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Physiological quality of cowpea bean (*Vigna unguiculata* L. Walp.) seed by the electrical conductivity and germination testing

Calidad fisiológica de la semilla de fríjol caupí (*Vigna unguiculata* L. Walp.) por conductividad eléctrica y prueba de germinación

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Cowpea bean seedlings.

Photo: H. Araméndiz-Tatis

ABSTRACT

Cowpea is the main legume of the Colombian Caribbean. It is cultivated by small producers, who use seeds from their own crops, stored under uncontrolled environmental conditions. The objective of this research was to evaluate the physiological quality of the seeds of five cowpea cultivars stored for 8-10 years. For the electrical conductivity test, six treatments were carried out (0, 4, 8, 12, 16, and 20 hours of imbibition), using a completely randomized design with five replicates of 50 seeds each per treatment. For the germination test in the greenhouse, a randomized complete block design was used with four repetitions of 50 seeds each per treatment. The results indicate that both methods were efficient in identifying reduction of seed viability due to storage effects, with the differential response of cultivars being due to their genetics. The most affected genotypes were L-026 and C-Tierralta, because they showed a greater amount of leachate 107.19±11.81 and 108.87±8.57 μS cm^{-1} g^{-1}, respectively at 16 h of imbibition, lower percentage of germination and rate of germination speed index.

Additional key words: seed preservation; seed deterioration; physiological potential; seed viability; vigor.

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RESUMEN

El fríjol caupí es la principal leguminosa del Caribe colombiano, cultivada por pequeños productores, quienes usan semillas de sus propios cultivos y almacenadas bajo condiciones ambientales no controladas. El objetivo de esta investigación fue evaluar la calidad fisiológica de la semilla de cinco cultivares de fríjol caupí almacenadas durante 8-10 años. Para la prueba de conductividad eléctrica se hicieron seis tratamientos (0, 4, 8, 12, 16 y 20 horas de imbibición), utilizando el diseño completamente aleatorizado con cinco repeticiones de 50 semillas cada una por tratamiento. Para la prueba de germinación convencional en invernadero se utilizó el diseño de bloques completos al azar con cuatro repeticiones de 50 semillas cada una por tratamiento. Los resultados indican que ambos métodos fueron eficientes para identificar la reducción de la viabilidad de la semilla por efectos del almacenamiento, con respuesta diferencial de los cultivares a causa de su genética. Los genotipos más afectados fueron L-026 y C-Tierralta, por acusar una mayor cantidad de lixiviados 107,19±11,81 y 108,87±8,57 μS cm^{-1} g^{-1}, respectivamente a las 16 h de imbibición, menor porcentaje de germinación e índice de velocidad de germinación.

Palabras clave adicionales: conservación de semillas; deterioro de semillas; potencial fisiológico; viabilidad de semillas; vigor.

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INTRODUCTION

Cowpea bean (*V. unguiculata* (L.) Walp.) is a tropical and subtropical plant of social, economic, and nutritional importance in the Caribbean region, as it is used as a source of protein, calories, fiber, minerals, and vitamins (Araméndiz-Tatis *et al*., 2019). It is characterized as being precocious and drought tolerant, since it can adapt to different agroclimatic conditions in the production systems of the semi-arid regions of the tropics (Xavier *et al*., 2017).

Brazil is the main producer in South America, with the northeast region being the most productive with 713,003 t with yields of 338 kg ha^{-1} (Silva *et al*., 2019a) and, in Colombia, the Caribbean region reports the yields of 600 kg ha^{-1}, very distant from Peru and the United States, which have yields of 1,363 and 2,251 kg ha^{-1}, respectively (FAO, 2021). This is due to the influence of abiotic and biotic factors which affect seed quality, mainly due to temperature and humidity fluctuations (Araméndiz-Tatis *et al*., 2019), which influence the speed, percentage of emergence of seedlings as well as their final population (Smiderle *et al*., 2017).

In the Colombian Caribbean, there are no entities that produce cowpea bean seeds, a situation that forces farmers to use commercial grain seeds or their own crops. In both cases, the seeds are stored under fluctuating environmental conditions in unsuitable packaging, which causes their deterioration because of the loss of physiological quality; therefore, the seed conservation is important for obtaining vigorous seedlings and good yields.

Studies conducted by Sarma *et al.* (2014) in India with cowpea bean seeds packed in metal or clay containers, polyethylene or jute bags for six months reported that the moisture content, the germination percentage, and the vigor index showed variation depending on the type of packaging. The above stated parameters recorded more significant reduction in the jute bag, since the humidity increased with the storage time, due to the bag hygroscopic characteristics. In this way, the absorption of moisture from the environment led to a high population of fungi as well as physiological and physicochemical alterations, which led to the loss of seed viability.

Electrical conductivity (EC) is a test that allows the quick detection of seed deterioration (FAO, 2019). It is based on the process of cellular deterioration and leachate release that occurs when seeds are soaked in water, due to loss of the cell integrity. Therefore, high EC values are associated with seeds of poor physiological quality, due to damage and disorder of cell membranes, a process that is irreversible (Xavier *et* *al*., 2017). Low EC values are associated with good seed quality (Arun *et al*., 2017). On the other hand, the conventional germination method is the most reliable, despite demanding more time and has been applied in cowpea to be compared with other methods (Bortey *et al*., 2016; Moura *et al.,* 2017).

It should be noted that the quality of the seed cannot be improved during storage, but it can be maintained when conditions are favorable for conservation (Oliveira *et al.*, 2016). Therefore, the objective of the present research was to evaluate the physiological quality of seeds from five stored cultivars of cowpea beans, through the methods of electrical conductivity and conventional germination testing.

MATERIALS AND METHODS

Location

This research was carried out during the months of April to May 2019 at the Faculty of Agricultural Sciences of the Universidad de Córdoba (Monteria, Colombia). Its geographical coordinates correspond to 8°31' N and 75°58' W, at an altitude of 13 m, with an average temperature of 28°C, average relative humidity of 80%, average annual rainfall of 1,200 mm, and annual solar brightness of 2,108.2 h (Palencia *et al*., 2006).

Genetic material

In the study, cowpea bean seeds stored in the germplasm bank of the Universidad de Córdoba on different dates, were evaluated. The five cowpea cultivars and year of storage are listed below: 1) L-026 (2010); 2) C- Valledupar (2011); 3) IT-86 (2012), 4) C- Tierralta (2012), and 5) C-001 (2010). The seeds were packed in plastic containers and stored in a cold room with temperatures between 5.0 and 5.5°C and relative air humidity of 60%.

Evaluation methods

The seed physiological quality was assessed by means of electrical conductivity and germination tests (Batista *et al*., 2012). The seeds were previously disinfected with 1% sodium hypochlorite for 1 min and washed with plenty of water before being evaluated: a) Electrical conductivity test: A completely randomized design was used with six treatments and five replicates per treatment. Each experimental unit consisted of 50 seeds per cultivar; six experiments were carried out corresponding to the imbibition periods of 0, 4, 8, 12, 16, and 20 h. The seeds were deposited in plastic containers with a volume of 100 mL of distilled water, kept at 25°C. The electrical conductivity readings were obtained with a Hanna brand conductivity meter, model HI 873, and the results were expressed in $\mu S\ cm^{-1}\ g^{-1}$ of the seed sample and b) Germination test: This test was performed in each mesh according to the methodology of Batista *et al*. (2012), and the evaluation 8 d after its assembly, at 29°C of relative temperature and humidity of 80%; the randomized complete block design was used, with five genotypes and four replicates of 50 seeds per treatment. Disposable aluminum trays measuring 50×9×2 cm were used per replicate, with disinfected coco peat substrate.

Response variables. The EC measured with the conductivity was divided by the weight of the 50 seeds of each experimental unit and was expressed in $\mu S\ cm^{-1} g^{-1}$ per seed; while for the germination test, the following variables were taken into account: Seed dry weight (SW) of 50 seeds, germination speed index (GSI), germination percentage (GP), seed moisture percentage (SM), seedling height (SH), percentage of abnormal seedlings (PAS) (Brasil, 2009) and seedling dry weight (SDW).

Seed dry weight (SW). Four replicates of 50 pure seeds per treatment (in g) were weighed on an OHAUS precision electronic analytical balance, to two decimal places.

Germination speed index (GSI). It was carried out simultaneously with germination. In determining the germination speed index, the formula recommended by Maguire (1962) was applied.

$$GSI = \sum_{i=1}^{n} \frac{p_i}{t_i} \qquad (1)$$

where, p_i is the number of normal seedlings germinated per day; t_i is the number of days elapsed since sowing; *i* is the number of counts, *i*=1,2, 3, ..., n.

Germination percentage (GP). Germinated seeds were counted daily until the 5th day, germinated seeds were those that presented a radicle of at least 2 mm long. For this, in each replicate, 50 seeds were placed to germinate on paper towels moistened with distilled water equivalent to 2.5 of their dry weight at a temperature of 27°C (Brasil, 2009).

Seed moisture percentage (SM). It was determined using 50 seeds for each replicate, by applying a non-destructive method using a Gehaka Agri model G600 equipment (Sao Paulo, Brazil).

Seedlings height (SH). Their height (cm) was measured in 10 seedlings, from the base of the stem to the insertion point of the true leaves, 10 d after emergence in each replicate.

Seedlings dry weight (SDW). It was determined in conjunction with the germination speed index. For this, 10 normal seedlings of each replicate, were subjected to drying for 72 h in an oven with circulating air at 60°C (Xavier *et al*., 2017) until reaching a constant weight (in g), measured on an OHAUS precision balance, to two decimal places.

Percentage of abnormal seedlings (PAS). They were quantified with the GSI test, considering those plants with some of their essential structures absent, malformed or with physiological disturbances that compromised the growth and normal development of the seedling (Brasil, 2009).

The data obtained were subjected to analysis of variance and the Tukey's mean comparison test at 5% probability. Likewise, for the variable electrical conductivity in each cultivar, regression analyses were carried out as a function of the imbibition time. Compliance with the assumptions of the parametric statistics for the additive model of the experimental design and for the regression analyses was verified; SAS v 9.1 software was used.

RESULTS AND DISCUSSION

Electrical conductivity test

The analysis of variance for EC is presented in table 1. The results show significant ($P<0.05$) and highly significant ($P<0.01$) differences in the imbibition times of 0, 4. 8, 12 and 20 h. These differences in EC between genotypes have also been reported in cowpea seeds (Smiderle *et al*., 2017) and in *Zeyheria tuberculosa* seeds (Gonzales and Valeri, 2011) and are due to the genetic variability of cultivars, as well as differences in harvest years, different storage times.

Studies carried out by Bortey *et al.* (2016) showed that loss of the quality attributes of the seed can occur faster in one genotype than another even when exposed to the same storage conditions, since the genotypes exude solutes at differential rates due to their ability to resist aging. As the imbibition time elapses, more solutes are released to the outside due to the level of deterioration of the cell membranes (Batista *et al*., 2012).

In the first 4 h of imbibition, the cultivars registered electrical conductivities between 51.60 μS cm^{-1} g^{-1}, in genotype C-001, and 68.78 μS cm^{-1} g^{-1} in L-026 (Tab. 2). These values are lower than those reported by Smiderle *et al*. (2017). Leachate release is associated with loss of cell membrane integrity (Moura *et al*., 2016), indicating a low physiological seed quality. It can be associated with fluctuations in factors such as temperature and relative humidity during storage, the type of packaging used, and genotype of the seeds as confirmed by Tesfay *et al.* (2016) and Zucareli *et al.* (2015) when studying the behavior of the

Table 1. Mean squares of the analysis of variance of the variable electrical conductivity (μS cm^{-1} g^{-1}) in cowpea beans.

FV	GL	Imbibition time (h)					
		0	4	8	12	16	20
Genotype	4	21.85*	284.32**	2071.38**	2684.49**	372.06^{ns}	3972.04**
Error	20	4.97	59.02	113.49	101.79	256.58	294.69
Mean		4.42	61.93	88.80	100.01	99.19	123.90
CV (%)		50.44	12.40	12.00	10.09	16.15	13,86
Normality	P<F	0.1002	0.7705	0.2654	0.7098	0.0520	0.7443
Homoscedasticity	P>F	0.0134	0.1740	0.0976	0.6275	0.0575	0.6585

* = Significant at 5% ($P<0.05$); ** = Significant at 1% ($P<0.05$); FV = Source of variation; GL = Degrees of freedom; CV = Coefficient of variation. Normality: Levene's test; Homoscedasticity: Shapiro-Wilk test.

physiological quality of common beans under different storage periods.

As the imbibition time increased (Tab. 2), the EC values increased in the five genotypes, because of a greater release of leachate, which varied according to the degree of deterioration of the seeds, as argued by Batista *et al.* (2012) and Silva *et al.* (2013) in cowpea beans and common beans. The progressive increase in EC is due to the effects of seed moisture (Albuquerque *et al*., 2009), the number of submerged seeds (Araméndiz-Tatis *et al.,* 2017), and ambient temperature fluctuations (Silva *et al*., 2013).

In this study, the greatest increase was observed in the first 4 h of imbibition, approximately 1,400%; from then on the increase was progressive with a tendency to decrease, 43.3% between 4 and 8 h; 12.6%, between 8 and 12 h and zero increase between 12 and 16 h of imbibition. It can then be considered that 4-12 h of imbibition are sufficient for determination of the physiological quality of cowpea seeds, which is in accordance with what was earlier reported by Dutra *et al.* (2006), who indicated that 16 h is a sufficient time to indicate about seed quality when there are small differences between batches.

Table 3 shows the regression models for the five cultivars evaluated in the leachate variable. These models are of high reliability for the values of the coefficients of determination (R^2) and the mean squares (MS), criteria considered when selecting the models of best fit. The linear effects indicate that the electrical conductivities fluctuated between 13.71 μS cm^{-1} g^{-1} for each hour of imbibition in 'C-Valledupar' and 21.13 μS cm^{-1} g^{-1} for each hour of imbibition in 'L-026', with deviations from the negative sign linearity and low magnitude.

The coefficients of the linear effect of the models allow them to be ordered from highest to lowest in terms of the release of leachates in the water during imbibition for 12 h and, consequently, due to a decrease in the physiological quality of the seeds in the following order: 'L-026', 'C-001', 'IT-86', 'C-Tierralta', and 'C-Valledupar'. The seeds of the last two cultivars turned out to be the ones with the best physiological quality due to the lower EC, that is, the lower rate of leachate release. These results are consistent with those reported by Smiderle *et al.* (2017), in this same species.

Table 2. Means and standard errors of electrical conductivity (μS cm^{-1} g^{-1}) of five genotypes of cowpea beans, between 0 and 16 h of imbibition.

Genotype	Imbibition time (h)				
	0	4	8	12	16
L-026	3.45±0.60 ab	68.78±3.72 a	123.71±6.71 a	137.13±4.79 a	107.19±11.81 a
C-Valledupar	5.70±1.53 ab	56.29±2.11 ab	75.72±2.62 b	89.20±4.47 c	98.19±5.35 a
IT-86	2.81±0.36 b	66.91±5.07 a	75.42±2.27 b	82.16±2.49 c	90.46±2.77 a
C-Tierralta	7.46±1.43 a	66.09±2.94 ab	89.73±6.15 b	108.02±5.24 b	108.87±8.57 a
C-001	2.67±0.30 b	51.60±2.53 b	79.44±4.31 b	83.56±5.02 bc	91.28±2.71 a
Mean	4.42±0.45	61.93±1.54	88.80±2.13	100.01±2.02	99.19±3.20

Means with different letters indicate significant difference according to the Tukey test ($P<0.05$); ± indicates the standard deviation.

Table 3. Regression models for electrical conductivity as a function of the imbibition time (0-12 h) of five cowpea cultivars.

Genotype	Model	R^2	MS	D
L-026	Y= 1.90 + 21.13**X - 0.81**X^2	0.9632	110.90	1.61
C- Valledupar	Y= 6.96 + 13.71**X - 0.58**X^2	0.9552	48.91	2.16
IT- 86	Y= 5.50 + 16.92**X - 0.90**X^2	0.9210	86.37	1.07
C- Tierralta	Y= 8.94 + 15.70**X - 0.63**X^2	0.9357	102.25	1.61
C- 001	Y= 2.54 + 15.16**X – 0.70**X^2	0.9483	59.31	2.72

Y = electrical conductivity (EC); X = imbibition time (h); R^2= coefficient of determination; MS= Mean square of the error; ** = statistical significance of the estimated model parameter ($P<0.01$); D = Durbin-Watson statistic.

Germination test

The level of statistical significance of the mean squares (MS) of the analysis of variance and mean values of variables related to the physiological quality of seeds and seedlings evaluated through the conventional germination test are shown in table 4; where the existence of significant differences for all variables is appreciated.

Seed dry weight (SW). The results were like those reported by Smiderle *et al*. (2017) and are attributed to genetic effects and mineral nutrition, which influence the size of the seed, in such a way that the heaviest ones accumulate a greater amount of dry matter (Marcos-Filho, 2015; Silva *et al*., 2019b). According to the values recorded in table 4, the C-001 cultivar presented a greater seed weight with respect to the other cultivars, indicating a greater accumulation of dry material.

Germination percentage (GP). The results of this variable coincide with those reported by Smiderle *et al.* (2017) and Chagas *et al.* (2018), who found significant differences (Tab. 4). Therefore, it is evidenced that during long storage periods, the effects of temperature, seed moisture and relative humidity greatly influence the deterioration and loss of seed viability (Bortey *et al*., 2016).

The mean values of the germination percentages (Tab. 4) indicate that all cultivars showed percentages between 51 and 79, below 85% required by the Instituto Colombiano Agropecuario regulation (Colombia, 2015) for this species. The cultivars C-Tierralta and L-026, expressed the lowest physiological potential due to their genetic constitution and the inherent ability of a cultivar to resist stress (Shaheen *et al*., 2019).

Germination speed index (GSI). Genotypes IT-86, C-001, and C-Valledupar yielded mean values between 12.45 and 13.15 and higher than cultivars L-026 and C-Tierralta, which presented values less than or equal to 9.85 (Tab. 4). The records are lower than those reported by Batista *et al*. (2012) due to a lower enzymatic capacity in protein breakdown and a gradual reduction in viability and vigour, known as seed aging (Marcos-Filho, 2015; Kirigia *et al.,* 2018). This resulted in a decline of physiological potential over time, due to the loss of proteins demanded by the embryo during seed germination (Shaheen *et al*., 2019). Therefore, the genotypes presented a differential deterioration due to fluctuations in temperature and humidity, which alter the integrity of the cell membranes and during imbibition release exudates that affect the vigor of the seed.

Seed moisture percentage (SM). The percentage of moisture of the seeds used registered statistical differences between the genotypes (Tab. 4), with values fluctuating between 8.45% for C-Tierralta and 13.77% in C-Valledupar, which is due to the differential hygroscopic capacity of these genotypes, in the gas exchange (Bortey *et al.*, 2016; Ruíz *et al*., 2017).

The percentages of moisture in the genotypes L-026 and C-Valledupar, were above 12%, probably due to a greater humidity accumulated in the gas exchange or being poorly dried at the time of harvest (Silva *et al.,* 2019a), regardless of the depth of drying, the seeds of these five genotypes were in the storage chamber for years and reached equilibrium with the relative humidity of the chamber, taking into account that their storage packages were semi-permeable. In this sense, Carvalho and Nakagawa (2012), argued that with values above this percentage, there is a greater intensity of respiration, leading to loss of vigor and

Table 4. Seed dry weight (SW), germination percentage (GP), germination speed index (GSI), seed moisture percentage (SM), seedling height (SH), seedling dry weight (SDW) and percentage of abnormal seedlings (PAS) of five cowpea seed cultivars.

Genotype	SW (g)	GP (%)	GSI	SM (%)	SH (cm)	SDW (g)	PAS (%)
C-001	7.76 a	79.0 a	12.67 ab	11.42 c	18.86 a	1.59 ab	5.50 ab
C-Valledupar	6.90 b	65.5 ab	12.45 ab	13.77 a	17.77 ab	1.65 a	10.50 a
L-026	6.86 bc	51.0 d	7.35 c	12.77 b	14.36 c	1.10 b	9.50 a
IT-86	6.64 bc	75.5 ab	13.15 a	11.32 c	14.76 bc	1.16 ab	2.00 b
C-Tierralta	6.60 c	55.0 cd	9.85 bc	8.45 d	17.68 ab	1.60 a	9.50 a
Significance level of the F-test	*	*	*	*	**	**	*

Means with different letters indicate significant difference according to the Tukey test, * and **: significance at 5 and 1% probability, respectively.

reduction of germination. However, the cultivar C-Tierralta with 8.5% (Tab. 4), showed a low percentage of germination and germination speed index and a high number of abnormal seedlings (Tab. 4). This is attributable to excessive desiccation, which is critical and causes damage to macromolecules, and is evidenced when they begin to germinate (Marcos-Filho, 2015).

Seedling height (SH). The results show highly significant differences for seedling height (Tab. 4), which is consistent with the studies by Tavares *et al.* (2016) in cowpea beans and Bahry *et al.* (2017) in soybeans. By correlating the germination percentage, germination speed index, and seed weight, the influence of these variables on plant height can be appreciated, as indicated by Ferreira and Novembre (2016), because the genotype C-001 showed the highest plant height (Tab. 4), due to having a greater weight and better use of the seed reserves (Ruíz *et al*., 2017).

Seedling dry weight (SDW). The dry weight of seedlings registered highly significant differences between the genotypes (Tab. 4), the results contrasted with those of Xavier *et al*. (2017), when seeds from different batches of the same cultivar were used. The dry weight was the same for cultivars C-001, C-Valledupar, C-Tierralta and IT86, but not with L-026, which is related to the loss of viability and lower growth, a situation that varies between species and cultivars within the same species, as argued by Shaheen *et al.* (2019).

Percentage of abnormal seedlings (PAS). Significant differences were detected in the number of abnormal seedlings (Tab. 4), and the results are consistent with that of Ferreira and Novembre (2016). The IT-86 genotype stood out with a single seedling, which might be related to its higher vigor and the hormonal balance in its seeds, allowing an adequate production of primary and secondary compounds (Elli *et al*., 2016). Likewise, it is associated with genetics, in such a way that cultivars with a greater number of abnormal seedlings lose their vigor due to the reduced activity of the enzyme's malate dehydrogenase, alcohol dehydrogenase, isocitrate lyase, esterase and superoxide dismutase (Carvalho *et al*., 2014). These enzymes are fundamental in the degradation of macromolecules, so that the seedling develops a root system capable of taking the nutrients from the soil (Carvalho and Nakagawa, 2012).

CONCLUSION

Prolonged storage generated a reduction in the physiological potential of the seeds in all cultivars, affecting vigor, due to the deterioration of the cell membrane and release of solutes, a situation that was confirmed by the methods of electrical conductivity and germination test, being the genotypes L-026 and C-Tierralta the most affected.

Conflicts of interest and authorship contributions: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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