Influence of Seed Moisture Content on Short Term Storage of Cowpea (*Vigna unguiculata* L. Walp) Seeds

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Summary

Seeds of four improved varieties of cowpea (*Vigna unguiculata* L. Walp), 'Modupe', 'Ife Brown', 'IT-69D-610' and 'TVX 3236', were adjusted to three moisture levels: 10%, 15% and 20% to determine the appropriate moisture level to maintain the viability and vigour of seeds and to investigate the extent to which duration of storage affects their viability and vigour. Response of cowpea seeds was assessed by standard germination test, accelerated ageing test, saturated salt accelerated ageing test and bulk conductivity test. The moisture contents of the seeds were determined gravimetrically, before being conditioned to 10%, 15% and 20% moisture levels. Cowpea seeds maintained at 10% had the highest viability and vigour irrespective of the duration of storage. Irrespective of genotype, there was a progressive significant decline in the germination potential during the storage period. While viability loss was negligible when seeds were stored at 10 and 15% moisture contents, germination of seeds stored at 20% decreased by 35.59% in the second month and by another 30.28% in the third month. Thus, there was an inverse relationship between moisture contents of cowpea seeds and the viability and vigour of the seeds in storage.

Key words

seed deterioration, germination, vigour, accelerated ageing, longevity

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Introduction

Cowpea is a crop widely cultivated in Nigeria due to its high protein content (Akande et al., 2012), which helps combat the problem of malnutrition among children. Although its cultivation is predominantly in the northern part of the country, its consumption is nationwide. Cowpea production is wholly dependent on seed as propagation material (Buleti et al., 2019). Being a leguminous crop, cowpea produces seeds contained in pods that split open at maturity and when dried. Pod and seed maturity in cowpea are assessed by pod colour, changing with age from dark green, through light green and yellow to finally brown. However, seeds are often harvested before the pods turn brown, since brown pods are prone to shattering resulting in significant seed loss. This early harvesting also presupposes relatively high moisture content within the seeds, requiring drying at an additional cost.

The amount of moisture in seeds is the most important factor influencing seed viability during storage (O'Hare et al., 2001). The combined effect of high moisture content and storage temperature are critical factors that affect storage potential and eventual quality of seed at sowing time. In humid tropical countries, high ambient relative humidity leads to an increase in moisture content of seeds held in open storage. Together with high ambient temperatures, these conditions result in rapid deterioration leading to a decline in seed vigor and ultimate loss of viability (Eliud et al., 2010). On the other hand, a combination of low seed moisture content and storage temperature slows down the rate of ageing (Coolbear, 1995). Seed ageing leads to deterioration in seed quality, and aged seeds show decreased vigour leading to weak seedlings that cannot withstand the vagaries of the environment under field conditions (Rokich et al., 2000).

The rate of decline in seed quality is largely dependent on storage temperature, relative humidity, seed moisture content, length of storage, type of seed and seed quality (Yin et al., 2000; Hung et al., 2001; Amjad and Anjum, 2002); however, there is lack of information of how these relate to cowpea seeds. Therefore, the objectives of this study were to determine the appropriate moisture level for maintaining high viability and vigour of cowpea seeds, and to investigate the extent to which duration of storage affects their viability and vigour.

Materials and Methods

Seeds of four varieties of cowpea: 'Modupe', 'Ife Brown', 'IT-69D-610' and 'TVX 3236' obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were used for the study. The seeds were adjusted to moisture contents of 10%, 15% and 20% through seed hydration and dehydration, after which they were subjected to standard germination, accelerated ageing, saturated salt accelerated ageing and bulk conductivity tests in the Seed Science Laboratory, Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife. The procedures were as follows:

Seed hydration and dehydration: A representative sample of known weight was taken from each variety, weighed and oven dried at 103°C until constant weight to determine the dry weight of the respective samples. The dry weights were used to estimate moisture requirement for the seeds to attain the desired experimental moisture contents of 10%, 15% and 20%.

Hydration was achieved by lightly sprinkling the seeds with estimated quantity of water for each moisture level. The seeds were then lightly mixed and covered for 24 hours to allow equilibration. For the lower content, seed dehydration was achieved by placing the seeds in the desiccator, and monitoring until the required moisture content was reached. Seeds were sampled at storage intervals of 1, 2 and 3 months and subjected to standard germination, accelerated ageing, saturated salt accelerated ageing and bulk conductivity tests.

Standard germination test: Germination test was conducted to evaluate seed viability using sand substratum. A hundred seeds in four replications from each cultivar were placed on moistened sterilized river-bed sand in seed bowls. The bowls were covered with transparent polythene nylon to reduce the moisture loss, and to allow light penetration under ambient conditions. Counts of emerged seedlings were performed daily from the 3rd to the 9th day after sowing (DAS) (ISTA, 2007).

Germination Percentage (GP) and Germination Index (GI) were calculated from the obtained data as follows:

GP = (Total No. of seedlings emerged on the final count / Total No. of seeds sown) x 100

GI = (Σ (Nx) (DAS)) / Total No. of seedlings emerged on the final count

where Nx represents the number of seedlings emerged on day x after sowing.

Accelerated ageing test: Fifty seeds in four replications were weighed for each variety and placed on a wire mesh suspended on 40 ml distilled water in accelerated ageing boxes. The set up was covered tightly and placed in the accelerated ageing chamber (BINDER GmbH Model ED 400, Germany) at 43°C for 72 hours, after which the seeds were weighed to determine the amount of absorbed moisture, and subsequently placed on moistened sand in seed bowls (Tekrony, 2005). The germination counts were performed daily from the 3rd to the 9th day after sowing (DAS). Accelerated Ageing Germination Percentage (AAPT) and Accelerated Ageing Index (AAI) were then calculated as follows:

AAPT = (Total No of Seedlings emerged at the final count / Total No of seeds sown) x 100

AAI = (Σ (Nx) (DAS)) / Total No of seedlings emerged at the final count

where Nx represents the number of seedlings emerged on day x after sowing.

Saturated salt accelerated ageing test: Fifty seeds in four replications were weighed for each cultivar and placed on a wire mesh suspended on 76% NaCl solution in accelerated ageing boxes. The set up was covered tightly and placed in the accelerated ageing chamber at 43°C for 72 hours, after which the seeds were weighed to determine the amount of absorbed or lost moisture, and subsequently placed on sterilized moistened sand in seed bowls. The germination counts were performed daily from 3rd to the 9th day after sowing (DAS) (McDonald, 1997). Saturated Salt Accelerated Ageing Germination Percentage (SSAPT) and Saturated Salt Accelerated Ageing Index (SSAAI) were calculated as follows:

SSAPT = (Total No of Seedlings emerged at the final count / Total No of seeds sown) x 100

SSAAI = (Σ (Nx)(DAS)) / Total No of seedlings emerged at the final count

where Nx represents the number of seedlings emerged on day x after sowing.

Conductivity test (COND): Fifty seeds in four replicates were weighed and placed into a beaker containing 100 ml of distilled water. A beaker with distilled water but no seeds was used as the control. The beakers were covered with aluminium foil and left upright for 24 hours. Conductivity meter (Jenway 4510, Staffordshire UK) was used to determine the amount of leached ions, expressed as μS/cm per gram of seeds.

Conductivity (μS/cm/g) = (Conductivity reading (μS/cm) – initial conductivity of distilled water) / Initial weight (g) of seeds

(ISTA, 2018)

Statistical analysis: Data collected were subjected to analysis of variance using generalized linear model (GLM) procedure of SAS statistical analysis package. Means were separated using Duncan's Multiple Range Test.

Results

Highly significant ($p < 0.001$) mean squares were detected for Germination Percentage (GP), Germination Index (GI), Accelerated Ageing Germination Percentage (AAPT), Accelerated Ageing Germination Index (AAI), Saturated Salt Accelerated Ageing Germination Percentage (SSAPT) and Saturated Salt Accelerated Ageing Germination Index (SSAAI) (Table 1). This implies that the variability found in GPCT, GI, AAPT, AAI, SSAPT and SSAAI was largely due to variety, seed moisture content and duration of storage. The variety x duration interaction was significant ($p < 0.05$) for GI, variety x moisture content x duration were also significantly different ($p < 0.01$). There was no significant difference in variety x moisture content and in the variety x duration for GI and SSAAI, with no significant difference for variety x moisture content x duration for SSAAI. The R^2 for the model used was highly significant ($p < 0.001$) for all tests (Table 1).

The performance of four cowpea varieties varied for the seed quality parameters (Table 2). Variety 'Modupe' and 'IT-69D-610' had similar germination values, significantly higher than those of other two varieties. However, 'Modupe' had the highest speed of germination (GI and AAI), accelerated ageing germination value and the lowest conductivity value, while 'Ife Brown' and

Table 1. Mean square values of the analysis of variance of the seed quality tests conducted on the cowpea seeds

	df	GP (%)	GI (days)	AAPT (%)	AAI (days)	SSAPT (%)	SSAAI (days)	COND (μS/cm/g)
Replication	3	40.24	0.25	118.18	2.61	16.99	0.15	444.94
Variety, Var	3	1228.22***	1.01**	3048.06***	9.59***	9520.55***	0.91**	25213.88***
Moisture, MC	2	9410.33***	7.49***	5016.79***	65.58***	26226.78***	2.44***	34825.75***
Duration, Dur	2	11666.31**	3.63***	3093.79***	17.71***	8744.36***	9.04***	131833.67***
MC x Dur	4	6352.33***	5.61***	399.43**	2.07	3648.61***	1.17***	17856.24***
Var x Dur	6	227.34***	0.61*	286.04*	8.50***	4346.88***	2.61***	2428.70***
Var x MC	6	232.67***	0.54	464.81***	3.59**	1349.19***	0.23	2632.83***
Var x MC x Dur	12	240.06***	0.61*	469.74**	4.32***	897.52***	0.14	972.02***
Error		31.78	0.26	113.08	1.22	124.90	0.19	188.35
Mean		73.75	4.54	14.63	7.09	57.74	4.76	103.47
CV (%)		7.64	11.20	72.66	15.59	19.36	9.12	13.26
R^2 (%)		95.85	69.70	76.15	72.81	92.34	78.46	96.36

*, ** and ***indicates significance at 0.05, 0.01 and 0.001 level of probability, respectively

GP: Germination Percentage
GI: Germination Index
AAPT: Accelerated Ageing Germination Percentage
AAI: Accelerated Ageing Germination Index
SSAPT: Salt Accelerated Ageing Germination Percentage
SSAAI: Saturated Salt Accelerated Ageing Index
COND: Conductivity Test
CV (%): Coefficient of Variation
R^2: (%) Coefficient of Determination
df: Degrees of Freedom

'TVX 3236' had the lowest values for the germination percentage, accelerated ageing test and saturated salt accelerated ageing test but the highest values for conductivity test (COND). Seeds at 10% and 15% moisture contents had similar germination percentage and speed of germination values (Table 3). However, there was a significant reduction of approximately 9% and 5% in accelerated ageing test and saturated salt accelerated ageing test respectively, when seed moisture increased from 10% to 15%. Also, a further increase in seed moisture content from 10% to 20% led to an approximately 24%, 20% and 43% reduction in germination percentage, accelerated ageing and saturated salt accelerated ageing tests, with about 40% increase in the amount of ions leaked.

Furthermore, along with the duration of storage a significant consistent gradual reduction in quality of the seeds occurred for all traits, seen as the lowest viability and vigour values obtained in the third month (Table 4).

Table 2. Mean values for germination, accelerated ageing, saturated salt accelerated ageing parameters and conductivity test for different variety of cowpea across moisture content and duration of storage

Variety	GP (%)	GI (days)	AAPT (%)	AAI (days)	SSAPT (%)	SSAAI (days)	COND (µ s/cm/g)
Modupe	78.19a	4.33b	28.33a	6.57c	73.56a	5.10a	73.74d
Ife brown	68.19b	4.49ab	7.78b	7.13b	49.33b	4.72b	117.68b
IT 69D 610	79.36a	4.71a	11.71b	6.88bc	68.94a	4.59b	90.09c
TVX 3236	69.25b	4.64a	10.56b	7.77a	39.11c	4.50b	132.39a
Mean	73.75	4.54	14.63	7.09	57.74	4.76	103.47

Means with different letters within column are significantly different ($P < 0.05$)

GP: Germination Percentage
GI: Germination Index
AAPT: Accelerated Ageing Germination Percentage
AAI: Accelerated Ageing Germination Index
SSAPT: Salt Accelerated Ageing Germination Percentage
SSAAI: Saturated Salt Accelerated Ageing Index
COND: Conductivity Test

Table 3. Mean values for germination, accelerated ageing, saturated salt accelerated ageing parameters and conductivity test for different levels of moisture content across cowpea varieties and duration of storage

SMC (%)	GP (%)	GI (days)	AAPT (%)	AAI (days)	SSAPT (%)	SSAAI (days)	COND (µ s/cm/g)
10	82.00a	4.31b	24.55a	6.46b	73.46a	4.54b	95.44b
15	81.66a	4.32b	15.23b	6.35b	68.88b	4.84a	81.47c
20	57.58b	5.00a	4.33c	8.43a	30.88c	4.99a	133.51a

Means with different letters within column are significantly different ($P < 0.05$)

SMC: Seed moisture content
GP: Germination percentage
GI: Germination index
AAPT: Accelerated Ageing Germination percentage
AAI: Accelerated Ageing Germination index
SSAPT: Salt Accelerated Ageing Germination Percentage
SSAAI: Saturated Salt Accelerated Ageing Index
COND: Conductivity Test

Table 4. Mean values for germination, accelerated ageing, saturated salt accelerated ageing parameters and conductivity test for different duration of storage across cowpea varieties and moisture contents

Duration (Months)	GP (%)	GI (days)	AAPT (%)	AAI (days)	SSAPT (%)	SSAAI (days)	COND (µ s/cm/g)
1	85.02a	4.38b	23.35a	7.57a	72.58a	4.44b	42.99b
2	80.27b	4.39b	13.29b	7.27a	46.21c	4.60b	132.03a
3	55.96c	4.86a	7.63c	6.39b	54.42b	5.34b	135.40a

Means with different letters within column are significantly different ($P < 0.05$)

GP: Germination Percentage
GI: Germination Index
AAPT: Accelerated Ageing Germination Percentage
AAI: Accelerated Ageing Germination Index
SSAPT: Salt Accelerated Ageing Germination Percentage
SSAAI: Saturated Salt Accelerated Ageing Index
COND: Conductivity Test

Discussion

The relative contribution of variety as a factor influencing the response of cowpea seeds to storage duration was minimal, compared to the contribution of seed moisture content and the duration of storage *per se*. The variations observed among the varieties might be attributed to the differences in their chemical composition and other inherent differences. Rao et al. (2006) reported that the relative effects of temperature and seed moisture content on seed longevity differ with species, as well as with structural and biochemical composition of seeds. Seeds at lower moisture content had higher viability and vigour, and vice versa. It is an established norm in seed biology that lower rate of metabolic activities slows down the rate of deterioration in seeds. However, in cowpea seeds, the effect of lower seed moisture was more pronounced for vigour than for viability. Despite seeds held at 10 and 15% having similar viability levels, the vigour levels were different. Namely, seeds held at 10% had higher vigour levels as measured by accelerated ageing and conductivity values. High conductivity values resulted from high leakage of metabolites from the seed tissues and this was associated with increase in seed moisture from 15% to 20% and with storage duration. Possible mechanisms responsible for increase leakage of metabolites are: increased metabolic activities, weakening of structural membranes, alteration of metabolic pathways resulting in the inability of seeds to carry out self-repair as suggested by Coolbear (1995). It was also observed that vigour was lost preceding viability within the seeds, across moisture contents and duration of storage. The loss was however greater between the first and the second month of storage, compared to between the second and third months of storage. It is also believed that regardless of storage conditions, the moisture content of seeds eventually comes into equilibrium with the surrounding moisture (Eluid et al., 2010).

Conclusions

Irrespective of genotype, a progressive significant decline was found in the germination potential during the storage period. Unlike viability, seed vigour was significantly reduced by the length of storage period irrespective of moisture content, while the rate of vigour loss was much lower for seeds held at 10%. An inverse relationship was found between moisture contents of cowpea seeds and the viability and vigour of the seeds in storage. Therefore, storage of seeds at 10% moisture content is the best to preserve the viability and vigour of cowpea seeds, but this can only be for a short period of time.

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