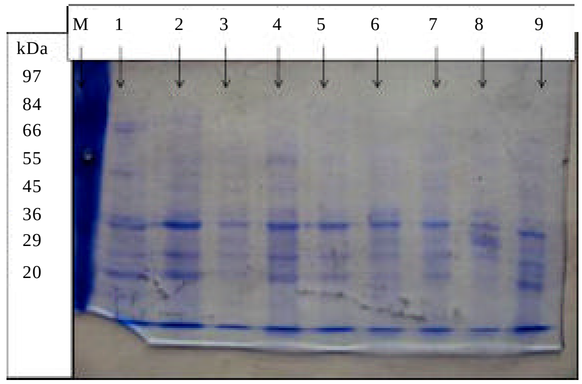

Fig. 1: Electrophoregrams of total seed protein profiles of accession NG/AO/APR/09/024 with intermediate life span. M- Marker, Lane 1 is 6 months old seed lots; Lane 2 is freshly harvested seeds, Lane 3-9 are profiles from seeds aged for 3, 6, 24, 27, 48, 51 and 72 h

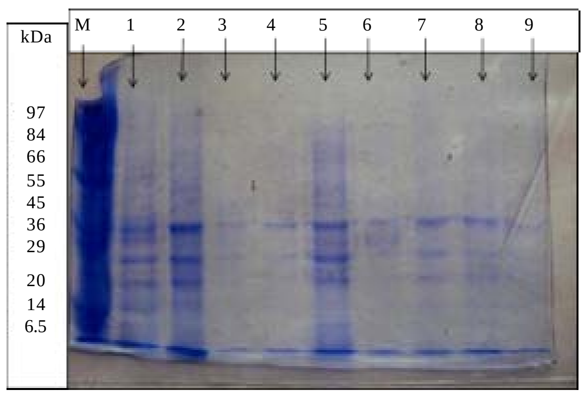

Fig. 2: Electrophoregrams of total seed protein profiles of accession NG/AO/11/08/123 with longest life span. M- Marker, Lane 1 is 6 months old seed lots; Lane 2 is freshly harvested seeds, Lane 3-9 are profiles from seeds aged for 3, 6, 24, 27, 48, 51 and 72 h

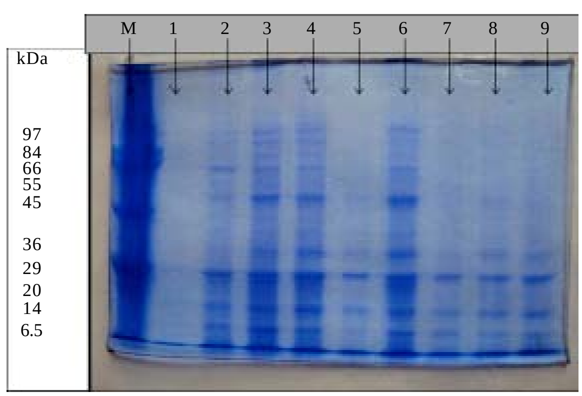

Fig. 3: Electrophoregrams of total seed protein profiles of accession NG/TO/AUG/09/007 with shortest life span. M- Marker, Lane 1 is 6 months old seed lots; Lane 2 is freshly harvested seeds, Lane 3-9 are profiles from seeds aged for 3, 6, 24, 27, 48, 51 and 72 h

Table 7: Summary of allelic frequency of total seed protein electrophoresis during artificial ageing of *Amaranthus* seeds, Data is protein activity profile in the region of 6.5-36 kDa equivalent to low molecular weight proteins

	Molecular weight (kDa)				
Seed ageing (h)	6.5	14	20	29	36
NG/AO/APR/09/024					
3	0	0	1	1	1
6	0	0	0	0	1
24	0	0	1	1	1
27	0	0	1	0	1
48	0	0	0	0	1
51	0	0	0	0	1
72	0	0	0	0	1
NG/AO/11/08/123					
3	1	1	1	1	0
6	1	1	1	1	0
24	0	0	0	1	1
27	1	1	1	1	1
48	0	0	0	1	0
51	0	0	0	1	0
72	0	0	0	1	0
NG/TO/AUG/09/007					
3	0	1	1	1	1
6	0	0	0	1	0
24	0	0	0	1	0
27	0	1	1	1	1
48	0	0	0	1	0
51	0	0	0	1	0
72	0	0	0	1	0

1: Sharp band, 0: Band fainting or absence

differences in band staining over time for the three seed lots. All seed lots also showed consistent fainting of band staining with seed ageing time in the regions of low molecular weight proteins (<36 kDa). Scoring banding pattern between freshly harvested and aged seeds within this region revealed that for all the three accessions, band fainting consistently commenced between 24-27 h of seed ageing (Table 7).

DISCUSSION

Several authors in the field of seed science have identified seed viability and seedling vigour index as components of any assessment of seed quality. In this research, considerable variation occurred among accessions, storage and ageing duration on seed viability, seedling vigour and potential longevity of amaranth seed.

The significant differences observed among the accessions were mostly due to genetic make-up of the accessions considered, suggesting there is an opportunity for selection of accessions with superior seed quality and longevity performance. This support earlier finding by Adebisi *et al*. (2003) in tropical soybean.

The result of the probit modelling of seed viability of amaranth seeds after AA revealed differences in estimates of intercepts and slopes, indicating significant reduction in seed longevity of amaranth irrespective of ageing duration examined.

Differences in estimates of K_i establish reports of varieties and seed lot differences in potential longevity of amaranth. This is in agreement with the reports of Zanakis *et al.* (1993) and Adebisi *et al.* (2003) in soybean varieties. Also, differences in the seed deterioration rates of amaranth accessions showed that initial seed germination before storage influenced seed deterioration parameters. Probit modelling had been widely applied in evaluation of seed quality i.e., potential longevity (Pieta-Filho and Ellis, 1992), seed deterioration (Daniel *et al.*, 1999) and predicting seed viability in storage under constant conditions for different species under different storage environments (Daniel *et al.*, 2003).

In this study, accession NG/AO/11/08/123 after AA showed significant vigour and longest storage life than other seed lots, while accession NG/AO/APR/09/024 behaved mid-way. Also, the result of the probit modelling of seed viability of amaranth seed after ambient storage conditions for 6 months revealed differences in estimates of intercepts and slopes. Earlier reports by Demir and Ellis (1992) pointed out that the higher the quality of seed moved into storage, the longer the expected seed storage life, since seed longevity cannot be improved upon during storage. Seed lots of accession NG/AO/11/08/123 had higher seed storage life values compared with other treatments after 6 months storage under ambient conditions.

In addition, significant decline in seed viability and seedling vigour was observed as the month progressed under ambient storage condition and became pronounced as from 120 days of storage. Significant higher seed viability and seedling vigour were maintained at 30 to 90 days of storage and a sharp decline was observed with prolonged storage period. Similar result was observed during AA. Significant decline in seed viability and seedling vigour was observed as the ageing duration progresses and became pronounced as from 27 h of ageing. However, accession NG/AO/11/08/123 maintained significant seed viability and seedling vigour throughout the storage period and AA.

Quantification of losses in genetic integrity as seeds age is of interest to biodiversity conservation in *ex-situ* gene banks. Earlier works showed that chromosomal aberrations occurred during seed ageing in storage and major breaks in chromatids imply point mutations while seeds age which may become fixed in the population causing genetic changes (Roberts, 1988; Malik and Shamet, 2009). More recent researches had involved the use of molecular genetic markers for investigating genetic integrity during seed storage (Ramiro *et al.*, 1995; Wang *et al.*, 2010; Awad *et al.*, 2009; Emre *et al.*, 2007). The results of this study based on total 6 seed protein markers largely confirms hypothesis of genetic deterioration with seed ageing in storage.

In this study, seed proteins studied showed a range of peptides varying from 6.5-97 kDa but most visible fainting of banding indicating reducing or removal of protein activity with seed aging were observed between 6.5 and 36 kDa which corresponds to the molecular weight of legume protein-vicilins (Machuka, 2001; Oladejo *et al.*, 2009). This suggests that protein activity in this region is possible marker for seed ageing.

Consistent loss of band intensity between 24 and 27 h of artificial ageing indicates losses in protein functions and suggests that amaranth seeds lost genetic integrity at this point of seed ageing. Correlating this period in artificial ageing to naturally ageing or controlled deterioration procedures is important to make a meaning out these results. Simply matching germination at these periods of ageing with loss of protein band intensity showed that percentage seed germination at 24 hours of ageing was 60% and vigour index was 3.5, which declined to 30% and vigour index of 2.3 at 27 h. Wang *et al.* (2010) also reported that low germination percentage (20-40%) as regeneration standard for *Medicago ruthenica* was not proper for maintaining the genetic integrity of the germplasm resource.

CONCLUSION

The implication of the results for seed physiological and genetic resources management is that information on germination tests could indicate threshold of deterioration that affects genetic integrity of amaranth seeds during storage. From this trial between 30-60% germination indicate threshold of germination capacity that impacts on genetic integrity, thus gene bank managers should multiply amaranth seed stocks before it reaches this level to preserve genetic integrity of seed in repositories.

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