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Research Paper

Physiological, morphological, and biochemical characterization of *Cratylia argentea* (Desv.) Kuntze seeds

Caracterización fisiológica, morfológica y bioquímica de semillas de Cratylia argentea *(Desv.) Kuntze*

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Abstract

Cratylia argentea is a shrub legume native to tropical regions of South America where it is used for animal feed and green manure. In the absence of germination guidelines, the key aim of this study was to define the most suitable temperature for conducting germination and accelerated aging tests. The biochemical attributes of seeds were also assessed. Seeds with 10 % moisture from 4 different seed lots were germinated using the between paper method in a germinator at temperatures of 20, 25, 30 and 35 °C and alternating temperatures of 20/30 °C (16/8 h), with daily counting until germination was stable (seven days without germination). For the accelerated aging test, two temperatures (41 and 45 °C) and six aging periods (0, 24, 48, 72, 96 and 120 h) for seeds with between 10–40 % moisture content were used. Carbohydrates (%), ethereal extract (%), crude protein (%) and macro and micronutrient contents of the seeds were measured. Results showed that *C. argentea* seeds consist predominantly of starch (22.67 %) and protein (26.45 %) reserves with a low percentage of lipids. For the germination test, the temperature of 30 °C is recommended, allowing greater percentage and speed of germination, with seedling evaluation at 10 and 20 days. For the accelerated aging test, aging for 48 h at 41 °C is recommended to discriminate *C. argentea* seed lots in terms of quality.

Keywords: Accelerated aging, dry environment plant, germination test, seed analysis, tropical shrub legume, unconventional forage crop.

Resumen

Cratylia argentea es una leguminosa arbustiva originaria de las regiones tropicales de América del Sur, donde se utiliza como alimento para animales y abono verde. En ausencia de pautas de germinación, el objetivo de este estudio fue definir la temperatura más adecuada para realizar pruebas de germinación y envejecimiento acelerado. También se evaluaron los atributos bioquímicos de las semillas. Semillas con 10 % de humedad de 4 lotes de semillas diferentes fueron colocadas entre hojas de papel toalla, en un germinador a temperaturas de 20, 25, 30 y 35 °C y alternando temperaturas de 20/30 °C (16/8 h), con conteo diario hasta que la germinación se estabilizó (siete días sin germinar). Para la prueba de envejecimiento acelerado se utilizaron dos temperaturas (41 y 45 °C) y seis períodos de envejecimiento (0, 24, 48, 72, 96 y 120 h) para semillas con un contenido de humedad entre 10–40 %. Se midieron carbohidratos (%), extracto etéreo (%), proteína cruda (%) y contenido de macro y micronutrientes de las semillas. Los resultados mostraron que

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las semillas de *C. argentea* contienen predominantemente reservas de almidón (22,67 %) y proteína (26,45 %) con un bajo porcentaje de lípidos. Para la prueba de germinación se recomienda la temperatura de 30 °C, que permite mayor porcentaje y rapidez de germinación, con evaluación de plántulas a los 10 y 20 días. Para la prueba de envejecimiento acelerado, se recomienda un envejecimiento de 48 h a 41 °C para discriminar los lotes de semillas de *C. argentea* en términos de calidad.

Palabras clave: Análisis de semillas, ensayo de germinación, envejecimiento acelerado, forrajes no convencionales, leguminosas tropicales, planta de ambiente seco.

Introduction

Cratylia argentea (Desv.) Kuntze is a shrub legume (Fabaceae) native to tropical regions of South America. It has multiple uses, especially as animal feed and as a green manure crop. It has a symbiotic relationship with nitrogen-fixing bacteria (Mattar et al. 2018) and is recommended for silvopasture systems (Valles-de la Mora et al. 2014). The use of *C. argentea* together with *Urochloa brizantha* has shown promise for feeding calves (F1 Holstein × Zebu) (Valles-de la Mora et al. 2017). The species has potential for control of parasitic diseases in ruminants (Silva et al. 2017), as well as having a low tannin concentration (Pereira et al. 2018). It shows potential for use as feed in swine production (Sarria and Martens, 2013).

Germination is the first step in the crop production cycle. Diverse intrinsic and extrinsic factors contribute to the success of seed germination, including initial physiological quality, hormonal balance, temperature and lighting (Lone et al. 2016; Ebone et al. 2019; Wu et al. 2020). Temperature is a key factor for germination because it determines the ecological limitations for geographic distribution and establishment of the species (Daibes and Cardoso 2018). Therefore, the definition of optimal temperature(s) for germination can be useful for expression of the maximum potential of a species, as has been observed in Fabaceae species such as *Diptychandra aurantiaca* (Oliveira et al. 2013), *Robinia pseudoacacia* (Giuliani et al. 2015) and *Calobota sericea* (Müller et al. 2019).

Seed vigor also effects seed quality and crop establishment in the field, governing the capacity of seeds to grow under different environmental conditions (Marcos-Filho 2015). Seed vigor can be defined as the sum of the properties of the seed that allows acceptable germination and the development of uniform seedlings under adverse conditions (Finch Savage and Bassel 2016). Tests can be used to estimate seed lot vigor, including the accelerated aging test, which is used to simulate the deterioration process by subjecting seeds to high temperatures and relative humidity over a defined period. It is an effective test for ranking seed lots for storage capacity and field emergence. Therefore, developing the correct methodology for this test in *C. argentea* is important for estimating the potential of seed lots, as has been observed in different species such as *Jatropha curcas* (Oliveira et al. 2014), *Leucaena leucocephala* (Araújo et al. 2017) and *Urochloa brizantha* (Oliveira et al. 2020).

Knowledge regarding the morphological aspects of seeds and seedlings in initial stages of development is relevant in development of standard methods and interpretation of results for the physiological analysis of seeds. Seed morphology is also useful to identify species under natural conditions (Abud et al. 2010). Knowledge of the chemical composition of seeds can assist in understanding the biochemical properties of the species and cellular processes involved in seed quality. Vaz Patto et al. (2015) report that the analysis of the mineral composition is also crucial when considering the nutritional quality of the seeds. Currently, this has been evaluated in different species, such as quinoa (Reguera et al. 2018), beans (Los et al. 2018) and watermelon (Lawal, 2011). These studies bring a new contribution to understanding seed germination and vigor in these species. Fang and Wang (2007) evaluated changes in biochemical composition and enzyme activity during dormancy release in *Cyclocarya paliurus* seeds. Information on lipid content is important because oilseeds generally have shorter longevity after harvest (Wiebach et al. 2019) related to lipid peroxidation during the deterioration process, being one of the main causes of loss of vigor in orthodox seeds (Ebone et al. 2019).

Information regarding seed quality, such as germination, vigor, morphology and chemical composition of *C. argentea* seeds is scarce. A better understanding of germination, storage, control of seed quality, species ecology, cultivation and commercialization can help producers develop better strategies for storage and planting as an alternative forage. This study aimed to define suitable temperature(s) for germination of *C. argentea* seeds and to characterize seed and seedling development, generating technical visual

support material. Other objectives were to chemically characterize seeds and define the best methodology for carrying out accelerated aging tests to discriminate seed lots with different physiological quality.

Materials and Methods

Seed sources for the study

Four different lots of manually harvested *C. argentea* seeds were used for the experiments. For the biochemical analyses, biometric characterization of seeds and classification of seedlings, a seed lot (approximately 0.5 kg of seeds) stored in a refrigerator at 10 ºC for 4 months post-harvest was obtained from the seed bank of Empresa Brasileira de Pesquisa Agropecuária (Embrapa Milho e Sorgo), Sete Lagoas, Minas Gerais, Brazil. For the emergence, germination, and accelerated aging tests, 4 different seed lots (approximately 1.8 kg of seeds each) were used (Table 1).

Characterization of seeds and development and classification of seedlings

The seeds were imbibed for 24 h on paper towel moistened with 2.5 times the weight of the dry paper with water and covered with a sheet of paper towel and rolled. After imbibition, seeds were cut longitudinally to use for the photographic record. The dimensions of 50 seeds were measured with a caliper and width (mm), length (mm) and thickness (mm) were recorded. An image was taken to illustrate seed structure.

A sample of 45 seeds was sown in sand in a polyethylene tray and maintained for 15 d at 25 °C. Three seeds were removed daily for observation. Some seeds were kept intact for external observation while others were cut longitudinally for internal observation of seedling development. A succession of images was taken daily of the individual seeds to track seedling emergence and development over a 15 d period. The seedlings were grouped into "normal" and "abnormal" and photographed independently as a reference set (Brasil 2009).

Seed biochemical analyses

A preliminary germination test using the methodologies described above conducted 90 days after harvest, showed that seed lot 3 had a germination of 89 % and emergence in sand of 91 % and considered to be of high physiological quality and suitable for the analysis. Carbohydrates were quantified by the methodology of Thompson (1990), ethereal extract and protein by the methodology of Brasil (2009) and fatty acids by the methodology of the Associations of Official Analytical Chemists (AOAC 2005). Seed samples were ground in a Wiley mill, passed through a 0.5 mm sieve, and subjected to nitric-perchloric acid digestion (Alvarez et al. 2001). Phosphorus (P) was determined by colorimetry, potassium (K) by flame emission photometry, calcium (Ca) and magnesium (Mg) by atomic absorption spectrometry, nitrogen (N) by titration after digestion by the Kjeldahl method, and sulfur (S) by turbidimetry (Alvarez et al. 2001).

Physiological characterization of the seed lots

A seedling emergence test in sand was conducted in a greenhouse. Four replications of 50 seeds were washed and sown in sterilized sand in polyethylene boxes. The sand was moistened to 60 % of water retaining capacity. Normal seedlings were counted daily until stabilization of emergence, defined as 7 d without emergence, for calculation of percentage of seedling emergence. After stabilization of emergence, the following variables were obtained:

Emergence speed index (ESI) according to the equation proposed by Maguire (1962):

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \ldots + \frac{En}{Nn}$$

where:

E1, E2, En = number of emerged seedlings at the first, second and last count;

N1, N2, Nn = number of days of sowing to the first, second and last count.

Table 1. *C. argentea* seeds used for the analyses.

Lot	Lot origin	Other information
1	Santa Rita Farm of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Prudente de Morais (MG), Brazil.	Seeds stored for 4 months after harvest in a refrigerator at 10 °C
2	From the private property of Caetanópolis (MG), Brazil.	Ibid
3	Embrapa Milho e Sorgo in Sete Lagoas (MG), Brazil	Ibid
4	Embrapa Milho e Sorgo in Sete Lagoas (MG), Brazil	Seeds stored for 16 months after harvest in paper bags at room temperature (mean of 22 °C day and 17 °C night).

Emergence speed (ES) according to the equation proposed by Edmond and Drapala (1958):

$$ES = \frac{[(N1E1) + (N2E2) + \ldots + (NnEn)]}{E1 + E2 + \ldots + En}$$

where:

E1, E2, En = number of emerged seedlings at the first, second and last count;

N1, N2, Nn = number of days of sowing to the first, second and last count.

Shoot dry matter (SDM) and root dry matter (RDM) of seedlings, length of the main root (RL) and shoot length (SL) were measured. Shoot height and main root length were measured for each seedling obtained in the emergence test using a caliper and the results were expressed in cm/seedling. After measurement, seedlings obtained from each replication of the last count of the emergence test were placed in paper bags and dried in a forced air circulation oven at a temperature of 65 ºC for 72 h. After this period, the material was weighed on a precision balance, with the results expressed in mg/seedling.

Germination at different temperatures

Five replications of 40 seeds of each lot were treated with fungicide Protreat® at a concentration of 200 mL/100 kg of seeds and each replicate placed on two sheets of paper towel moistened with 2.5 times the weight of the dry paper with water and covered with a sheet of paper towel and rolled. The rolls of paper were kept in seed germinators at constant temperatures of 20, 25, 30 and 35 °C (with a photoperiod of 8 h) and at alternating temperatures of 20/30 °C (8h at 30 °C light/16h at 20 °C dark) until stabilization of germination of all treatments (around 30 days). For the constant temperatures (20, 25, 30 and 35 °C), the rolls were maintained in a germination chamber (Mangelsdorf) and for the alternating temperatures (20/30 °C), the seeds were placed in plastic bags and kept in a BOD (Biochemical Oxygen Demand) incubating chamber without supply of oxygen. The BOD was used for alternating temperatures because it changes temperatures and light regimes automatically. The use of plastic bags prevents the loss of water and makes it possible to compare the results with constant temperatures conducted in a germination chamber. Evaluations of the number of normal seedlings were performed daily up to stabilization of the number of normal seedlings germinating.

For all treatments, on the date of final count, the final percentage of normal seedlings was calculated. The ESI and ES were calculated based on the number of normal seedlings obtained daily (as described above for the emergence test), according to the equations of Maguire (1962) and Edmond and Drapala (1958), respectively.

Accelerated aging test

Five replications of 40 seeds of each lot were placed on suspended metal screens inside plastic boxes (gerboxes) containing 40 mL of distilled water at the bottom. Lids were placed on the boxes and they were kept in BOD (Biochemical Oxygen Demand) incubators regulated at two temperatures (41 and 45 °C) for six periods of aging (0, 24, 48, 72, 96 and 120 h) (Araújo et al. 2021). After each period, a germination test was conducted on the seeds as described above, using a constant temperature of 25 °C with evaluations at 10 days after sowing. The number of normal seedlings, abnormal seedlings and dead seedlings were counted and percentages calculated. After seed aging, moisture content was determined according to the method described in Brasil (2009).

Experimental design and statistical analysis

The experiments were conducted in a completely randomized design. The replicates were randomly distributed inside the germinators and BODS, and all the temperatures were conducted simultaneously. The germination and accelerated aging tests were conducted in a double factorial function. In the germination test, one factor corresponded to the seed lots and another to the temperatures. In the accelerated aging test, one factor corresponded to the seed lots and another to the aging periods. Analysis of variance was conducted on the data. The means were compared by the Tukey test at 5 % probability with use of the R software (R Core Team 2020).

Results

Biochemical analyses of the seeds

C. argentea seeds consist of predominantly starch and proteins as reserves and have a low percentage of lipids (Table 2).

The nutritional composition was nitrogen (5.59 dag/kg), phosphorus (0.38 dag/kg), potassium (0.56 dag/kg), calcium (0.13 dag/kg), magnesium (0.18 dag/kg), sulfur (0.21 dag/kg), copper (9.90 mg/kg), iron (58.80 mg/kg), zinc (39.95 mg/kg), manganese (25.30 mg/kg) and boron (9.07 mg/kg).

Table 2. Biochemical composition of *C. argentea* seeds.

Analyses	Unit	Results
Crude protein	%	26.45
Ethereal extract	%	0.74
Starch	%	22.67
Palmitic acid (C16:0)	%	0.07
Stearic acid (C18:0)	%	0.02
Elaidic acid (C18:1n9c)	%	0.11
Linoleic acid (C18:2n6c)	%	0.21
Linolenic acid (C18:3n3)	%	0.12
Polyunsaturated fats	%	0.33
Trans fats	%	0.00
Monounsaturated fats	%	0.11
Saturated fats	%	0.12
Unsaturated fats	%	0.44
Fructose	mg/kg	<50.001
Raffinose	mg/kg	<50.00
Maltose	%	<50.00
Free glucose	%	0.06
Lactose	mg/kg	<50.00
Total carbohydrates	%	41.46
Fucose	mg/kg	<50.00
Arabinose	mg/kg	<50.00
Galactose	%	2.72
Glucose	%	31.17
Xylose	%	1.71
Rhamnose	%	5.86
Mannose	mg/kg	<50.00
Sucrose	%	1.00
Free fructose	%	0.01
Butyric acid (C4:0)	%	0.02

Carbohydrate results expressed as <50.00 are below the limit of quantification.

Characterization of seeds, seedling development and classification

The *C. argentea* seeds sampled had a mean width of 9.5 mm, mean length of 11.35 mm, and mean thickness of 3.16 mm. Based on the observations, the seeds were considered exalbuminous, where there is no endosperm and the cotyledons comprise the key storage organ. The cotyledons are greenish-white (Figure 1A) and externally, the seeds have a smooth brown seed coat and a visible hilum (Figure 1A-b). There is also a caruncle, a whitened tissue in the region of the hilum (Figure 1A- a and b). The longitudinal section in the central part of the seed revealed the hypocotyl-radicle axis located in the region near the hilum and the cotyledons (Figure 1A-c).

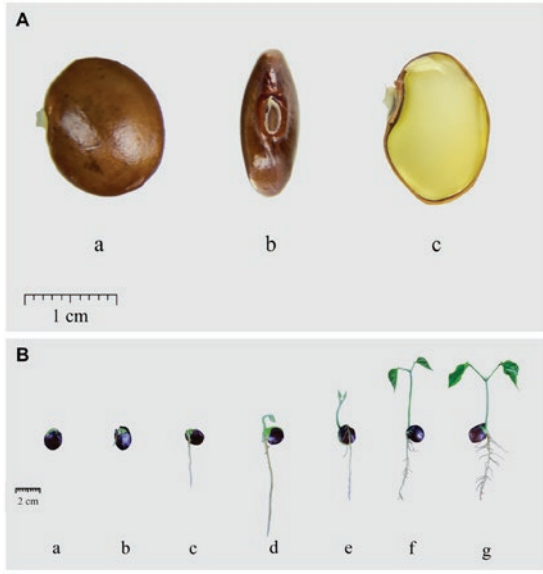

Figure 1. A. Seed in lateral (a), frontal (b), and internal (c) perspectives. B. Development of the *C. argentea* seedling: Root emergence at one to two days after sowing (a); development of the primary root from three to five days after sowing (b-c); normal seedling with formation of secondary roots, opening of the cotyledons, epicotyl growth and leaf primordia at approximately six days after sowing (d); from seven to eight days after sowing (e): from 10 to 11 days after sowing (f); and from 12 to 13 days after sowing (g). Source: own elaboration.

Germination began by day 2 after sowing at room temperature (Figure 1B-a). From 3 to 5 days (Figure 1B-b and c), the primary root elongated and from day 6 on, secondary root formation began. Growth of the epicotyl to around 2 cm length was also seen at this stage (Figure 1B-d). Plumules or leaf primordia appeared at the tip of the epicotyl and a normal seedling can already be observed (Figure 1B-d). Normal seedlings obtained on days 7 and 8 are also shown (Figure 1B-e) with a more developed epicotyl (around 3 cm). At 10 and 11 days (Figure 1B-f) and at 12 and 13 days (Figure 1B-g), the plumules or already developed primary leaves can be seen. Germination is hypogeal, with the cotyledons remaining under the soil surface. No dormancy was identified in the seeds. The normal seedlings (Figure 2) consist of a primary root, along with the emergence of secondary roots, and upright or slightly curved hypocotyl, with two primary leaves at the upper tip. A well-developed primary root was also observed, an important factor for development of a vigorous plant in the field.

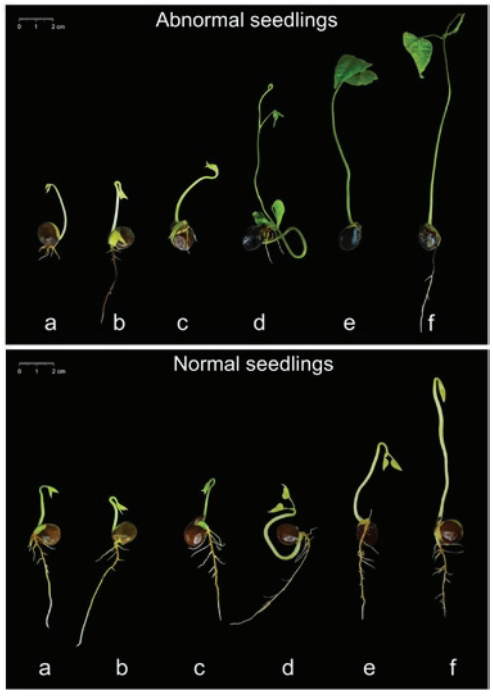

Figure 2. Abnormal *C. argentea* seedlings: poorly developed root system (a, c, e); damaged root system (b, f); malformation of the root and shoot system (d). Normal *C. argentea* seedlings: all essential structures present and good root-shoot ratio (a, b, c); curvature in the hypocotyl without affecting formation of a normal seedling (d); slightly less developed root system, but without affecting formation of a normal seedling (e, f).

Physiological characterization of the seed lots

The 4 seed lots exhibited significant differences in initial physiological quality. Lots 1 and 3 exhibited 89 and 94 % of emergence, respectively; being higher than lots 2 (75 %) and 4 (62 %) (Table 3). In general, seedling emergence began on day 9 after sowing and stabilized on day 30. Lots 1 and 3 exhibited superior values of ESI, indicating a mean number of approximately 4 emerged seedlings per day. For lots 2 and 4, these values were approximately 3 seedlings. For ES, higher values are found for lot 4 (16 days), i.e. lower physiological quality compared to the other lots. Lot 1 exhibited a lower value (around 3 days) than the others, confirming the faster germination of this lot (Table 3).

Higher values for RDM were observed for lots 2 and 3, which were superior to lot 4, but did not differ significantly from lot 1. For RL, there was no significant difference among the lots. SDM and SL showed a higher value for lot 3, which was significantly superior to lot 4 (Table 3).

Germination at different temperatures

Germination percentage (%GERM), germination speed index (GSI), and germination speed (GS) at the different temperatures were similar for lots 1, 2 and 3 (Table 4).

For these seed lots, there was no difference in %GERM at the temperatures of 20, 25, 30 and 20/30 °C, but significant reduction at 35 °C. Lot 4 was less vigorous and germination was superior at the temperatures of 30 and 20–30 °C, followed by the temperatures of 20 and 25 °C, with the lowest value at the temperature of 35 °C (Table 4 and Figure 3).

The GSI and the GS under the different temperature conditions generally showed a similar pattern in the four lots analyzed. The GSI, which is based on the mean number of seeds germinated per day, was higher at 30 °C, followed by 25 and 20/30 °C, which were higher than 20 and 35 °C (Table 4). For GS, the lowest value found was at 30 °C, confirming faster germination at that temperature.

Accelerated aging

The accelerated aging test showed that 41 °C allowed a less drastic reduction in germination (Table 5). At 45 °C there was significantly reduced germination, with germination reduced to 0 % after 48 h in lots 1 and 4. This was observed as lower percentages of normal seedlings (NS1 and NS) and higher percentages of dead seeds (DS). Analyzing the results of the combinations of temperatures and periods of seed exposure to aging, at the temperature of 41 °C at the times of 48 h, 72 h

Table 3. Characterization of physiological potential of seeds from four lots of *C. argentea* using the emergence test in sand.

Lot	E (%)	ESI	ES (days)	RDM (mg/seedling)	SDM (mg/seedling)	RL (cm/seedling)	SL (cm/seedling)
1	89a	3.78a	12.02c	2.93ab	9.56ab	11.61a	6.52ab
2	75b	2.73b	14.60b	3.34a	8.07bc	11.33a	6.75ab
3	94a	3.51a	13.80b	3.48a	10.24a	11.25a	7.29a
4	62b	2.08c	16.02a	2.27b	11.25a	11.09a	6.23b

E = total emergence (%); ESI = emergence speed index; ES = emergence speed; RDM = root dry matter; SDM = shoot dry matter; RL = root length; SL = shoot length (cm/seedling). Mean values followed by the same lowercase letters in the columns do not differ from each other according to the Tukey test at 5% probability.

Table 4. Germination percentage (%GERM), germination speed index (GSI) and germination speed (GS) of four seed lots of *C. argentea* incubated at different temperatures (°C).

Variable	(°C)	Lot 1	Lot 2	Lot 3	Lot 4
%GERM	20	90abA	81bA	93aA	37cB
	25	89aA	83aA	93aA	46bB
	30	89aA	87aA	92aA	58bA
	35	29aB	29aB	27aB	4bC
	20/30	93aA	84aA	88aA	58bA
GSI	20	3.16aC	2.45bC	2.91abC	0.92cC
	25	4.74aB	3.60bB	4.27abB	1.83cB
	30	7.04aA	5.73bA	6.42aA	3.77cA
	35	1.83aD	1.67aD	1.59aD	0.15bD
	20/30	4.37aB	3.39bB	3.95abB	2.09cB
GS	20	14.67cA	17.96bA	16.24bcA	23.25aA
	25	9.60bBC	12.61abBC	11.15abB	13.14aB
	30	6.51aC	8.55aD	7.45aC	8.20aC
	35	8.07bBC	9.49bCD	8.79bBC	14.62aB
	20/30	10.84bB	13.30abB	11.57bB	14.76aB

Mean values followed by the same uppercase letters in the columns (among temperatures) and the same lowercase letters in the rows (among lots) do not differ from each other according to the Tukey test at 5% probability.

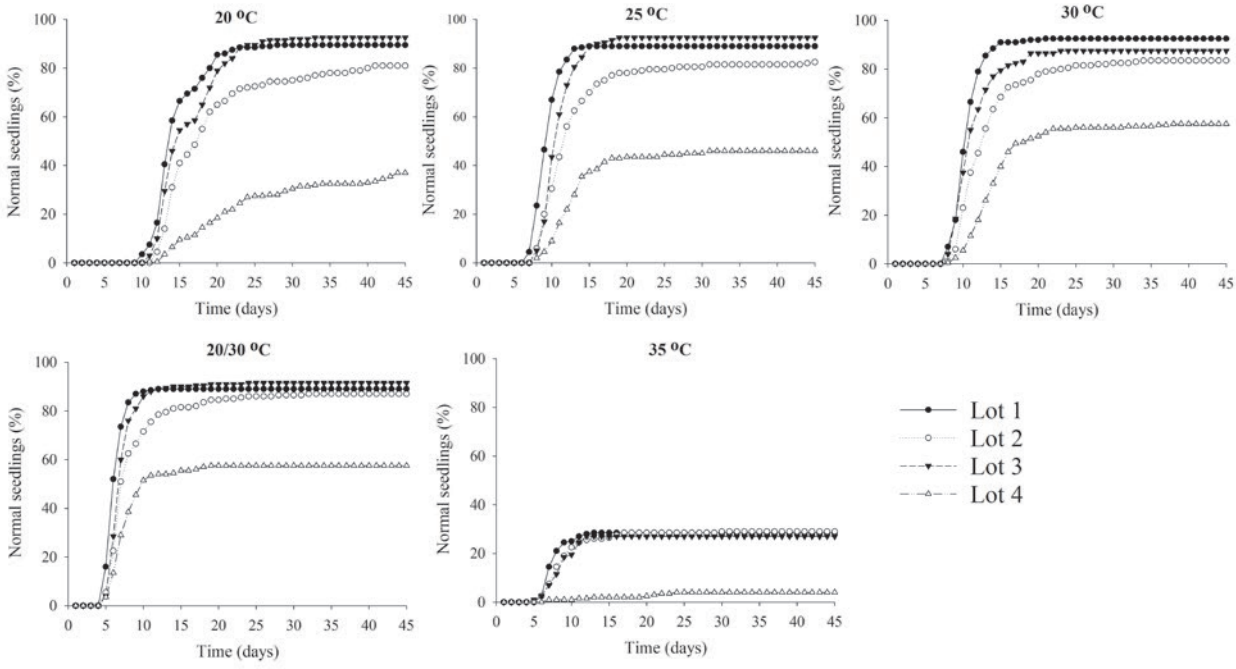

Figure 3. Germination curves of four lots of *C. argentea* seeds at different temperatures. The curves represent 5 replicates of 40 seeds of each of 4 seed lots.

and 96 h, seed lots 2 and 3 maintained their germination rates at 0 h (control) and 24 h. However, for lots 1 and 4, there was a significant reduction in germination of the seeds that had been placed under accelerated aging under these conditions. There were increases of up to 30 % in seed moisture content with the aging times at both temperatures (41 and 45 °C). However, the variation in seed moisture content was generally low (around 3 percentage points) among different lots at the same temperature.

Table 5. Normal seedlings at first count (NS1), normal seedlings (NS), dead seeds (DS) and moisture content (MC) in 4 seed lots of *C. argentea* not under the accelerated aging test (0 h; control) and under the accelerated aging test at two temperatures (41 and 45 °C) and five aging times (24, 48, 72, 96 and 120 h).

Variable	Lot	Conventional test	Accelerated aging tests									
		0 h (control)	41 °C					45 °C				
			24 h	48 h	72 h	96 h	120 h	24 h	48 h	72 h	96 h	120 h
NS1(%)	1	27.0a*	35.5abA	26.5aAB	34.0aA	20.0cB	17.0aB	29.0abA	17.5abB	0.0cC	0.0C	0.0aC
	2	18.5a	25.5bBC	29.5aB	44.5aA	47.5aA	14.0abC	21.5bA	20.5aA	9.0bB	0.0C	0.0aC
	3	16.5ab	37.5aA	29.5aAB	35.5aAB	33.0bAB	24.0aB	32.5aA	19.5aB	21.5aB	3.5C	0.5aC
	4	6.5b	25.0bA	18.0aAB	17.5bABC	8.0dBC	5.0bC	0.0cB	10.5bA	4.5bcAB	0.5B	0.0aB
CV(%)		30.01			24.07					43.39		
NS(%)	1	70.5a	72.5aA	48.5bB	52.5bB	26.5bC	19.0bcC	61.0bA	25.5bB	0.5cC	0.0aC	0.0aC
	2	72.0a	72.5aA	65.5aA	71.0aA	75.5aA	24.0bB	61.0bA	51.0aA	36.0aB	0.0aC	0.0aC
	3	72.0a	71.0abA	63.0aAB	68.0aA	66.5AB	53.5aB	71.5aA	60.5aB	44.0aC	5.0aD	1.0aD
	4	39.0b	60.0bA	24.0cB	28.0cB	10.0cC	8.0cC	0.5cB	16.0bA	13.5bA	0.5aB	1.0aB
CV(%)		10.53			13.63					23.06		
DS(%)	1	7.5b	11.0abC	17.5bC	16.0bC	32.0bB	53.0aA	13.0bD	44.0aC	75.5aB	95.5aA	96.5aA
	2	9.0b	8.0abB	8.0bcB	12.0bcB	8.5cB	34.0bA	7.5bC	21.0bB	27.0cB	96.0aA	98.5aA
	3	4.5b	3.5bA	3.5cA	5.0cA	8.0cA	9.5cA	6.0bC	8.0cC	24.0cB	59.0bA	72.0bA
	4	23.5a	16.0aC	30.0aB	28.5aB	48.5aA	41.0bA	54.0aB	45.0aB	55.5bB	85.0aA	83.0bA
CV(%)		21.55			27.25					12.29		
MC (%)	1	9.9	12.9	38.9	35.8	28.6	40.9	14.4	37.1	26.8	22.8	42.6
	2	10.4	13.3	33.1	26.9	28.0	41.5	14.3	30.0	32.5	30.1	41.9
	3	10.1	9.4	31.5	28.3	24.4	39.7	13.9	34.1	29.7	26.6	43.8
	4	10.3	10.3	22.7	31.7	27.2	42.5	17.0	38.6	31.5	30.9	41.4

*Mean values followed by the same lowercase letters in the columns (among lots) and the same uppercase letters in the rows (among aging times) do not differ from each other according to the Tukey test at 5% probability.

Discussion

C. argentea seeds were found to have protein-carbohydrate reserves, following the general pattern of Fabaceae (Marcos-Filho 2016). The low concentration of lipids observed in *C. argentea* seeds is favorable for longevity because lipids have lower chemical stability compared to other reserve components due to the lipid peroxidation process (Parkhey et al. 2012). The high concentration of proteins is an important factor for development of the embryonic axis and formation of the seedling (Verma et al. 2015; Finch-Savage and Bassel 2016). However, proteins have high affinity with water, which contributes to reduction in storage potential (Marcos-Filho 2016). For that reason, storage is recommended under controlled temperature and relative humidity conditions (Ramos et al. 2003). The composition of *C. argentea* seeds is similar to the composition of pea (*Pisum sativum*) seeds with a similar quantity of proteins and a low percentage of lipids (Marcos-Filho 2016).

Seeds of *C. argentea* did not show dormancy as previously observed by Montoya et al. (2009), who evaluated the effects of cryopreservation in *C. argentea* seeds. These authors observed a high initial physiological quality of seeds and lack of physical or physiological dormancy which drastically reduced germination during ambient storage conditions. Although the tegument hardness may be responsible for dormancy and contribute to longevity during seed storage, from a practical point of view these results confirm that planting can be carried out directly after harvest. In legume species (such as *C. argentea*) the permeability of the seed coat can be related to some pigments, such as proanthocyanidins (Smýkal et al. 2014). According to these authors, the seed coat provides not just structural and protective functions,but has a decisive role in the timing of seed germination by regulating water uptake. Another aspect to be considered is the seed size. *C. argentea* seeds are generally larger than the seeds of other tropical legumes of economic importance, such as *Leucaena leucocephala*, *Crotalaria* species, *Flemingia macrophylla*, *Tephrosia vogelii*, *Stylosanthes* species, *Calopogonium mucunoides* and tropical kudzu (*Pueraria phaseoloides*). *C. argentea* seeds have considerable nutrient reserves (as observed for starch and proteins), which are important characteristics for ensuring greater survival in the field. Seed size can be related to a higher amount of nutrients, as observed in bean seeds (Perin et al. 2002). These authors stated that large seeds increased the plant height, the leaf area index and the shoot and root biomass. Plants originating from large seeds accumulated more N and K in shoots and roots. Specific work on the relationship of seed size and nutrients should be conducted with seeds of *C. argentea* to clarify this.

The germination test is fundamental for determining the value of seeds for sowing and vigour of different seed lots (Marcos-Filho 2016). Although the seed lots exhibited high germination percentages, the constant temperatures of 20 and 25 °C and alternating temperatures of 20/30 °C led to lower GSI compared to the constant temperature of 30 °C. Therefore, the constant temperature of 30 °C can be considered better for germination of *C. argentea* seeds. These results indicate *C. argentea* is a truly tropical species and best suited to sowing in the summer months if grown in the subtropics. Based on the germination curve at this temperature, day 10 can be defined as ideal for the first germination count (attaining 50 % of normal seedlings) and day 20 for final evaluation of germination (total stabilization of germination) for the 4 seed lots analyzed. The temperature of 35 °C led to low germination percentages in all the seed lots analyzed. Temperatures above the ideal during germination can cause thermoinhibition, with related oxidative stress and protein degradation (Liu et al. 2015; Mittler 2017). These apparent deleterious effects were more pronounced in lot 4, which was related to the initial lower germination capacity of low vigor seeds. *C. argentea* is present in the Amazon, Cerrado, and Caatinga biomes (Queiroz and Coradin 1996) and these results were similar to other tree species collected from the same region in Brasil (Brancalion et al. 2010). This suggests that germination tests for Brazilian tree species from the Cerrado and Atlantic Forest should be conducted at 25 °C and from the Amazon at 30 °C.

Accelerated aging at 41 °C for 48 h, 72 h and 96 h effectively discriminated between seed lots of different vigor status with lots 2 and 4 showing lower vigor. At high temperatures, respiratory rates are increased, causing higher reactive oxygen species (ROS) production (such as hydrogen peroxide and superoxide anion), which are free radicals produced during normal metabolism and are involved in enzymatic reactions, mitochondrial electron transport and signal transduction (Mittler 2017). When above the basal levels, they cause damage, such as protein denaturation and lipid peroxidation (Mittler 2017; Ebone et al. 2019). It is important to highlight the difference in seed moisture content in the accelerated aging test, especially when comparing 24 and 120 hours of exposure. Along with high temperatures, high humidities accelerate seed respiration and contribute to cellular changes relating to seed deterioration (Shu et al. 2020; Pinheiro et al. 2021).

This is evident when observing the significant reduction of normal seedlings and significant increase of dead seeds mainly in 96 and 120 h aging times.

The observed results were similar to results with seeds of *L. leucocephala*, where the accelerated aging test conducted at 41 °C for 96 h was efficient in differentiating seed lots (Araújo et al. 2017). Araújo et al. (2021) concluded the accelerated aging test with saturated NaCl solution (76 % RH) conducted at 41 °C for 48 h is effective for evaluation of chickpea seed vigor. These test conditions can be used to identify differences in storage potential and seedling emergence in the field between seed lots (Marcos-Filho 2016). Although 48, 72 and 96 h at 41 °C were all efficient for the accelerated aging test, considering the practical implications, the time of 48 h is recommended for *C. argentea* seeds because it provides faster results.

Conclusions

Temperature of 30 °C with the first germination count at 10 days and final germination count at 20 days is recommended for conducting germination tests of *C. argentea*. The combination of the temperature of 41 °C with the period of exposure of 48 h is recommended for conducting the accelerated aging test for this species. *C. argentea* seeds are exalbuminous, have hypogeal germination and do not exhibit seed dormancy. *C. argentea* seeds predominantly accumulate starch and protein as reserves and contain a low percentage of lipids. All this information is important to optimize the use of the species as a forage.

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