Seed Science and Biotechnology ©2011 Global Science Books

Cowpea (*Vigna unguiculata* L. Walp) Seed Germination Indices and Yield as Affected by Length of Storage

Johnson Adedayo Adetumbi[1*] • Jaime A. Teixeira da Silva[2] • Olabisi Alamu[3]

[1] Grain Legume Improvement Programme, Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, P.M.B. 5029 Moor Plantation, Ibadan. Nigeria

[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Ikenobe, 761-0795, Kagawa, Japan

[3] Plant Genetic Resources Department, National Centre for Genetic Resources and Biotechnology (NACGRAB), P.M.B. 5382, Moor Plantation, Ibadan. Nigeria

Corresponding author: * **jaadetumbi@yahoo.com**

ABSTRACT

Due to the deleterious effects of regeneration on the genetic variability of stored seed, this research was carried out to investigate the effect of long-term storage on some germination indices and yield of cowpea (*Vigna unguiculata*). Seeds of two elite cowpea varieties (Ife Brown and Ife BPC), previously conserved and freshly produced, were evaluated for seed germination quality and yield. Ten kg each of stored seed were kept in 45 × 35 cm transparent polyethylene bags in the gene bank of NACGRAB, and maintained at -20°C and 10% relative humidity for 7 years. A standard germination test was conducted on the seed lot before storage, after storage and after field cultivation. The field evaluation was conducted at the NACGRAB research farm during the 2010 cropping season. Long-term storage reduced viability by between 4 and 12% in cowpea seed regardless of the temperature and relative humidity of the storage environment. However, for the purpose of regeneration from a seed bank, cowpea seed could be successfully stored for 7 years without a significant loss in viability or yield potential of the crop. Seed germination indices play an important role in seed quality evaluation of cowpea to complement the germination test.

Keywords: cowpea seed regeneration, germination indices, length of storage

INTRODUCTION

The aim of this study was to examine the effect of long-term storage on some germination indices and yield of cowpea (*Vigna unguiculata* L. Walp; Fabaceae). Cowpea is regenerated by seed and is largely self-pollinating but up to 2% outcrossing has been reported (Fatokun and Ng 2007). Seed regeneration is too demanding in term of resources and time. Usually, regeneration is done when the quantity of seed is below the required amount and when seed viability has fallen below the required germination percentage i.e. 85% for most crops (Lerotholi-Qhobela and Marandu 2009). Seed regeneration is also a tool for evaluating germplasm by breeders to understand the genetic diversity resident in accessions for selection of important traits into a breeding program (Kivuva 2004). The need to regenerate seed bank accessions is frequently mentioned in the literature (Breese 1989; Sackville-Hamilton and Chorlton 1997). However, regeneration has been found to have deleterious effects on the genetic variability of stored seeds (Stoyanova 1992; Diaz *et al*. 1997). It is recommended that regeneration be reduced to a minimum by emphasizing the efficiency of seed preservation systems (Anonymous 1994). Usually, emphasis has always been on germination capacity before regeneration is considered. Other germination parameters, like seed vigour that measure the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence, are equally important. The vigor and viability of seeds stored even at low temperatures declines over time due to ageing. Seed ageing and deterioration affects both seed germination and seed vigor (Mohammadi *et al.* 2011). Standardized tests of germination conducted in the laboratory include seed emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil (ISTA 2006).

MATERIALS AND METHODS

Experiment to determine changes in cowpea seed quality during storage

1. Seed collection and storage

10 kg each of two elite cowpea varieties (Ife Brown and Ife BPC) produced in Ballah (Sudan Savannah agroecology) station of the Institute of Agricultural Research and Training in the 2002 cowpea cropping season (August to October) was obtained from a processed seed lot and kept in 45 × 35 cm transparent polyethylene bags in the gene bank of NACGRAB. The storage room was maintained at -20°C and 10% relative humidity throughout the 7 years (2003 to 2010) of storage. A standard germination test conducted on the seed lot before storage revealed a baseline germination of 95 and 89% for Ife Brown and Ife BPC, respectively.

Treatment seed for the experiment were as follows:

i. Seed previously conserved for 7 years at the National Gene bank (old seed);
ii. Freshly produced seed of same variety produced in same station in 2009 (new seed).

2. Seed quality evaluation

A standard germination test was conducted on seed samples (old and new) by placing 25 seeds on Whatman filter paper (9 mm diam.) inside a Petri dish (one dish = one replicate). The test was replicated four times. The filter paper of each Petri dish was moistened with 2 ml of distilled water to imbibe the seed (ISTA 1996). Petri dishes were placed at room temperature (approx. 25 ± 2°C

Received: 6 June, 2011. Accepted: 25 August, 2011.

Original Research Paper

for 7 days. The standard germination test was used to evaluate germination while speed of germination was used to evaluate seed vigour.

First germination count was performed three days after planting and at 2-day intervals (i.e., day 5 and 7). Germination was evaluated as the percentage of seeds that produced normal seedlings (ISTA 1993). Germination indices such as germination percentage (G%), germination index (GI) and germination rate index (GRI) were calculated from germination data according to Olisa *et al.* (2010), as follows:

$$G\% = \frac{\text{No of emerged seedlings at the final count}}{\text{Total number of seeds planted}} \times 100 \quad (1)$$

$$GI = \frac{\sum(Nx)(DAS)}{\text{Total number of seedlings that emerged at the final count}} \quad (2)$$

where Nx is the number of seedlings that emerged on day X after planting and DAS is the day after planting.

$$GRI = \frac{GI}{G\%} \quad (3)$$

3. Field evaluation

Seed of two cowpea varieties (Ife Brown and Ife BPC) stored for 7 years (old seed) and freshly harvested seed (new seed) were sown in a randomized complete block design (RBCD) in a two-row plot with three replicates at the NACGRAB Experimental and Research Farm, Ibadan, Nigeria (lat. 7° 22' N, long. 3° 50' E) during the 2010 cropping season. Each row was 2.5 m long. Crops were spaced at 75 cm between rows and 25 cm within rows with two seeds per hole to give a plant population of 40 plants per plot.

Planting was performed after ploughing and harrowing on the field and the plot was sprayed with Galex 500EC (Metachlor 250 g/ai plus metobromuron 250 g/ai) manufactured by Syngeta™ as the pre-emergent selective herbicide. This was followed by manual weeding at 4 weeks after planting. Two weeks before anthesis, plants were sprayed weekly with Nuvacron (400 g/ai Monocrotophos) manufactured by Ciba-Geigy™ to control insect pests until the plants reached physiological maturity. All chemicals were sourced from a reputable agro dealer in Ibadan, Nigeria. At maturity, agro-morphological and physiological data were collected mainly from 12 tagged inner plants in the two rows of each plot to avoid any border effect. Data collected are pod length, pod width, no of seed per pod, total seed weight/plot and 100 seed weight. Data collected in the laboratory and on the field were subjected to analysis of variance (ANOVA) while the means were separated using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Changes in germination quality during storage

In this experiment there was a reduction in the G% of both Ife Brown and Ife BPC by 4 and 12%, respectively during storage (**Table 1**). Ife Brown showed 95% G% before storage while Ife BPC recorded 89%. After 7 years' storage, germination was reduced to 91 and 77% for Ife Brown and Ife BPC, respectively (**Table 1**). Despite the low temperature and relative humidity conditions of the storage environment, there was reduction in the viability status of both varieties. Seed deterioration is inevitable but the rate can be reduced by paying due attention to the temperature and relative humidity during storage (Ellis and Roberts 1981; Mettananda *et al*. 2001; Adetumbi *et al*. 2009).

Effect of long-term storage on germination indices after field production

The G% and GI of both varieties of cowpea (Ife Brown and Ife BPC) and the type of seed (old and new) were significantly different from each other at $P < 0.05$ before field planting (**Table 2**). Also, a significant GRI was observed between old and new seed before field planting although there was no significant difference between both varieties (**Table 2**). However, after field production, there was no significant difference between the varieties and the type of seed in all parameters. The significant difference recorded between old and fresh seed for G%, GI and GRI in both varieties is an indication that the longer the seed is stored the higher the seed ageing effect on seed germination indices regardless of the storage conditions. However, once seedlings have become established in the field during regeneration, seed ageing effect would have been removed. Akhter (1992) observed that the frequency of abnormal cells is a sign of low seed vigour and that it increases as the seeds age.

Table 1 Germination percentage loss of cowpea seed after seven years storage.

Variety	Germination % before storage	Germination % after storage	Germination % loss
Ife Brown	95	91	4
Ife BPC	89	77	12

Effect of long-term storage on agronomic yield of cowpea

Some yield parameters (pod length, pod width, number of seed/pod and 100-seed weight) of both varieties were not significantly different from each other ($P < 0.05$). However, total seed weight/plot of the varieties differed significantly between the two (**Table 3**). Similarly, yield of old seed was not significantly different from new seed ($P < 0.05$). The significant total seed weight recorded between the varieties is an indication that variability exists in the yield potentials of the two cowpea varieties used in this study, similar to what was reported by Awonaike *et al.* (1990) and Akande and Balogun (2009). In those studies, the performance of cowpea, in terms of flower initiation, pod formation and yield, varied due to differences in genetic components when all the varieties are subjected to same conditions.

Mean germination indices and agronomic yield of cowpea varieties as shown by mean separation

Mean separation from the ANOVA for significant parameters by DMRT shows that both the G% and GI of Ife Brown (94.17% and 3.54, respectively) were significantly higher than the G% and GI of Ife BPC (85.0% and 3.16, respectively) before field planting (**Table 4**). Also, the total seed weight of Ife Brown (281.87 g) was significantly higher than that of Ife BPC (128.68 g). Differences observed between the varieties in terms of G%, GI and total seed weight could be due to variations in their genetic constitution. Variations in genetic make-up of crops have been reported as one of the major causes of differences observed in seed germination quality and yield (Adebisi *et al.* 2006; Okelola *et al.* 2007).

Mean germination indices and agronomic yield of cowpea types as shown by mean separation

Similarly, G% of new seed (95.0%) was significantly higher than that of old seed (84.17%) before field planting (**Table 5**). However, the GI and GRI of old seed were significantly higher than those of the new seed (**Table 5**). The observed higher rate of germination (GI and GRI) recorded for old seeds indicates the time required to attain 100% germination if all seeds had germinated. Therefore, old seeds, regardless of the variety, require significant longer days to germinate than new seeds. This performance of old seed is expected because legumes generally have low vigour (Olisa

Table 2 Mean square value of cowpea seed germination indices before and after field planting.

Source of variation	df	Before field planting			After harvest from field		
		Germination percentage	Germination index	Germination rate index	Germination percentage	Germination index	Germination rate index
Variety	1	252.08*	0.42*	0.04	2.08	0.04	0.07
Type	1	352.08*	0.30*	1.99*	18.75	0.18	0.09
V × T	1	102.08*	0.12	0.13	2.08	0.06	0.08

*Significant at $P < 0.05$ according to the *F*-test.

Table 3 Mean square value of agronomic yield of cowpea.

Source of variation	df	Pod length	Pod width	No. of seed/pod	Total seed weight/plot	100-seed weight
Variety	1	0.37	0.40	2.08	70395.4*	1.27
Type	1	0.07	0.00	2.08	4290.3	1.4
V × T	1	0.00	0.40	2.08	36.4	0.37

Table 4 Germination indices and agronomic character of the tested cowpea varieties.

	Before planting			After harvest			Pod length	Pod width	No of	Total seed	100-seed
Variety	G%[a]	GI[b]	GRI[c]	G%[a]	GI[b]	GRI[c]			seed/pod	weight	weight
Ife BPC	85.00 b	3.16 b	3.77	90.83	3.18	3.43	12.8	7.12	10.83	128.68 b	13.48
Ife Brown	94.17 a	3.54 a	3.76	90.00	3.22	3.58	13.15	6.75	11.67	281.87 a	12.83

Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$)
[a] Germination percentage; [b] Germination index; [c] Germination rate index

Table 5 Germination indices and agronomic characters of the tested cowpea types.

	Before planting			After harvest			Pod length	Pod width	No of	Total seed	100-seed
Type	G%	GI	GRI	G%	GI	GRI			seed/pod	weight	weight
New	95.00 a	3.19 b	3.36b	91.67	3.16	3.42	13.05	6.93	11.67	224.18	13.50
Old	84.17 b	3.51 a	4.17a	89.17	3.24	3.60	12.90	6.93	10.83	186.37	12.82

Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$)
[a] Germination percentage; [b] Germination index; [c] Germination rate index

et al. 2010) which, coupled with a long storage period, causes seed deterioration regardless of storage conditions (Daniel 2007).

Interaction effect of variety and type of seed on germination indices

In both varieties, the germination indices of new seeds were better than those of the old seeds. G% of new Ife Brown (96.67%) seed was higher than that of Ife BPC (93.33%) before field planting, whereas after harvesting the germination percentages recorded by both varieties was the same (91.67%) (**Table 6**). This result further confirmed that after field establishment, the effect of seed ageing caused by long-term storage is removed regardless of the variety (Akhter 1992).

Furthermore, G% of new Ife Brown (96.7%) was higher than that of new Ife BPC (93.3%) before field planting, and new seed of Ife BPC consistently recorded lower GI and GRI before field production (3.11 and 3.33, respectively) and after field production (3.14 and 3.37, respectively) (**Table 6**). Usually, high G% is accompanied with smaller GI and GRI. The smaller value recorded for GI and GRI in Ife BPC indicated that its rate of germination was faster, although final G% was low. The smaller the GI value, the faster the germination of the seed. This result corroborates the importance of seed vigour and places emphasis on germination potentials only as a measure of seed quality. A study by Marli and Denise (2006) stressed the fact that seed vigour characteristics are important not only for crop physiologists and seed technologists, but also for ecologists because it is possible to predict the degree of success of a crop species based on the capacity of the harvested seed to speed germination over time.

Table 6 Germination indices and yield of cowpea varieties as affected by long term preservation.

Parameters	Ife Brown		Ife BPC	
	Old	New	Old	New
[a]G % before planting	91.67	96.67	76.67	93.33
[b]GI before planting	3.80	3.28	3.22	3.11
[c]GRI before planting	4.13	3.39	4.21	3.33
[a]G % after field harvest	88.33	91.67	90.00	91.67
[b]GI after field harvest	3.26	3.18	3.22	3.14
[c]GRI after field harvest	3.69	3.47	3.50	3.37
Pod length	13.23	13.67	12.73	12.87
Pod width	6.57	6.93	6.93	7.30
No of seed/pod	11.67	11.67	10.00	11.67
Total seed weight (g/plot)	264.70	299.03	108.03	149.33
100-seed weight	12.67	13.00	12.97	14.00

[a] Germination percentage; [b] Germination index; [c] Germination rate index

CONCLUSIONS

From the results obtained in this experiment, seed germination indices, including GI and GRI, played an important role in seed quality evaluation of cowpea to complement the germination test. Long-term storage reduces seed vigour in cowpea seed regardless of temperature and relative humidity of the storage environment. It is hereby concluded that for the purpose of regeneration in a seed bank, cowpea seed can be successfully stored for 7 years under low temperature and relative humidity without significant loss in viability or yield potential of the crop.

REFERENCES

Adebisi MA, Ajala MO, Daniel IO, Fasan KO (2005) Pre-sowing treatment for improving seed quality in West African rice varieties seedling emergence and seedling growth. *Nigerian Agricultural Journal* **37**, 159-167

Adetumbi JA, Odiyi AC, Olakojo SA, Adebisi MA (2009) Effect of storage environments and storage materials on drying and germination of maize (*Zea mays* L.) seed. *Electronic Journal of Environmental, Agricultural and Food Chemistry (Ejeafche)* **8 (11)**, 1140-1149

Akande SR, Balogun MO (2009) Multi-locational evaluation of cowpea grain yield and other reproductive characters in the forest and southern guinea savannah agroecologies of Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry (EJEAFChe)* **8 (7)**, 526-533

Akhter FN, Kabir G, Mannan MA, Shaheen NN (1992) Aging effect of wheat and barley seeds upon germination mitotic index and chromosomal damage. *Journal of the Islamic Academy of Sciences* **5 (1)**, 44-48

Annonymous (1994) *Genebank Standards*, Food and Agriculture Organization, International Board for Plant Genetic Resources, Rome, pp 1-46

Awonaike KO, Kumarasingbe KS, Danso SKA (1990) Nitrogen fixation and yield of cowpea (*Vigna unguiculata*) as influenced by cultivar and *Bradyrhizobium* strain. *Journal of Field Crops Research* **24 (3-4)**, 163-171

Breese EL (1989) Regeneration and multiplication of germplasm resources in seed genebanks: The scientific background, International Board for Plant Genetic Resources (IBPGR), Rome, 165 pp

Daniel IO (2007) Longevity of maize (*Zea mays* L.) seeds during low input storage under ambient conditions of South Western Nigeria. *Journal of Tropical Agriculture* **45 (1-2)**, 42-48

Diaz O, Gustafsson M, Astley D (1997) Effect of regeneration procedures on genetic diversity in *Brassica napus* and *B. rapa* as estimated by isozyme analysis. *Genetic Resources and Crop Evolution* **44**, 523-532

Duncan DB (1955) Multiple range and multiple *F* tests. *Biometrics* **11**, 1-42

Ellis RA, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* **9**, 373-409

Fatokun CA, Ng Q (2007) Outcrossing in cowpea. *Journal of Food, Agriculture and Environment* **5**, 334-338

ISTA (1996) *Handbook of Vigor Test Methods* (2nd Edn), International Seed Testing Association, Zurich, Switzerland, pp 28-37

ISTA (2006) *International Rules for Seed Testing*, International Seed testing Association, Zurich, 333 pp

Kivuva BM (2004) An overview of crop germplasm characterization and evaluation, regeneration and multiplication. In: *Country Training Report on Sound Principles and Practices of Plant Genetic Resource Conservation and Management in Gene Banks*, 29th November – 3rd December 2004, Nairobi, Kenya, pp 89-95

Lerotholi-Qhobela L, Marandu WYF (2009) Seed regeneration practices at the Southern African Development Cooperation (SADC) Plant Genetic Resources Centre. *African Crop Science Conference Proceedings* **9**, 289-291

Marli AR, Denise G (2006) How and why to measure the germination process? *Revista Brasileira de Botânica* **29 (1)**, 1-11

Mettananda KA (2001) The viability of okra (*Hibiscus esculentus* L.) seeds under different storage conditions. *Journal of the Sri Lanka Department of Agriculture* **7**, 183-189

Mohammadi H, Soltani A, Sadeghipour HR, Zeinali E (2011) Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. *International Journal of Plant Production* **5 (1)**, 65-70

Ng NQ, Hughes Jd'A (1998) Theoretical and practical considerations in the regeneration of cowpea germplasm at IITA. In: Engels JMM, Ramanatha RR (Eds) *Regeneration of Seed Crops and Their Wild Relatives. Proceedings of a Consultation Meeting*, 4–7 December 1995, ICRISAT, Hyderabad, India and IPGRI, Rome, Italy, pp 76-80

Okelola FS, Adebisi MA, Kehinde OB Ajala MO (2007) Genotypic and phenotypic variability for seed vigour traits and seed yield in West African rice (*Oryza sativa* L). *The Journal of American Science* **3 (3)**, 34-41

Olisa BS, Ajayi SA, Akande SR (2010) Physiological quality of seeds of promising African yam bean (*Sphenomstylis stenocarpa* (Hoechst. Ex A. Rich) Harms) and pigeon pea (*Cajanus cajan* L. Mill sp.) landraces. *Research Journal of Seed Sciences* **3 (2)**, 93-101

Sackville-Hamilton NR, Chorlton KH (1997) *Regeneration of Accessions in Seed Collections: A Decision Guide. Handbooks for Genebanks. Number 5*, International Plant Genetic Resources Institute (IPGRI), Rome, p 7 (75 pp)

Stoyanova SD (1992) Effect of seed ageing and regeneration on the genetic composition of wheat. *Seed Science and Technology* **20**, 489-496

Tenebe VA, Yusuf Y, Kargama BK, Asenime IOE (1995) The effect of sources and levels of phosphorus on the growth and yield of cowpea (*Vigna unguiculata* (L.) Walp) varieties. *Tropical Science* **35**, 223-228