

Effect of Storage Temperature and Moisture Content on Seed Quality, Plant Establishment and Grain Yield of Cowpea

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Abstract

Low grain yield of cowpea (*Vigna unguiculata* L. Walp) undermines its potential as a source of vegetable protein, staple and food security crop in Africa. Among factors militating against increased cowpea production is poor seed quality resulting from storage under warm and humid environments under farmers' circumstances, where over 95% of seeds are produced. Seed storage experiment was conducted at Kwadaso in the Ashanti Region of Ghana. Two popular cowpea seeds in Ghana, a pigmented (IT82E-32) and an unpigmented (IT81D-1951), were stored at 8%mc/10 °C, 8%mc/26 °C, 12%mc/10 °C and 12%mc/26 °C for 13 months, after which seed quality and field performance were assessed. Seed vigour (as indicated by decreasing percentage germination, vital staining and increasing levels of electrical conductivity, P and K contents of leachate), seedling emergence, number of plants harvested and grain yield decreased in the order of 8%mc/10 °C, 12%mc/10 °C, 8%mc/26 °C and 12%mc/26 °C, with the white-seeded variety, suffering the most deterioration and agronomic performance particularly at 12%mc/26 °C. The mean grain yields of 1.50, 1.29, 1.44, and 0.52 t ha⁻¹ for 8%mc/10 °C, 12%mc/10 °C, 8%mc/26 °C and 12%mc/26 °C respectively observed, provided options among the first three treatments for seed storage, depending on facilities available, the value of the seed and period of storage. Agronomic performances, including grain yields were better in the transition zone than in the forest and also better in the minor season than the major season. Correlation coefficients indicated highly dependence of grain yield on number of seedlings and plants harvested, which intend depended on the seed vigour indices, ecology and season.

Keywords: Deterioration, Seed, Storage, Vigour, Yield

Effet de la température de stockage et de la teneur en humidité sur la qualité des semences, l'établissement végétal et le rendement en grains du niébé

Résumé

Le faible rendement céréalier du niébé (*Vigna unguiculata* L. Walp) sape son potentiel en tant que source de protéines végétales, de cultures de base et de sécurité alimentaire en Afrique. Parmi les facteurs qui militent contre l'augmentation de la production de niébé, il y a la mauvaise qualité des semences résultant du stockage dans des environnements chauds et humides dans des conditions agricoles, où plus de 95% des semences sont produites. Une expérience de stockage de semences

a été menée à Kwadaso dans la région Ashanti du Ghana. Deux graines de niébé populaires au Ghana, une pigmentée (IT82E-32) et une non pigmentée (IT81D-1951), ont été stockées à 8 % mc/10 °C, 8 %mc/26 °C, 12 %mc/10 °C et 12 %mc/26 °C pendant 13 mois, après quoi la qualité des semences et les performances au champ ont été évaluées. La vigueur des semences (comme indiqué par la diminution du pourcentage de germination, la coloration vitale et l'augmentation des niveaux de conductivité électrique, les teneurs en P et K de lixiviat), la levée des semis, le nombre de plantes récoltées et le rendement en grains diminué de l'ordre de 8 %mc/10 °C, 12 %mc/10 °C, 8 %mc/26 °C et 12 %mc/26 °C, avec la variété à graines blanches, c'est la plus grande détérioration et les performances agronomiques, en particulier à 12%mc/26 °C. Les rendements céréaliers moyens de 1,50, 1,29, 1,44 et 0,52 t ha⁻¹ pour 8 %mc/10 °C, 12 %mc/10 °C, 8 %mc/26 °C et 12 %mc/26 °C respectivement observés, ont fourni des options parmi les trois premiers traitements pour le stockage des semences, en fonction des installations disponibles, de la valeur des semences et de la période de stockage. Les performances agronomiques, y compris les rendements céréaliers, ont été meilleures dans la zone de transition que dans la forêt et aussi meilleures pendant la saison mineure que la saison majeure. Les coefficients de corrélation indiquaient une forte dépendance du rendement en grains par rapport au nombre de plants et de plantes récoltées, ce qui dépendait des indices de vigueur des semences, de l'écologie et de la saison.

Mots-clés: détérioration, semences, stockage, vigueur, rendement

Introduction

Seed longevity differs among species and varieties and may be influenced by post-maturation climatic factors, seed moisture content and storage temperature (Domergue *et al.*, 2019; Asiedu *et al.*, 2002). In many tropical climates, sun or mechanical drying of seeds to moisture levels below 12% is rather difficult due to the prevalence of high relative humidity during most parts of the year (Asiedu *et al.*, 2002). The rule of thumb suggests that 1.0% decrease in seed moisture content or 10 ° F decreases in storage temperature would double the storage life of seeds (Harrington, 1972). This implies that seed moisture content, which is a function of relative humidity of the storage environment, is more important than temperature in determining seed longevity. Thus, farmer-saved seeds, which constitute 95% of cowpea seeds planted annually in Ghana (SRID-MOFA, 2004) and in most African countries may be stored under unfavourable conditions of high relative humidity with monthly maximum of 80 to 97% and minimum of 38 to

72% depending on the season and ecology, and mean daily minimum and maximum temperatures of 22 °C and 35 °C, respectively (Asiedu *et al.*, 2000). As a result of these harsh conditions, cowpea seeds deteriorate rapidly under non-conditioned storage and consequently, emerge poorly, contributing significantly to low grain yields (Awosanmi *et al.*, 2020; Asiedu, *et al.*, 2009).

The objectives of these studies were to determine firstly, the effect of storage conditions and seed moisture content on cowpea seed quality and secondly, to determine the effect of seed storage quality on plant establishment and grain yield.

Materials and Methods

Study location and experimental design

The experiment was conducted in the Ashanti region of Ghana at Kwadaso (245 m asl N 06° 40. 696 W 001 40, 321°) in the Forest Agro-Ecological Zone. Seeds of two most popular early maturing (60 days to harvest maturity) cowpea cultivars, IT82E-32 (red seeded) and

IT81D-1951 (white seeded), which were developed at the International Institute of Tropical Agriculture (IITA) were planted and harvested for the storage trial. The studies on seed storage started in December 2002 and terminated in December 2003, after which agronomic performance and yields were determined in the field.

Sample preparation

After harvesting, dehumidification of seed from 12% to 8% moisture content was done using a Munter's model MX 1500E dehumidifier. This equipment takes 300 kg seed at a time. Harvested seeds were dried in a conventional dryer to 12% moisture contents after they had been threshed and cleaned to remove extraneous materials. Three hundred kilograms (300 kg) of each cultivar was then conditioned in the dehumidifier for 5h to further reduce the moisture contents to 8%, while the remaining (also 300 kg) were maintained at 12%. Portions of 1 kg of the dehumidified and non-dehumidified seeds of each cultivar were packaged in moisture-proof polythene bags, 10x10 cm and 0.2 mm thick, and heat-sealed. Sets of dehumidified and non-dehumidified packs of each cultivar were stored in a cold room at 10 °C, and a replica stored in a warehouse at a mean ambient temperature of 26 °C, for 13 months.

Experimental design

The experimental design was 2x2x2 factorial (two cultivars, two moisture levels and two temperature regimes) in a randomized complete block, replicated four times. Four hundred and sixteen (416) of seeds were therefore stored, and samples withdrawn monthly for seed quality assessments.

Data collection

Germination test

Four replicates of 50 seeds were put in moist sand (sterilized by heating at 105 °C for 24 h) in 30 cm diameter trays and kept in polythene

bags (to reduce moisture loss) at 27-32 °C. Germination counts were made on 5th (first) and the 8th (final) day. Seeds were considered germinated if the seedlings were normal (AOSA, 2001).

Tetrazolium Test

At the end of the storage period tetrazolium test was done to measure the viability of the seeds. The living tissues of the seed's embryo were stained using 2,3,5 triphenyl tetrazolium chloride (TTC), after soaking 50 seeds per replica in 100 ml water in Petri-dishes for 24 h at room temperature (26 °C). One hundred milliliters (100 ml) of 1% (w/v) TTC solution in distilled water was added to each set of 50 seeds in a Petri-dish after the testa had been removed. The seeds were then held in the dark for 3 hours at 30 °C. In living tissues, the TTC reacts with the dehydrogenase enzymes in the cotyledons to produce a red stain called formazan (Asiedu and Powell, 1998). Tissues of the cotyledons damaged during imbibition remain unstained. Cotyledons were then categorized as (1) $x=100$, (2) $100 > x > 50$, (3) $50 > x > 1$ or (4) 0% stained, where x stands for the extent of staining of each embryo. The percentage of each category was then calculated for each experimental treatment.

Electrical Conductivity Test

This was done as a measure of seed membrane integrity and vigour at the end of the storage period. A 100 ml deionised water was used to soak 3.5 g of each seed lot for 24 h into which cell content of the seed embryo had leached. The measurement was done under laboratory condition (26 °C), using the conductivity meter-dip cell. This instrument measures the electrical current passing through the water as a result of the leakage of electrolytes from weak or damaged embryonic cells into the surrounding water. The measurement was expressed in micro-seconds per centimeter per gram of seed ($\mu S cm^{-1} g^{-1}$).

Phosphorus (P) Leachates

Contents of Phosphorus (P) in the seed leachate were estimated as additional measures of vigour differences between the cowpea cultivars at the end of the period of storage. Five (5) milliliters of the seed leachate from each seed sample, together with blanks and standard series were pipetted into test tubes, after which 10 ml of color reagent was added slowly and mixed. A pinch of L-ascorbic acid was then added and thoroughly mixed. Absorbance was measured with 10 mm cuvettes at 880 nm after 30 min, but within 24h with a spectrophotometer. A graph relating the absorbance to the amount of phosphate present was plotted, and P was estimated as a product of sample extract in ppm and extracting ratio. The estimation was expressed in milligram phosphorus per kilogram of seed.

Potassium (K) Leachate

The amount of Potassium (K) was determined by flame photometry by comparing the intensities of radiation emitted by K atoms in the seed leachate to series of standard solutions. Seed samples were weighed and 3.5 g, put into 250 ml glass bottles and 100 ml of deionized water added. The bottles and their contents were incubated for 24 h and then filtered through a fine filter paper. Extracts were taken to the flame analyzer and the emission readings taken. A calibration curve of K-emission against concentration was then drawn. Potassium was estimated as the product of sample in ppm and the extracting ratio. Potassium extract content was expressed in milligram of potassium per kilogram of seed (mg K kg^{-1}).

Data was analysed using MSTAT-C statistical package. The Duncan Multiple Range Test was used to determine differences in parameter measured.

Yield and Yield Related Experiment

After 13 months storage, field performance of

the cowpea seeds were assessed in a trial conducted at two locations, the Forest (Kwadaso) and Transition (Ejura) zones for two seasons namely major and the minor seasons. The experimental design was randomized complete block, replicated four times. Freshly harvested seeds of the two varieties were used as checks. An experimental plot consisted of 4 rows, each measuring 5 m. Row spacing was 60 cm, within row spacing was 20 cm and two seeds per hill planted with expected target population of 166,667 plants per hectare. Data taken on the two middle rows included seedling count, days to flower, plant stand at harvest and grain yield. Total rainfall was recorded for the year and the growing periods (June-July for the major season and September-October for the minor season)

Results and Discussion

Results of 13 months seed storage studies, showed high germination above 80% in seeds stored under all conditions, except the white-seeded variety, IT81D-1951, stored at 12% mc/26 °C beyond 6 months (Table 1). The variety, IT81D-1951, showed significant ($P < 0.05$) decreases in germination after 6 months to levels far below Ghana's acceptable standard of 75% for certified cowpea seeds (Ocran, *et al.*, 1998). This revealed a difference in seed longevity between the pigmented and the un-pigmented seeds in agreement with similar observations (Zaman *et al.*, 2019; Asiedu *et al.*, 2009; Odindo, 2007). All the seeds dehumidified, and/or stored in the cold-room exhibited high levels of vital staining comparable to the freshly harvested checks, indicating that most of their embryonic tissues were viable (Table 2). Seeds stored at 12% mc in the ambient, however, showed significant reduction in complete vital staining, particularly in the white-seeded variety, which had earlier shown significant reduction in germination. Despite the high level of germination and vital

staining of dehumidified, and/or seed stored in the cold room, significant reductions in vigour occurred ($P < 0.05$, DMRT) as indicated by increased electrical conductivity, phosphorus, and potassium contents of seed leachate in the order of 8% mc/10 °C, 12% mc/10 °C, and 8% mc/26 °C and 12% mc/26 °C, particularly in the white-seeded variety (Tables 3 and 7). In the pigmented variety, however, no significant differences occurred between 12% mc/10 °C and 8% mc/26 °C. The indications of vigour level as measured in electrical conductivity, P, and K contents of the seed leachate occurred in the order of freshly harvested checks, dehumidified seeds stored in cold room, non-dehumidified seeds stored in the cold room, dehumidified seed stored in the ambient, and non-dehumidified seeds stored in the ambient (Table 3). Thus, at each temperature, low seed moisture content was more important in determining the longevity of cowpea seeds in conformity with earlier observation by Harrington (1972). Low storage temperature was an added advantage to both dehumidified and non-dehumidified seeds (Kandil *et al.*, 2013). An immediate and rapid leakage of potassium and electrolytes are detected in low vigour seeds as an increase in conductivity of the surrounding water during imbibition which is an essential step towards germination of

legume seeds (Zaman *et al.*, 2019; Odindo, 2007). A similar rapid leakage of sugars, organic acids, and proteinacious substances occurred in legume seeds with weak cells, as a result of damage caused by rapid inrush of water into the embryonic cells (Demidchik *et al.*, 2014). The difference between the red-seeded, and the white-seeded varieties in the present study agrees well with several others (Siddiqui *et al.*, 2010; Asiedu *et al.*, 2000), where in some species of legumes, susceptibility to imbibition damage was associated with differences in seed coat color, and with difference in storage conditions (Mandizvo and Odindo, 2019; Zaman *et al.*, 2019).

In bambara groundnut seeds with white coats imbibed water more rapidly than pigmented ones, and therefore showed higher levels of imbibition damage as evident by high leachate conductivity and reduced germination when compared to pigmented cultivars (Chibarabada *et al.*, 2014). Thus, unpigmented or stored seeds often exhibit low viability and poor seedling vigour as observed in the study, particularly if storage conditions are unfavorable. However, this result is in contrast to Mandizvo and Odindo (2019) who reported slow water imbibition in light coloured seed coat of bambara groundnut.

Table 1. Effects of seed moisture content and storage temperature on percentage germination of cowpea after 13 months storage

Seed moisture content and storage temperature	Initial Germination		Germination (%) after 6 months storage		Germination after 13 months (%)	
	IT82E-32 (Red)	IT81D-1951 (White)	IT82E-32 (Red)	IT81D-1951 (White)	IT82E-32 (Red)	IT81D-1951 (White)
8%mc - 10°C	97.25 a	91.00 a	90.50 a	90.25 a	94.50 a	91.75 a
8%mc - 26°C	97.25 a	91.00 a	90.00 a	87.25 ab	86.25 ab	88.00 ab
12%mc - 10°C	97.25 a	91.00 a	92.25 a	91.00 a	89.25 ab	90.75 a
12%mc - 26°C	97.25 a	91.00 a	84.50 b	69.88 b	80.50 ab	15.50 c

Means of columns and rows followed by different letters differ significantly ($P < 0.05$). DMRT

Table 2. Effects of seed moisture content and storage temperature on vital staining of cowpea seed embryos after 13 months storage

Seed moisture content and storage temperature	100% Staining of Embryo (%)		99-50% Staining of Embryo (%)		49-1% Staining of Embryo (%)		0% Staining of Embryo (%)	
	IT82D-32 (Red)	IT81D-1951 (White)	IT82D-32 (Red)	IT82D-32 (White)	IT82D-32 (Red)	IT81D-1951 (White)	IT81D-1951 (Red)	IT82D-32 (White)
Freshly harvested check	100.0 a	99.0 a	0.0 e	0.0 c	0.0 b	1.0 b	0.0 b	0.0 b
8 % m c - 10 ° C	98.0 a	94.0 b	1.0 e	6.0 c	0.0 b	0.0 b	0.0 b	0.0 b
8 % m c - 26 ° C	99.0 a	96.0 b	2.0 e	4.0 d	0.0 b	0.0 b	0.0 b	0.0 b
12 % m c - 10 ° C	99.0 a	96.0 b	1.0 e	4.0 d	0.0 b	0.0 b	0.0 b	0.0 b
12%mc - 26°C	89.0 b	17.0 c	11.0 b	45.0 a	0.0 b	35.0 a	0.0 b	3.0 a

Means of columns and rows followed by different letters differ significantly. DMRT

Phosphorus is an essential part of many sugar phosphates involved in photosynthesis, respiration, and other metabolic processes and it is also part of nucleotides such as ribonucleic acid (RNA), adenosine diphosphate (ADP), adenosine triphosphate (ATP), and pyrophosphate (Rychter and Rao, 2005). Adenosine triphosphate (ATP) synthesized from ADP through respiration and photosynthesis, contains a high energy phosphate group that drives most biochemical processes requiring energy (Malhotra *et al.*, 2018), such as germination. In addition, P is an essential component of deoxyribonucleic acid (DNA), the seat of genetic inheritance, and of the various forms of RNA needed for protein synthesis (Malhotra *et al.*, 2018), which is an essential process in germinating seedlings. For example, after the increase in the volume of the radicle during imbibition of water that causes it to emerge from the testa, a phase of rapid growth, which involves increases in RNA, and DNA contents, an indication of cell division, follows (Malhotra *et al.*, 2018). Potassium is an activator of several enzymes responsible for energy metabolism, starch synthesis, nitrate reduction and sugar degradation, as well as for

photosynthesis, and protein synthesis (Malhotra *et al.*, 2018). During the period of storage, when vigour and later germination decline, many physiological and biochemical changes occur (Marthandan and Jerlin, 2017). It has been found that a decrease in the integrity of the cell membrane is the first change to occur during ageing as revealed by the increase in solute leakage that occurs from aged but living tissues. This deterioration of cell membranes is thought to result from changes in membrane phospholipids (Shereena and Salim, 2006), which may result from hydrolysis, and/or peroxidation of the lipids.

Decreases in the activities of enzymes, such as pentose phosphate pathway dehydrogenase, peroxidases and succinate dehydrogenase occur in stored seed (Shaheen *et al.*, 2019). A decrease in enzymatic activities with ageing has been observed in onion (Brar *et al.*, 2019); and a decrease in the activities of acid phosphatase, phosphomonoesterase and dehydrogenase has been observed during ageing of maize seeds (Shaheen *et al.*, 2019). Evidence of

Table 3. Effects of seed moisture content and storage temperature on electrical conductivity, phosphorus and potassium contents of seed leachate of cowpea seeds after 13 months storage

Seed moisture content and storage temperature	Electrical Conductivity of Leachate ($\mu\text{S cm}^{-1} \text{g}^{-1}$)		Phosphorus Content of Leachate (mg P/kg)		Potassium Content of Leachate (mg K/kg)	
	IT82D-32	IT81D-1951	IT82D-32	IT81D-1951	IT82D-32	IT81D-1951
	(Red)	(White)	(Red)	(White)	(Red)	(White)
Freshly harvested check	102.03 a	198.53 c	38.78 a	45.88 a	879.25 a	1923.75 d
8% mc - 10 °C	185.63 b	304.98 e	66.40 b	78.20 c	967.75 a	2207.00 ead
8% mc - 26 °C	229.43 c	397.60 g	81.40 c	279.50 f	1178.00 b	3590.25 g
12% mc - 10 °C	207.75 c	379.60 f	75.45 bc	141.38 e	1118.75 b	2257.03 f
12%mc - 26°C	289.90 e	606.00 h	118.50 d	690.00 g	1291.50 c	4473.00 h

Means of columns and rows followed by different letters differ significantly. DMRT

respiratory changes during ageing has also been strongly linked with vigour levels in seeds during ageing. A decline in early respiratory activity, increased leakage of electrolytes, and loss in seed dry weight were observed in soybean seeds subjected to accelerated ageing and in soybean seeds stored at 25 °C, respiration rates of mitochondria from axes decreased as leachate conductivity increased (Marthandan and Jerlin, 2017). Thus, the reduction in vigour observed in the stored cowpea seeds was related to the damage to weak embryonic cells, and therefore destruction of enzymes, genetic constitution, respiratory activities and protein synthesis required for germination and seedling growth.

Total rainfall during the 60-day growing period in the major season were 400 mm and 295 mm and in the minor season were 385 and 190 mm for the Forest and Transition zones respectively (Figure 1). Mean minimum temperatures were 22 and 24 °C and maximum were 33 and 35 °C for the Forest and Transition zones respectively. Results from the field trial further demonstrated the effect of storage on seedling emergence, number of plants harvested and grain yield

(Tables 4, 5 and 6); thus these results showed reductions in the order of 8%mc/10 °C, 12%mc/10 °C, 8%mc/26 °C and 12%/26 °C, which reflected the trends in germination, vital staining, leachate conductivity and P and K contents of leachate. Significant reduction in seedling count, number of plants harvested and grain yield occurred, during both seasons, particularly in the white-seeded cultivar, IT81D-1951 stored at 12%/26 °C; this treatment had earlier shown indices of poor storage quality (Tables 1, 2, 3 and 9). Seedling count and number of plants harvested, as well as grain yield were higher in the Transition zone than in the Forest zone during the major and the minor seasons (Tables 4, 5 and 6), possibly as a result of reduced levels of biotic stresses, including damages by soil and foliar insect pests and diseases, as well as reduced vegetative growth (due to low rainfall) in favour of pod formation.

Although, data was not taken on pests and diseases, it is generally known that pests and disease pressures are more prevalent in the forest zone. Mean grain yields were 1.59 and 0.90t ha⁻¹ for the Transition and Forest zones respectively and 1.67 and 0.83t ha⁻¹ for IT82E-32 and IT81D-1951 respectively,

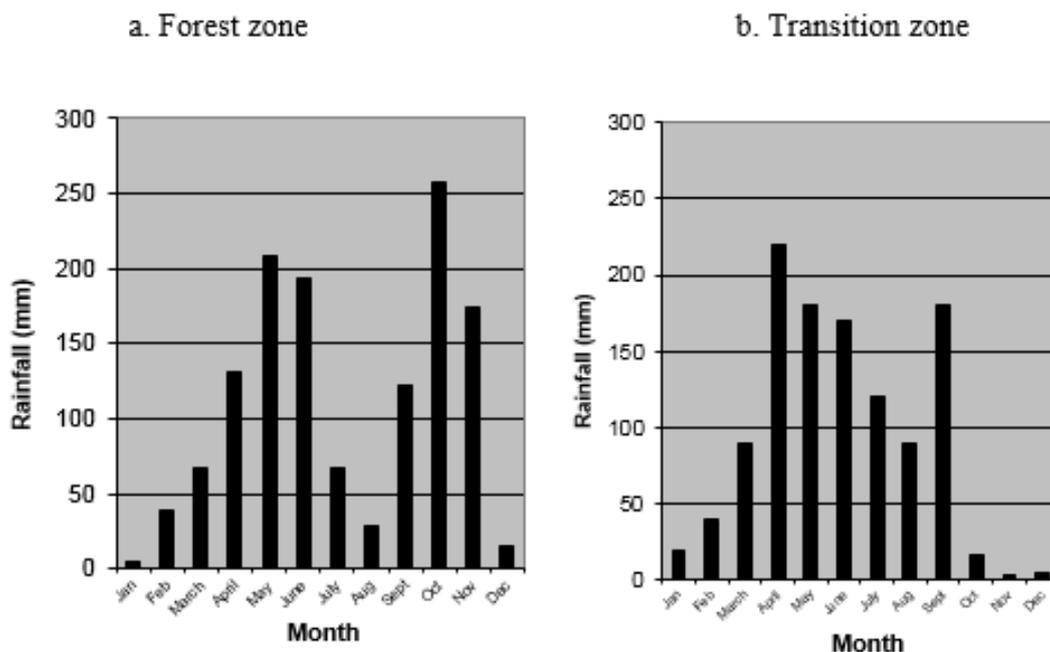


Figure 1. Annual rainfall pattern in (a) Forest zone and (b) Transition zone where the field trials were conducted.

which were reflections of seedling emergence and number of plants harvested. Yields were higher in the minor season than in the major season (Table 6 and 7), which also reflected the differences in seedling emergence (Table 4), number of plants harvested (Table 5) and seed quality indices (Tables 1, 2 and 3). Thus, storage conditions of seeds significantly affected seed quality and consequently, agronomic performance and grain yield of cowpea. Seeds storage at 8%mc/10 °C, 8%mc/26 °C and 12%mc/10 °C offered viable options of storing seeds in the humid environment with 8%mc/26 °C being possibly more cost effective. In addition to storage conditions, ecology, season and variety had significant influences on agronomic performances of cowpea.

Highly significant correlations occurred between the indices of seed vigour and agronomic performance (Table 8). For

instance, highly significant ($P < 0.001$) correlation occurred between germination on one hand, and complete vital staining, P content of leachate, number of seedling and plants harvested per hectare on the other; between complete vital staining and P content of seed leachate, number of seedlings and plants harvested per hectare; between electrical conductivity and P and K contents of leachate and number of seedlings ha⁻¹. Also negative and highly significant ($P < 0.001$) correlation occurred between P content of leachate and number of plants harvested. In addition, significantly ($P < 0.5$) negative correlations were observed between germination on one hand, and electrical conductivity, P and K contents of seed leachate on the other; between complete vital staining on one hand and electrical conductivity, and P content on the other. Also significant correlations occurred between electrical conductivity, P and K contents of

Table 4. Effects of seed moisture content and storage temperature on number of seedlings per hectare of cowpea seeds planted in the Major and minor seasons after 13 months storage.

Seed moisture content/temperature	Number of seedlings established per hectare								Mean
	IT82E-32				IT81D-1951				
	Major season		Minor season		Major season		Minor season		
	Forest	Transition	Forest	Transition	Forest	Transition	Forest	Transition	
Fresh seed	98,333	154,861	131,250	155,417	110,000	146,666	85,000	97,500	122,378
8%mc - 10°C	97,750	150,830	122,083	145,417	99,583	124,583	83,333	96,250	114,979
8%mc - 26°C	89,250	122,083	116,667	94,167	84,583	117,500	43,333	73,750	93,229
12%mc - 10°C	93,750	129,193	122,083	114,167	98,750	121,250	56,667	80,417	102,035
12%mc - 26°C	61,583	97,500	62,500	56,417	0	0	0	0	34,771
Mean	88,133	130,893	112,250	113,117	78,583	101,999	53,666	69,583	93,478
Lsd (0.05)	18,559	21,245	18,940	28,891	18,559	21,285	18,940	28,891	
CV (%)	20.17	24.23	22.98	31.62	20.15	24.23	22.98	31.62	

Means of columns and rows followed by different letters differ significantly. DMRT

Table 5. Effects of seed moisture content and storage temperature on number of plants harvested per hectare of cowpea seeds planted in the major season after 13 months storage

Seed moisture content/temperature	Number of seedlings established per hectare								Mean
	IT82E-32				IT81D-1951				
	Major season		Minor season		Major season		Minor season		
	Forest	Transition	Forest	Transition	Forest	Transition	Forest	Transition	
Fresh seed	91,250	153,000	127,500	141,350	102,917	137,900	82,083	122,481	119,811
8%mc - 10°C	91,666	145,000	121,250	155,321	96,250	117,100	77,500	105,364	113,681
8%mc - 26°C	82,916	119,600	106,250	134,121	81,667	115,400	38,750	66,258	93,121
12%mc - 10°C	90,000	126,000	114,167	138,200	92,500	112,100	65,833	98,450	104,657
12%mc - 26°C	84,583	92,500	56,667	96,246	0	0	0	0	41,250
Mean	88,083	127,220	105,167	131,847.6	74,667	96,500	52,833	78,510.6	94,504
Lsd (0.05)	16,818	21,285	23,140	20,300	16,818	21,285	23,140	20,300	
CV (%)	28.10	17.51	18.282	19.02	28.10	17.51	18.282	19.02	

Means of columns and rows followed by different letters differ significantly. DMRT

Table 6. Effects of seed moisture content and storage temperature on grain yield at harvest of cowpea seeds planted during the major and the minor seasons after 13 months storage

Seed moisture content/ temperature	Grain yield (t/ha)								Mean
	IT82E-32				IT81D-1951				
	Major season		Minor season		Major season		Minor season		
Forest	Transition	Forest	Transition	Forest	Transition	Forest	Transition		
Fresh seed	1.50	2.36	1.66	2.50	1.00	1.20	1.18	1.78	1.64
8%mc - 10°C	1.48	2.06	1.34	2.50	0.86	1.18	0.86	1.64	1.50
8%mc - 26°C	1.28	2.04	1.06	2.40	0.50	0.78	0.70	1.46	1.29
12%mc - 10°C	1.46	2.06	1.34	2.40	0.82	1.0	0.76	1.62	1.44
12%mc - 26°C	0.42	0.8	0.90	2.10	0.00	0.00	0.00	0.00	0.52
Mean	1.16	1.86	1.26	2.38	0.64	0.83	0.74	1.3	1.28
Lsd (0.05)	0.21	0.36	0.15	.025	0.21	0.36	0.15	0.25	
CV (%)	24.35	35.43	33.63	17.20	24.35	35.43	33.63	17.20	

Table 7. Comparison of mean seed quality and agronomic indices between cowpea varieties, seed moisture contents and storage temperatures

Seed Quality and Agronomic Indices	Variety		Seed moisture content (%)		Storage temperature (°C)	
	IT82E-32 (Red)	IT81D-1951 (White)	8	12	10	26
	Germination (%)	90.86 ± 10.5	82.50 ± 14	91.25 ± 18	82.52 ± 13	92.23 ± 16
Vital Staining (%)	97.00 ± 10.2	80.40 ± 11	98.50 ± 14	94.00 ± 14	99.00 ± 17	93.50 ± 14
Leachate Conductivity ($\mu\text{S cm}^{-1} \text{g}^{-1}$)	202.90 ± 24.6	377.60 ± 52	279.40 ± 66	370.80 ± 72	269.49 ± 68	380.73 ± 74
P Content of Leachate (mg P/kg)	76.20 ± 2.3	247.00 ± 45	126.38 ± 32	256.30 ± 58	90.36 ± 9	292.35 ± 62
K Content of Leachate (mg K/kg)	1,346.00 ± 221	2,890.20 ± 312	2,036 ± 225	2,285 ± 241	1,637 ± 149	2,633 ± 268
No. of Seedling/ha – Major Season	109,513.00 ± 10,003	61,625.00 ± 6,023	111,333 ± 10,542	75,254 ± 6,000	114,462 ± 12,425	72,125 ± 5,023
No. of Seedling/ha – Minor Season	112,684.00 ± 12,000	85,584.00 ± 6,500	96,876 ± 7,521	61,532 ± 5,002	102,553 ± 9,576	55,854 ± 4,520
Plants Harvested/ha – Major Season	105,652.00 ± 9,523	59,324.00 ± 4,520	106,200 ± 10,842	74,711 ± 6,544	108,827 ± 10,223	72,081 ± 6,320
Plants Harvested/ha – Minor Season	112,684 ± 11,852	61,625 ± 5,263	100,602 ±	71,196 ± 6,423	109,511 ± 10,521	62,287 ± 4,963
Grain Yield (t/ha) – Major Season	1.51 ± 0.32	0.73 ± 0.10	1.23 ± 0.29	0.82 ± 0.11	1.33 ± 0.31	0.76 ± 0.09
Grain Yield (t/ha) – Minor Season	1.82 ± 0.41	0.92 ± 0.13	1.49 ± 0.23	1.14 ± 0.27	1.56 ± 0.29	1.08 ± 0.26

Table 8. Correlation coefficients (r) among cowpea seed vigour indices and field performances after 13 months storage

	Germination	Vital staining	Electrical conductivity	P content of leachate	K content of leachate	Seedling count	Number of plants harvested	Grain Yield
Germination	-----	0.98***	-0.85**	-0.91***	-0.87**	0.90***	0.99***	0.65*
Vital staining		-----	-0.85**	-0.95***	-0.87**	0.91***	0.98***	0.66*
Electrical conductivity of leachate			-----	0.91***	0.94***	0.94***	-0.79**	-0.85**
P content of leachate				-----	0.88**	0.74**	-0.93***	-0.76*
K content of leachate					-----	0.84**	-0.68*	-0.80**
Seedling count						-----	0.96***	0.90***
Number of plants harvested							-----	0.99***
Grain yield								-----

*, $P > 0.05$; **, $P < 0.01$; ***, $P < 0.001$

seed leachate, as well as between P and K. Field seedling emergence correlated significantly with laboratory germination, vital staining, number of plants harvested and grain yield at $P < 0.001$. It also correlated negatively and significantly with leachate conductivity ($P < 0.001$), P and K contents of leachate ($P < 0.05$). Number of plants harvested correlated positively and significantly with grain yield, germination, seedling count and vital staining ($P < 0.001$) and negatively with P content ($P < 0.001$), as well as with leachate conductivity and K content ($P < 0.05$). Grain yield correlated positively and significantly with germination and vital staining ($P < 0.05$) and with seedling count and number of plants harvested ($P < 0.001$). It also correlated negatively and significantly with conductivity and K content of leachate ($P < 0.01$), as well as with P content ($P < 0.05$). Thus, grain yield was more dependent on seedling count and number of plants harvested, which were also dependent on the seed quality indices.

Conclusions

Cowpea seeds stored at 8%mc/10 °C,

8%mc/26 °C, 12%mc/10 °C and 12%mc/26 °C showed quality decline in order of 8%mc/10 °C, 12%mc/10 °C, 8%mc/26 °C and 12%mc/26 °C as indicated by decreasing percentage germination, vital staining and increasing levels of leachate conductivity, P and K contents, with the white-seeded variety, IT81D-1951 suffering more deterioration. In the field, seedling emergence, number of plants harvested and grain yield followed similar trends as the quality indices and under all conditions, the pigmented cultivar IT82E-32 performed better than the unpigmented, IT81D-1951, which failed to emerge to produce grain at 12%mc/26 °C. Correlation coefficients indicated highly dependence of grain yield on numbers of seedlings that emerged and of plants harvested, which also depended on the seed vigour indices, ecology and season. Thus, the pigmented seed maintained higher vigour throughout the storage period and consequently, emerged better and yielded higher than the unpigmented seed. All seeds, including the unpigmented, stored at 8%mc/10 °C, 12%mc/10 °C and 8%/26 °C showed acceptable performances over those stored at

12%mc/26 °C. These therefore provide options for seed storage depending on facilities available, the value of the seed and duration of storage; for short (0-6 months) and medium (0-13 months) storage of certified seeds, 8%/26 °C may seem the most economical.

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