

Effect of seed storage duration and temperature on germination and vigor of cowpea [*Vigna unguiculata* (L.) Walp. (Fabaceae)]

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Abstract

Cowpea is a traditional crop in Africa grown primarily for its seeds. It plays a very important role in food and is an essential asset for contributing to sustainable food and nutritional security in Côte d'Ivoire. Despite its importance, its yield is negatively impacted by the deterioration of seed quality during storage. Thus, this study was conducted to determine the temperature and storage duration for good germination and vigour of cowpea seeds. The seeds were stored in a freezer at -20°C, in a greenhouse between 29 and 30°C, and at room temperature between 25 and 35°C, for 30 days, 60 days, and 90 days before planting. Among the three storage temperatures compared in this study, freezer storage at -20°C recorded the best germination rate at 30, 60 and 90 days. Room temperature storage recorded the second-best germination rate for all three durations. However, the germination rate decreases over time regardless of storage temperature. Regarding vigor, seeds stored at room temperature gave the most vigorous plants after 30 and 60 days. These results could contribute to setting up an optimal conservation mode to ensure good germination and vigorous plants.

Keywords: *Vigna unguiculata*; Cowpea; Germination; Vigor; Temperature and storage duration

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.], is a traditional crop grown in West Africa, usually in association with other food crops such as maize, millet, sorghum etc. [1]. Cultivated mainly for its seeds in several regions of Côte d'Ivoire, cowpea plays a very important role in the diet. Its seeds are an important source of protein, vitamins and minerals. This makes cowpea an essential asset in the fight against food insecurity [2].

In addition to its use in the diet as a source of protein, cowpea is used as a cover crop. It helps to improve biodiversity and soil fertility. Undemanding, it develops both on fertile soils and on degraded soils. It is an essential component of cropping systems in savannah areas [3]. Despite its many nutritional, agronomic and socio-economic advantages, cowpea remains a marginal crop in Côte d'Ivoire with low yields [1]. The low yields observed are the result of several constraints, including deterioration in seed quality during storage. Cowpea seed storage is still traditional. While a quality seed would optimize yield by 40%, with better resistance to disease, adaptation to environmental conditions, and better commercial value. It is therefore necessary to set up a suitable conservation system to guarantee the quality of cowpea seeds. The present study was initiated at the Université Nangui Abrogoua in Abidjan, southern Côte d'Ivoire. The aim of the study is to assess the effect of seed storage temperature and duration on cowpea germination and vigor.

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2. Material and Methods

2.1. . Plant Material

Plant material used in this study consists of seeds from a cowpea variety from Nangui Abrogoua University in Abidjan, Côte d'Ivoire (**Figure 1**).



Figure 1 Cowpea seeds used as plant material

2.2. Methods

2.2.1. Study site

The trial was conducted at the experimental farm of Nangui Abrogoua University (UNA) in the district of Abidjan. Abidjan is in southern Côte d'Ivoire between 5°17' and 5°31' north latitude and between 3°45' and 4°31' west longitude. Nangui Abrogoua University is located on the Abobo-Adjamé axis. It is bordered to the north by the municipality of Abobo, to the south by Adjamé, to the west by the Banco Forest, and to the east by the municipality of Cocody (Figure 1).

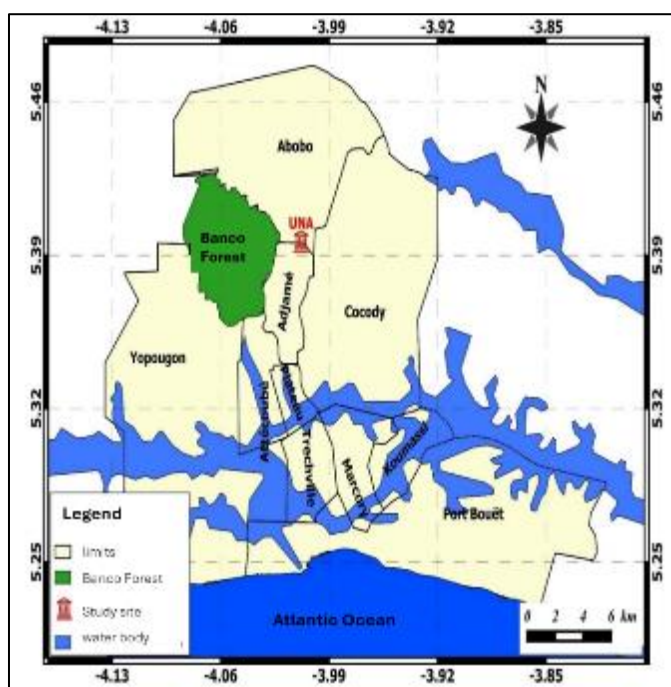


Figure 2 Study site map

2.2.2. Vegetation and soil

Abidjan is characterized by three types of vegetation: rainforest on tertiary sand (Banco and Anguédédou forests), hydromorphic formations (swamp forests and mangroves), and low coastal savannas [4,5]. In addition, there is cultivated vegetation, consisting of intra-urban food crops, fruit crops, market gardening, and horticulture. Located in a sedimentary basin north of Abidjan, it features sandy-clay high plateaus in the continental terminal, while to the south there is a series of low sandy-clay plateaus. Geologically, Abidjan consists of clayey sands, medium sands, and coarse sands. Ferralitic soils and hydromorphic soils are found in marshy areas. The soil pH is more acidic on the surface than at depth, with an organic matter content of between 2 and 3%. These soils owe their hydromorphic nature to heavy rainfall [6].

2.2.3. Rainfall and temperature

The climate of the study area is humid tropical, with temperatures varying between 25 and 35°C. Southern Côte d'Ivoire receives abundant rainfall, with approximately 2,000 mm of rain per year [5]. The precipitation and temperatures for the Abidjan area for the year of our trial (2022), obtained from the National Agricultural Research Center, were used to produce the following ombrothermic diagram (Figure 3). Our trial took place during the main rainy season.

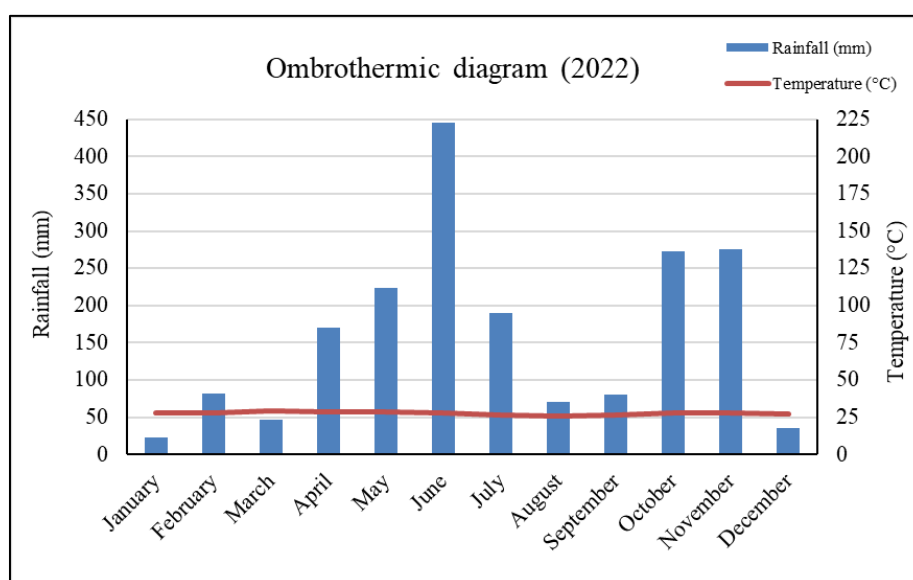


Figure 3 Ombrothermic diagram of the study site

2.2.4. Seed storage

To determine the effect of temperature and storage duration on germination and vigor, *Vigna unguiculata* seeds were stored under the following conditions:

- Seeds were stored in a freezer at -20 °C, in a greenhouse between 29 and 30 °C, and in at room temperature between 25 and 35 °C.
- Seeds were stored at these temperatures for 30 days, 60 days, and 90 days before planting.

2.2.5. Experimental design

The trial was conducted from April to June 2022 at the experimental farm on a plot measuring 5 m long and 5 m wide, covering an area of 25 m². A Fisher block design was adopted. The plot was divided into three blocks, each measuring 4 m x 1 m. The blocks were spaced 1 m apart and arranged perpendicular to a slight slope (heterogeneity gradient) observed on the plot. Each block was divided into 6 elementary plots, with 2 elementary plots per storage temperature (freezer at -20 °C, greenhouse between 29 and 30 °C, and room temperature between 25 and 35 °C). Each elementary plot (1 m x 0.30 m) received 20 seeds at a rate of 2 seeds per hole, for 10 holes, or 120 seeds for the 6 elementary plots per block. Thus, 360 seeds were needed for one planting. The elementary plots were spaced 30 cm apart. The seeds were planted at a density of 20 cm x 20 cm in holes 3 cm deep. Three plantings were made during the trial, depending on the storage times. The first planting took place after 30 days of storage (April 4). The second and last plantings were carried out after 60 and 90 days of storage, respectively (May 4 and June 3). The trial was conducted during the rainy

season, so we did not need to irrigate the plot. Similarly, no fertilizer was applied. To control weeds, manual weeding was carried out throughout the experiment. As for pests, no major or significant damage requiring special treatment was observed. Therefore, no specific pest control measures were taken.

2.2.6. Data Collection and Analysis

Data collection was carried out 15 days after sowing for each of the three treatments. Data were collected on all plants per elementary plot. Ten parameters were measured. The method used to measure these parameters is shown in Table 1. Measurements were taken on at least three leaves and three roots per plant, and average values were assigned to each plant. To determine the germination rate, the following formula was used:

$$\text{Germination rate} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

The germination rates obtained were subjected to a Khi 2 test to determine the relationship between temperature and shelf life of cowpea seeds. The data collected to evaluate the vigor of cowpea plants were subjected to a hypothesis checking, the following hypotheses: independence of observations, normality and equality of variances. Similarly, the statistical power required (80%) to detect a real effect was considered a priori to calculate the minimum number of seeds or plants to be included in our study. Thus, 320 cowpea seeds divided into three groups (storage duration) were used for the study. With this sample size, the statistical power analysis for an ANOVA or Kruskal-Wallis test calculated with R software using the pwr library is greater than 80% (power > 80 %, k=3, n=120, f=0.25, sig.level=0.05). Also, with the three other groups according to storage temperature, the statistical power is always greater than 80%. The data collected does not verify the assumptions of an ANOVA, thus, the non-parametric test of Kruskal-Wallis was carried out for each factor studied (duration and temperature). When a significant difference is found, multiple comparisons between groups using Dunn's Test were carried out in order to identify the groups concerned. These analyses were performed using R software [7].

Table 1 Parameter measured to determine the vigor of cowpea plants based on temperature and storage duration

Parameters	Code	Descriptions
Weight of the plant	WePl	Mass of a plant 15 days after sowing
Plant height	PlHe	Maximum vertical distance reached by leaves, measured 15 days after sowing
Leaf length	LeLe	Length of the sheath at the end of the leaf blade, measured 15 days after sowing
Leaf width	LeWi	Length of the widest part of the sheet, measured 15 days after sowing
Root length	RoLe	Length of the root, measured 15 days after sowing
Diameter at the collar	DiCo	Diameter of the stem at the transition zone between the roots and the trunk, measured 15 days after sowing
Number of leaves	NuLe	Number of leaves 15 days after sowing
Emergence time	EmTi	Number of days from sowing to seedling emergence
Length of petiole	LePe	Length of the sheath at the point of contact between the petiole and the leaf blade, measured 15 days after sowing
Germination rate	GeRa	Percentage of germinated seeds

3. Results

3.1. Germination of cowpea seeds according to the storage duration and temperature

The germination rates of cowpea [*Vigna unguiculata* (L.) Walp.] seeds obtained showed no association between storage duration and temperature (Khi 2, p.value = 0.15). Germination rates have similar decreasing variations depending on the shelf life (30D, 60D and 90D) for the three temperatures (-20°C; between 25 and 35°C; between 29 and 30°C) considered. However, the seeds stored in the freezer at -20°C had higher germination rates (76.25%, 65.62%, 50%) than the seeds stored at room temperature between 25 and 35°C (68.75%, 42.5%, 42.5%) and under Greenhouse

between 29 and 30°C (39.37%, 23.75%, 10.62%). Conservation under greenhouse between 29 and 30°C recorded the lowest germination rates (Table 2).

Table 2 Germination rate of cowpea seeds according to the storage duration and temperature

	30D	60D	90D	p.value (Khi 2)
Freezer at -20°C	76.25%	65.62%	50%	0.15 > α
Room temperature between 25 and 35°C	68.75%	42.5%	38.75%	
Greenhouse between 29 and 30°C	39.37%	23.75%	10.62%	

D: days, α = 0.05

3.2. Vigor of cowpea plants based on their storage temperature

3.2.1. Vigor of cowpea plants based on temperature at 30 days storage

Cowpea plants obtained from seeds at 30 days of storage showed varied vigour (p.value < 0.05). This variation was influenced by the storage temperature of the seeds (freezer at -20°C, room temperature between 25 and 35°C, greenhouse between 29 and 30°C). Except for the length of the roots (RoLe), all measured parameters allowed the cowpea plants to differentiate according to the storage temperature (p.value < 0.01). The seeds stored at room temperature between 25 and 35°C gave the cowpea plants with the best vigor. Those kept in the freezer at -20°C and under glass between 29 and 30°C produced cowpea plants with statistically identical vigours (Table 3).

Table 3 Difference in vigour of cowpea plants from seeds according to the temperature at 30-day storage

	Freezer at -20°C	Room temperature between 25 and 35°C	Greenhouse between 29 and 30°C	p.value
WePl (g)	3.63±1.57 ^b	5.36±2.15 ^a	3.66±2.06 ^b	***
PlHe (cm)	24.24±4.95 ^b	27.58±5.15 ^a	23.74±4.77 ^b	***
LeLe (cm)	13.58±2.68 ^b	15.69±3.22 ^a	13.42±2.96 ^b	***
LeWi (cm)	12.33±2.43 ^b	13.99±2.52 ^a	11.84±2.62 ^b	***
RoLe (cm)	7.66±1.97	7.43±1.37	7.99±1.64	ns
DiCo (mm)	2.48±0.63 ^b	2.73±0.57 ^a	2.49±0.61 ^b	**
NuLe	3.22±0.46 ^b	3.74±0.82 ^a	3.54±0.74 ^b	***
EmTi (day)	3.10±0.30 ^b	3.06±0.25 ^b	4.81±0.40 ^a	***
LePe (cm)	5.69±1.28 ^b	6.45±1.39 ^a	5.62±1.32 ^b	***

WePl: weight of the plant, PlHe: plant height, LeLe: leaf length, LeWi: leaf width, RoLe: root length, DiCo: diameter at the collar, NuLe: number of leaves, EmTi: emergence time, LePe: length of petiole, ns: not significant, ***: significant p.value < 0.001, **: significant p.value < 0.01, cm: centimeter, mm: millimeter, g: gram, a-b-c: ranking in descending order of the different averages

3.2.2. Vigor of cowpea plants based on temperature at 60 days storage

Cowpea plants obtained from seeds stored for 60 days in the freezer at -20°C, at room temperature between 25 and 35°C, and under greenhouse between 29 and 30°C showed varied vigor (p.value < 0.05). This variation was influenced by the storage temperature of the seeds. Except for diameter at the collar (DiCo), all measured parameters differentiated the vigour of cowpea plants according to storage temperature (p.value < 0.01). Similarly to 30-day storage, seeds stored at room temperature between 25 and 35°C for 60 days gave cowpea plants with the best vigor. Seeds stored in the freezer at -20°C and under glass between 29 and 30°C produced cowpea plants with statistically identical vigor (Table 4).

Table 4 Difference in vigour of cowpea plants from seeds according to the temperature at 60-day storage

	Freezer at -20°C	Room temperature between 25 and 35°C	Greenhouse between 29 and 30°C	p.value
WePl (g)	3.42±1.20 ^b	4.80±1.49 ^a	3.10±1.39 ^b	***
PlHe (cm)	23.12±4.73 ^b	26.70±4.02 ^a	21.62±4.60 ^b	***
LeLe (cm)	13.55±2.37 ^b	15.55±2.41 ^a	12.81±2.97 ^b	***
LeWi (cm)	11.76±2.35 ^b	13.46±2.30 ^a	10.97±2.82 ^b	***
RoLe (cm)	8.39±1.82 ^b	9.58±1.96 ^a	9.64±1.99 ^a	***
DiCo (mm)	2.50±0.61	2.67±0.68	2.39±0.60	ns
NuLe	3.71±0.51 ^b	4.03±0.24 ^a	3.59±0.51 ^b	***
EmTi (day)	3.03±0.17 ^b	3.00±0.00 ^b	4.94±0.24 ^a	***
LePe (cm)	5.71±1.08 ^b	6.28±1.18 ^a	5.02±1.02 ^c	***

WePl: weight of the plant, PlHe: plant height, LeLe: leaf length, LeWi: leaf width, RoLe: root length, DiCo: diameter at the collar, NuLe: number of leaves, EmTi: emergence time, LePe: length of petiole, ns: not significant, ***: significant p.value < 0.001, **: significant p.value < 0.01, cm: centimeter, mm: millimeter, g: gram, a-b-c: ranking in descending order of the different averages

3.2.3. Vigor of cowpea plants based on temperature at 90 days storage

Cowpea plants obtained from seeds after 90 days of storage showed similar vigour for most measured parameters (p.value > 0.05). Only three parameters allowed to differentiate the plants according to the three storage temperatures (freezer at -20°C, room temperature between 25 and 35°C, greenhouse between 29 and 30°C) (Table 5).

Table 5 Difference in vigour of cowpea plants from seeds according to the temperature at 90-day storage

	Freezer at -20°C	Room temperature between 25 and 35°C	Greenhouse between 29 and 30°C	p.value
WePl (g)	3.00±0.68	2.85±0.50	3.09±0.94	ns
PlHe (cm)	24.41±3.85 ^a	25.50±3.78 ^a	22.42±4.54 ^b	***
LeLe (cm)	12.87±2.00	12.60±1.81	12.73±2.32	ns
LeWi (cm)	12.34±1.92	12.12±1.80	11.58±2.06	ns
RoLe (cm)	7.89±1.52	7.68±1.22	7.21±1.85	ns
DiCo (mm)	2.23±0.53	2.39±0.51	2.32±0.66	ns
NuLe	3.01±0.19	2.95±0.28	3.00±0.00	ns
EmTi (day)	3.83±0.41 ^a	3.97± 0.18 ^a	3.18±0.39 ^b	**
LePe (cm)	4.84±0.88 ^b	4.73±0.76 ^b	5.27±0.92 ^a	**

WePl: weight of the plant, PlHe: plant height, LeLe: leaf length, LeWi: leaf width, RoLe: root length, DiCo: diameter at the collar, NuLe: number of leaves, EmTi: emergence time, LePe: length of petiole, ns: not significant, ***: significant p.value < 0.001, **: significant p.value < 0.01, cm: centimeter, mm: millimeter, g: gram, a-b-c: ranking in descending order of the different averages

4. Discussion

This study was conducted to determine the effect of temperature and shelf life on germination and vigor of cowpea [*Vigna unguiculata* (L.) Walp.] The main objective is to determine the ideal storage mode of cowpea seeds for good germination and growth. The data obtained from the measured parameters made it possible to evaluate the variations between the three durations and the three conservation temperatures. These variations are well marked for both the germination and the vigour of cowpea.

Our work has shown that the best germination rates were recorded in seeds stored in the freezer at -20°C and at room temperature between 25 and 35°C according to the three storage durations (30D, 60D and 90D). However, seeds stored under glass between 29 and 30°C gave the lowest germination rates of the three storage durations. These low germination rates could be explained by a rapid loss of the germinal capacity of cowpea seeds stored under greenhouse conditions (29 to 30 °C). Our results showed that the rate of germination of cowpea seeds decreases with duration for the three storage temperatures. Thus, the rate of germination of cowpea seeds would decrease depending on the duration. These results are consistent with those of Bamba *et al.* [8]. According to these authors, cowpea seeds lose their germinative power within 12 months after harvest.

The analysis of the measured parameters made it possible to differentiate the vigour of cowpea plants according to storage duration and temperature. During 30 and 60 days of storage, seeds stored at room temperature between 25 and 35°C gave cowpea plants the best vigor. This would mean that room temperature storage is the best conservation condition to obtain vigorous cowpea plants in a short period (60 days). During 90 days of storage, the seeds stored at room temperature between 25 and 35°C, in the freezer at -20 and under glass between 29 and 30°C gave plants with similar vigor. This could be explained by the natural degradation of cowpea grains based on shelf life. Thus, a shelf life of frains for 90 days would significantly affect the vigour of cowpea. Beyond 90 days of storage, the storage temperature would no longer have a differentiating effect on the vigour of cowpea plants. The plants grow with approximately the same vigor, regardless of the storage temperature. This explanation is shared by several authors including Bortey *et al.* [9]. These authors have shown in their study carried out in Ghana that storage duration significantly affects vigor, even under ambient temperatures. However, the present study having been conducted with a single variety of cowpea, at a single site during a single season, would limit its generalization. It would be necessary to redo the study in other environments with several varieties of cowpea to refine the results.

5. Conclusion

A quality seed is an essential criterion that guarantees good germination, vigorous growth and optimal yield. Setting up conservation methods capable of preserving their quality is essential. The present study on the effect of seed storage duration and temperature has allowed to determine the appropriate mode of conservation to ensure good germination and vigorous growth. The results showed variations between the rate of germination and vigour of cowpea as a function of storage duration and temperature. Among the three storage temperatures compared in this study, freezer storage at -20°C recorded the best germination rate for the three durations (30D, 60D, 90D). It is followed by storage at ambient temperature between 25 and 35°C. Conservation at ambient temperature recorded the second-best germination rate for the three durations (30D, 60D, 90D). However, the germination rate decreases over time regardless of the storage temperature. Regarding vigor, seeds stored at room temperature gave the most vigorous plants at 30 and 60 days. At 90 days, no significant difference was observed between the vigour of the plants regardless of the seed storage temperature. Thus, the best storage temperature to ensure good germination would be -20°C in the freezer according to this study. And the ambient temperature between 25 and 35°C would be the best condition to guarantee vigorous growth for a 30 to 60 days storage. However, the results obtained are specific to the conditions of this study. Thus, it would be necessary to repeat this study with other cowpea varieties and other temperature and shelf life.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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