

Optimal temperature for germination and seedling development of cowpea seeds

Temperatura óptima para la germinación y el desarrollo de plántulas de semillas de frijol caupí

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Diversity the cowpea cultivars
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ABSTRACT

Temperature is one of the climatic elements that affect most the germination process and seedling development. Thus, the objective of this study was to evaluate the germination and seedling development of cowpea cultivars under increasing temperature. The seeds of the cultivars Acauã, Carijó, Guariba, Gurguéia, Itaim, Juruá, Pajeú, Potengi, Pujante, Rouxinol, Tapahium, and Tumucumaque were kept in germination chamber at temperatures of 20, 25, 30, 35 and 40°C, with photoperiod of 12 h. Cowpea seeds have optimal germination performance and seedling development at temperatures in the range of 30.49 - 35.48°C. The cultivars presented germination percentage above 91% under 40°C, however, the seedling were abnormal. The temperature of 20°C provided germination above 96%, but with a longer period to germinate. The temperature range between 30 - 35°C favored the germination speed index, average time, and seed germination speed,

with optimum temperatures varying between the cultivars. The highest rate of normal seedlings was observed at temperatures between 25 and 35°C.

Additional key words: germination; seedling development; thermal stress; *Vigna unguiculata*.

RESUMEN

La temperatura es uno de los elementos climáticos que afectan la mayoría del proceso de germinación y el desarrollo de las plántulas. Por lo tanto, el objetivo de este estudio fue evaluar la germinación y el desarrollo de plántulas de los cultivares de frijol caupí a temperatura creciente. Para este propósito, las semillas de los cultivares Acauã, Carijó, Guariba, Gurguéia, Itaim, Juruá, Pajeú, Potengi, Pujante, Nightingale, Tapahium y Tumucumaque se mantuvieron en la cámara de germinación a temperaturas de 20, 25, 30, 35 y 40°C, con fotoperiodo de 12 horas. Las semillas de frijol caupí tienen un rendimiento óptimo de germinación y desarrollo de plántulas a temperaturas en el rango de 30.49 - 35.48°C. Los cultivares presentaron un porcentaje de germinación superior al 91% por debajo de 40°C, sin embargo, no se observó formación de plántulas normales. La temperatura de 20°C proporcionó una germinación superior al 96%, pero con un período más largo para germinar. El rango de temperatura entre 30 y 35°C favoreció el índice de velocidad de germinación, el tiempo promedio y la velocidad de germinación de la semilla, con temperaturas óptimas que varían entre los cultivares. La tasa más alta de plántulas normales se observó a temperaturas entre 25 y 35°C.

Palabras clave adicionales: germinación; desarrollo de plántulas; estrés térmico; *Vigna unguiculata*.

INTRODUCTION

The cowpea (*Vigna unguiculata* (L.) Walp.), is a legume of great importance, not only in Brazil but also for other countries, especially in the African continent. In recent years, this crop presented advances in the national and international context, with increased production and market expansion (Vale *et al.*, 2017). According to data from the Brazilian National Supply Company - CONAB (2019), in the 2018/2019 crop year, cowpea occupied an area of 1,327.5 thousand hectares in Brazil, with an estimated production of 651.8 thousand tons.

However, even with the cowpea importance, its yield is still considered low in Brazil. This low yield is associated with inadequate crop management and climatic conditions (Saboya *et al.*, 2013). Temperature, for example, is one of the most influential climatic elements on crops, as it can directly affect plant growth and yield, seed germination and can also reduce seedling growth (Martinez *et al.*, 2015).

Studies conducted by the Intergovernmental Panel on Climate Change (IPCC, 2013) showed that the average atmosphere temperature increased around 0.85°C from 1880 to 2012. Additionally, climate change scenarios also point to an increase in average temperature of the planet from 2.6 (most optimistic scenario) to 4.8°C (most pessimistic scenario), until 2100. Experts say that this increase will not be uniform, occurring inter-annual and regional variations, lead to extreme climatic events, such as droughts and heavy rainfall (IPCC, 2013). According to Vale *et al.* (2017) the cowpea development occurs in a wide temperature range between 18 and 37°C. However, the Brazilian Legislation for seed analysis indicates a temperature of 25°C as optimal for seed germination (Brazil, 2009). Thus, the predicted increase in temperature may cause problems for the seed germination.

Djanaguiraman *et al.* (2018) report that plants will not only be exposed to higher average temperatures, but also to more frequent short episodes of high temperatures. According to the authors, arid and semi-arid regions are more vulnerable to climate change and extreme temperature events. Thus, vigorous seed germination and uniform seedling establishment are highly desirable, especially in these regions (Parmoon *et al.*, 2015).

The early phase of plant life is considered one of the most important, as plant establishment will depend on the ability of seeds and seedlings to resist to adverse environmental conditions (Melo Junior *et al.*, 2018). Each species has a minimum, maximum, and optimal temperature for germination, and these cardinal temperatures characterize the limit over which germination of a particular species can occur (Mesgaran *et al.*, 2017; Felix *et al.*, 2018). The optimal temperature for maximum seed germination tends to differ between crops, and it is important to identify seed tolerance to high temperatures (Motsa *et al.*, 2015). According to this and to the future scenario of climate change, the selection of plants tolerant to temperature increase is of great importance, especially during the germination process, since it will contribute to the establishment of seedlings

and to uniformity of the crop. Thus, the objective of this study was to evaluate the germination and seedling development of cowpea cultivars under increasing temperature.

MATERIALS AND METHODS

The experiment was carried from April to May 2018. Germination tests were performed in BOD (Biochemical Oxygen Demand) chambers, manufactured by Eletrolab Equipamentos para Laboratório, located in Brazil, equipped with white fluorescent lamps, Philips 20 W. The experiment was carried out in a completely randomized design, with four replicates of 50 seeds, in a 12×5 factorial scheme, with twelve cowpea cultivars (Acauã, Carijó, Guariba, Gurguéia, Itaim, Juruá, Pajeú, Potengi, Pujante, Rouxinol, Tapahium, and Tumucumaque) which were stored in an air-conditioned place, which reduced changes in seed quality such as dormancy, moisture content and germination percentage (Brazil, 2009) and five temperatures (20, 25, 30, 35, and 40°C), with light regime of 12/12 h (dark/light), respectively.

Initially a superficial disinfection of the seeds was carried out using a fungicide of the Alkylfenobis (dithiocarbamate) chemical group in a ratio of 4 g (fungicide) to 1000 g of seeds. The seeds were arranged in a paper roll moistened with distilled water, at a ratio of 2.5 times the dry paper weight. Germinated seeds were counted daily for a period of 12 d, with the root protrusion equal to or greater than 2 mm as a parameter. After this period, normal and abnormal seedlings were counted, according to the rules for seed analysis (Brazil, 2009).

Germination percentage and kinetics indexes estimated were: The parameters evaluated were: germination percentage (G), germination speed (VG), germination speed index (GSI) and average germination time (AGT) using equations 1 to 4 respectively. For review e instructions on how to apply and calculate these indexes see Santana and Ranal (2000); Santana and Ranal (2004) and Ranal *et al.* (2009). Germination percentage (G) was obtained by the daily counting of germinated seeds (Eq. 1). The average germination time (AGT) refers to the sum of the number of germinated seeds multiplied by the incubation time in days divided by the sum of germinated seeds per day (Eq. 2). Germination speed (GS) was calculated by the inverse of the average germination time by daily counting (Eq. 3). The germination speed index (GSI) was calculated from the sum of the number of germinated seeds per day, divided by the respective number of days elapsed from

sowing, corresponding to the number of germinated seeds over time, expressed in seed / day (Eq. 4).

$$G = \frac{\sum_{i=1}^k ni}{A} * 100 \quad (1)$$

$$TMG = \frac{\sum_{i=1}^k ni.ti}{\sum_{i=1}^k ni} \quad (2)$$

$$VMG = \frac{\sum_{i=1}^k ni}{\sum_{i=1}^k ni.ti} \quad (3)$$

$$IVG = \sum_{i=1}^k \frac{Ni}{ti} \quad (4)$$

In which K is last day of observation, A total number of seeds placed to germinate, ni non accumulated number of germinated seeds, ti number of days, Ni accumulated number of germinated seeds.

Were considered normal seedlings those with potential to continue their development and generate normal plants, with developed shoot and root system, while abnormal seedlings were considered those with absent or damage of any of their essential structures (shoot and root system), according to the Brazilian Legislation for seed analysis (Brazil, 2009).

Data were submitted to variance analysis (ANOVA) and means compared by Scott Knott's test at 5% of significance using the software Sisvar v. 5.6 (Ferreira, 2011). The averages obtained for germination and germination speed index were compared by regression analysis.

RESULTS AND DISCUSSION

For all evaluated parameters: germination percentage (G%) germination speed index (GSI), average germination time (AGT), average germination speed (AGS), normal (NS) and abnormal (AS) seedlings. The variance analysis presented significant F values for cultivar, temperature and for the interaction cultivar $\times$ temperature (Tab. 1).

Table 1. Summary of variance analysis by mean square for germination percentage (G), germination speed index (GSI), average germination time (AGT), average germination speed (AGS), and percentage values of normal (NS) and abnormal (AS) seedlings of different cowpea (*Vigna unguiculata*) cultivars, submitted to temperature increase.

Variation source	Mean square						
	DF	G%	GSI	AGT	AGS	NS	AS
Temperature (T)	4	76.93**	5013.06**	9.26**	1.71**	39581.67**	36690.60**
Cultivar (C)	11	12.34**	122.65**	0.32**	0.05**	2602.84**	2537.27**
T × C	44	5.55**	50.314**	0.12**	0.02**	3199.53**	3210.55**
Residue	180	2.53	5.64	0.014	0.0023	90.83	97.18
CV%		1.61	7.2	6.84	7.7	19.3	19.96

DF = degree of freedom; CV = coefficient of variation; ns = not significant, ** significant at 1% of probability according to Scott-Knott's test.

The increase in temperature did not affect the germination of cowpea seeds, and the twelve cultivars presented germination percentage above 91% for all temperatures. Significant difference was observed only between the cultivars Acauã, Carijó, Gurguéia, Pujante and Rouxinol maintained at 40°C, where the lowest germination rate was observed in the seeds of the cultivars Acauã and Gurguéia, with germination of 93 and 91%, respectively (Fig. 1).

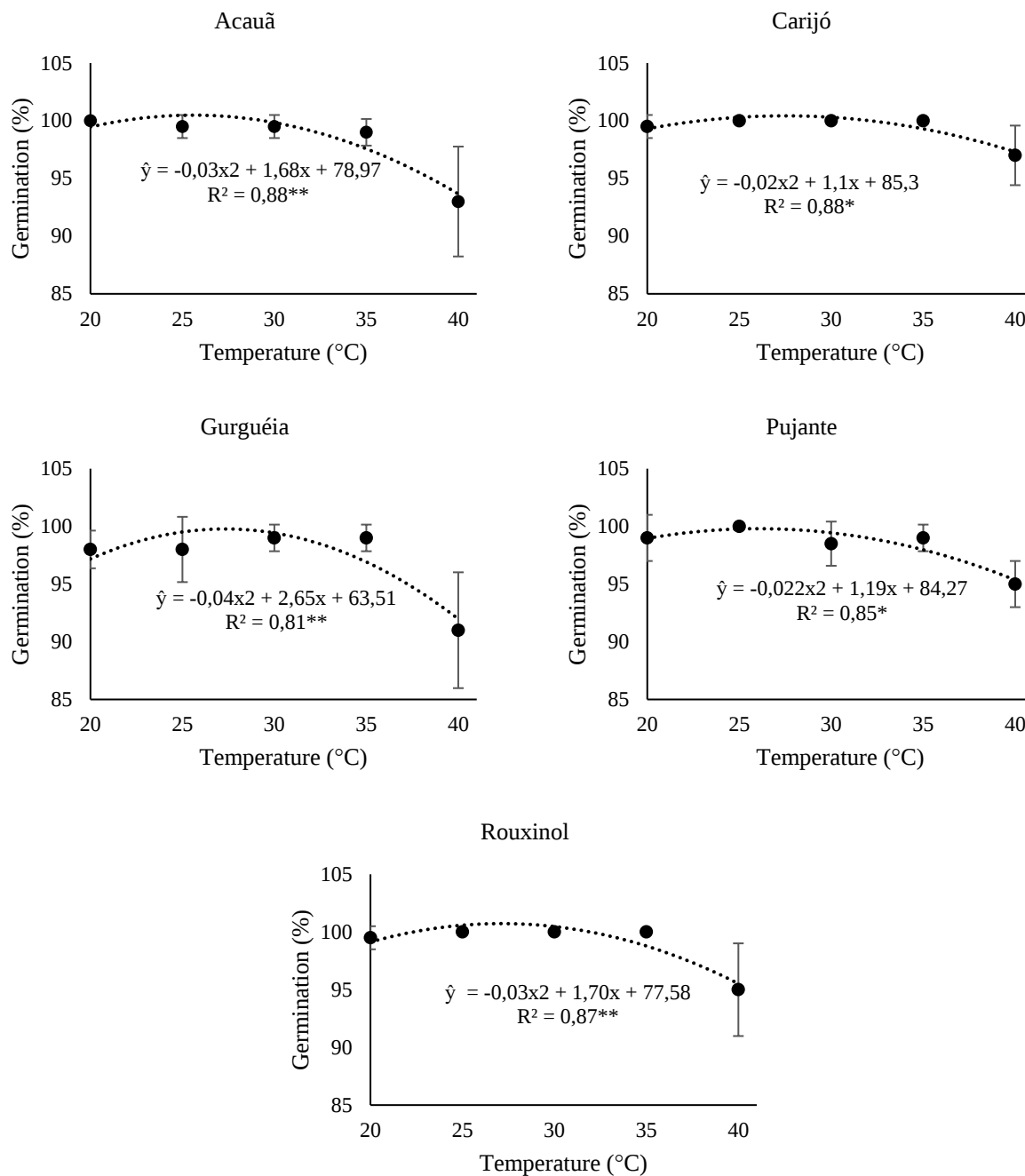


Figure 1. Percentage of seed germination of different cowpea (*Vigna unguiculate* L.) cultivars submitted to temperature increase. Significant regression coefficients with $P < 0.01$ () and $P < 0.05$ (*).**

In the present study, according to the average data in the regression analysis, the highest germination rates occurred in a temperature ranging from 25.55 to 27.50°C, where the germination percentage was between 99.75 to 100% for the cultivars Acauã, Carijó, Gurguéia, Pujante and

Rouxinol (Fig. 1). Although the temperature of 20°C provided germination percentage above 96%, the seeds took a longer period to germinate, which meant lower GSI, lower AGS and higher AGT. The seed germination percentage of Guariba, Itaim, Juruá, Pajeú, Potengi, Tapahium, and Tumucumaque cultivars was not influenced by the temperature increase.

Temperatures below the optimum result in a delay of seed germination due to reduced respiration-related enzyme activity and cellular metabolism (Taiz *et al.*, 2017). Each species has a minimum temperature and a limit temperature below and above which germination does not occur (Motsa *et al.*, 2015). In addition, increasing temperature may reduce the percentage of seed germination by causing thermal stress, leading to inhibition of germination, and may also cause thermal dormancy, affecting seed viability (Bewley *et al.*, 2013). This climatic element regulates enzymatic activities by promoting or inhibiting the synthesis of hormones, such as abscisic acid and ethylene that affect seed germination (Gao-Takai *et al.*, 2019). This occurs because, elevated temperatures increase the levels of abscisic acid and promote the inhibition of genes responsible for the synthesis of gibberellin, an essential hormone for activation of the germinative process (Miransari *et al.*, 2014). In addition, high temperature can inhibit the synthesis of ethylene, another hormone responsible for seed germination.

In table 2, it can be observed from the average data of the regression analysis, that the temperature range between 30-35°C favored the germination speed index, with optimum temperatures varying according to the cultivar. The highest average of the GSI was observed in seeds of cultivar Carijó, with a value of 48.61 at the optimal temperature of 30.40°C (Tab. 2).

Table 2. Means of regression analysis of the optimum temperature for the germination speed index of different cowpea (*Vigna unguiculate* L.) cultivars (GSI).

Cultivars	Optimum temperature (°C)	GSI
Acauã	31.37	36.37
Carijó	30.49	48.61
Guariba	30.73	46.01
Gurguéia	30.54	46.07
Itaim	35.48	36.09
Juruá	30.56	46.52
Pajeú	31.64	39.66
Potengi	31.61	39.53
Pujante	31.75	36.40
Rouxinol	30.96	40.93
Tapahium	31.80	38.13
Tumucumaque	30.51	47.82

The cultivar Itaim presented the temperature of 35.48 as the best for the GSI. This same temperature range favored AGT, since the higher the germination speed index, the shorter the time for seed germination (Tab. 3).

Table 3. Average germination time and average germination speed of different cowpea (*Vigna unguiculata* L.) cultivars submitted to temperature increase.

Germination time												
Temperature	Acauã	Carijó	Guariba	Gurguéia	Itaim	Juruá	Pajeú	Potengi	Pujante	Rouxinol	Tapahium	Tumucumaque
20°C	2.23 aC	2.12 bC	1.13 bD	2.08 bC	2.21 aC	2.11 bC	2.06 bC	2.21 aD	2.32 aD	2.27 aC	2.27 aD	2.17 bC
25°C	2.05 bC	1.57 eB	1.72 dC	1.83 cB	1.92 cB	1.48 eB	2.12 bC	1.97 cC	2.29 aD	1.86 cB	2.15 bD	1.49 eB
30°C	1.78 bB	1.10 cA	1.22 cB	1.13 cA	2.23 aC	1.33 cB	1.57 bB	1.60 bB	1.67 bB	1.70 bB	1.66 bB	1.20 cA
35°C	1.18 aA	1.03 aA	1.03 Aa	1.11 aA	1.11 aA	1.02 aA	1.02 aA	1.06 aA	1.22 aA	1.06 aA	1.10 aA	1.09 aA
40°C	2.14 aC	2.08 aC	2.04 aD	2.01 aC	1.75 bB	2.00 aC	2.00 aC	2.00 aC	2.09 aC	2.17 aC	2.00 aC	2.00 aC
Average germination speed												
Temperature	Acauã	Carijó	Guariba	Gurguéia	Itaim	Juruá	Pajeú	Potengi	Pujante	Rouxinol	Tapahium	Tumucumaque
20°C	0.45 aB	0.47 aB	0.47 aB	0.48 aB	0.45 aB	0.47 aB	0.48 aB	0.45 aB	0.43 aB	0.44 aB	0.44 aB	0.46 aB
25°C	0.49 dB	0.64 aC	0.58 bC	0.55 cB	0.52 cC	0.68 aC	0.47 dB	0.51 cB	0.44 dB	0.54 cC	0.47 dB	0.68 aC
30°C	0.56 dC	0.91 aD	0.82 bD	0.89 aC	0.46 eB	0.75 cD	0.64 dC	0.62 dC	0.60 dC	0.59 dC	0.60 dC	0.84 bD
35°C	0.85 cD	0.97 aD	0.97 aE	0.90 bC	0.90 bE	0.98 aE	0.97 aD	0.94 aD	0.82 cD	0.94 aD	0.90 bD	0.91 aE
40°C	0.45 bB	0.48 bB	0.49 bB	0.50 bB	0.62 aD	0.50 bB	0.50 bB	0.50 bB	0.48 bB	0.46 bB	0.50 bB	0.50 bB

Means followed by the same lowercase letter in the row and uppercase in the column do not differ from each other by the Scott-Knott test at 5% probability.

During the germination process, temperature interferes with water soak time and chemical reactions, thereby changing the germination speed. For common beans, the optimal temperature for GSI was 25-30°C (Zabot *et al.*, 2008). Other genera of Fabaceae plants such as fava beans and peas present temperatures of 28.5 and 28.1°C as optimal for seed germination (Tribouillois *et al.*, 2016). In this way, common beans and these other legumes may be more sensitive to temperature increase when compared to cowpea. This can be inferred because in the present study it was observed an optimal seed performance of the cultivars at temperatures from 30.49 to 35.48°C (Tab. 2).

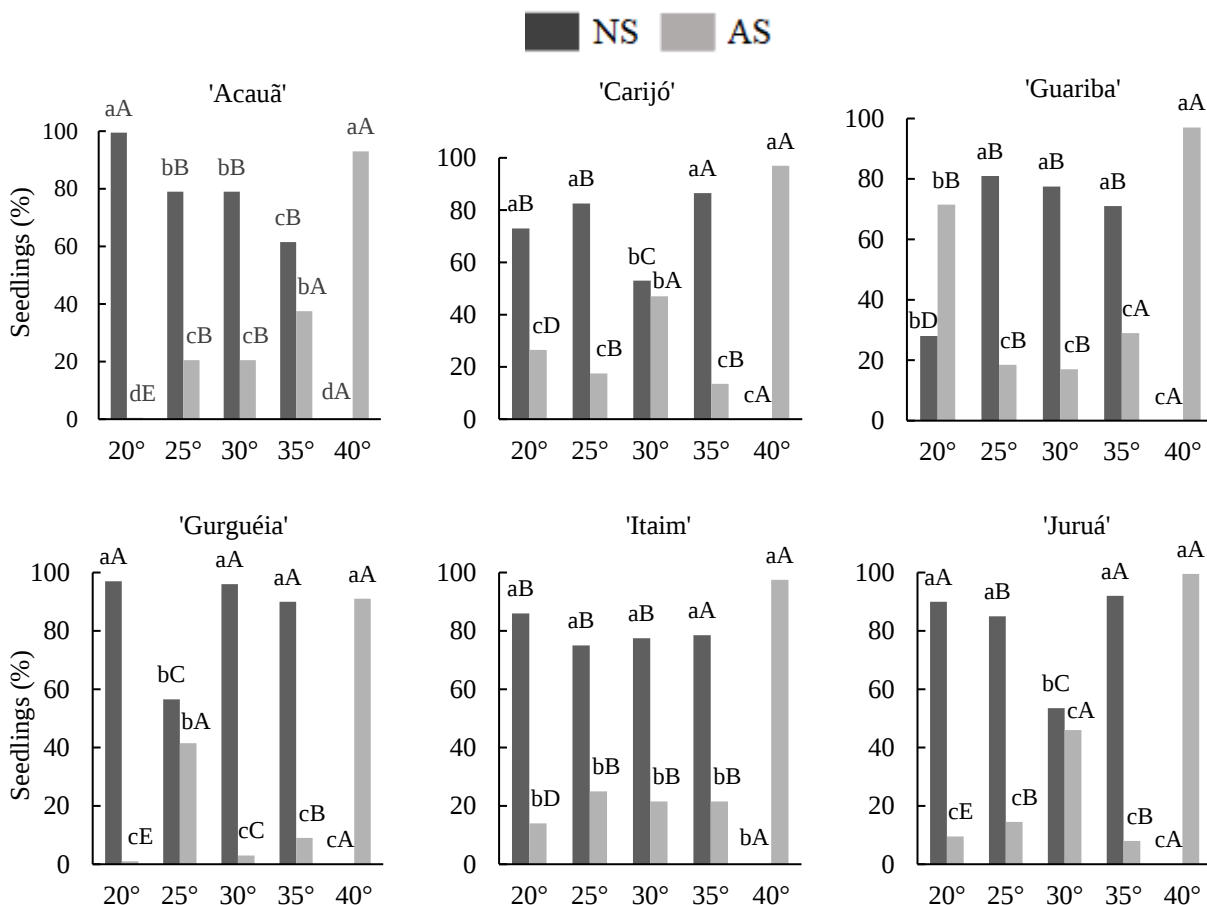
The Brazilian Northeast presented a total annual production of cowpea of 417.9 thousand tons in the 2018/2019 crop year (CONAB, 2019), above the means for other producing regions. In the Brazilian semiarid region, this is the main subsistence crop, being planted practically throughout the year, either in monoculture or intercrop system, in dry or irrigated areas (Vale *et al.*, 2017). This region is characterized by average temperatures of 26-28°C. Thus, a scenario of temperature increase of 4.8°C, as predicted by the IPCC, will not affect the germination and establishment of cowpea seedlings. In addition, the result of this research may also update the optimal temperature used by the Seed Analysis Rules (SAR), which determined the value of 25°C (Brazil, 2009).

It is also observed that at sub and supra optimum temperatures of 20 and 40°C, the seeds took longer to germinate, with a reduction in AGS (Tab. 3). This reduction indicates a physiological decline of the seed that can occur either by reducing or standstill seed metabolism at suboptimal temperatures, or even by protein denaturation, causing loss of enzymatic activity due to elevated temperatures (Orzari *et al.*, 2013).

It is noteworthy that the germination speed is a good index to evaluate the occupation of a species in a given environment, because rapid germination is characteristic of species whose strategy is to settle in the environment as quickly as possible, taking advantage of favorable environmental conditions (Melo Junior *et al.*, 2018). Thus, through the germination speed index, the optimal temperature for cowpea germination is between 30.49-35.48°C, varying according to the cultivar (Tab. 3). Orzari *et al.* (2013) confirmed that the optimal temperature is that in which the highest germination percentage was obtained within the shortest time. Temperature interferes with water soaking time and chemical reactions, changing the speed of germination (Gordin *et al.*,

2012). This speed may be related to the physiological quality of the seeds, because the greater the vigor, the greater the speed and the germination rate (Pádua *et al.*, 2010).

Even under thermal stress of 40°C, all cultivars reached germination percentage from 91 to 100%. However, this was not a good result, since under this condition there was no formation of normal seedlings in the evaluated cultivars (Fig. 2). Thus, environments with temperatures that are not optimal for each species can delay seedling emergence and reduce the stand due to the formation of small seedlings, confirming that environmental conditions are decisive in the process of crop establishment (Santos and Zonetti, 2009).



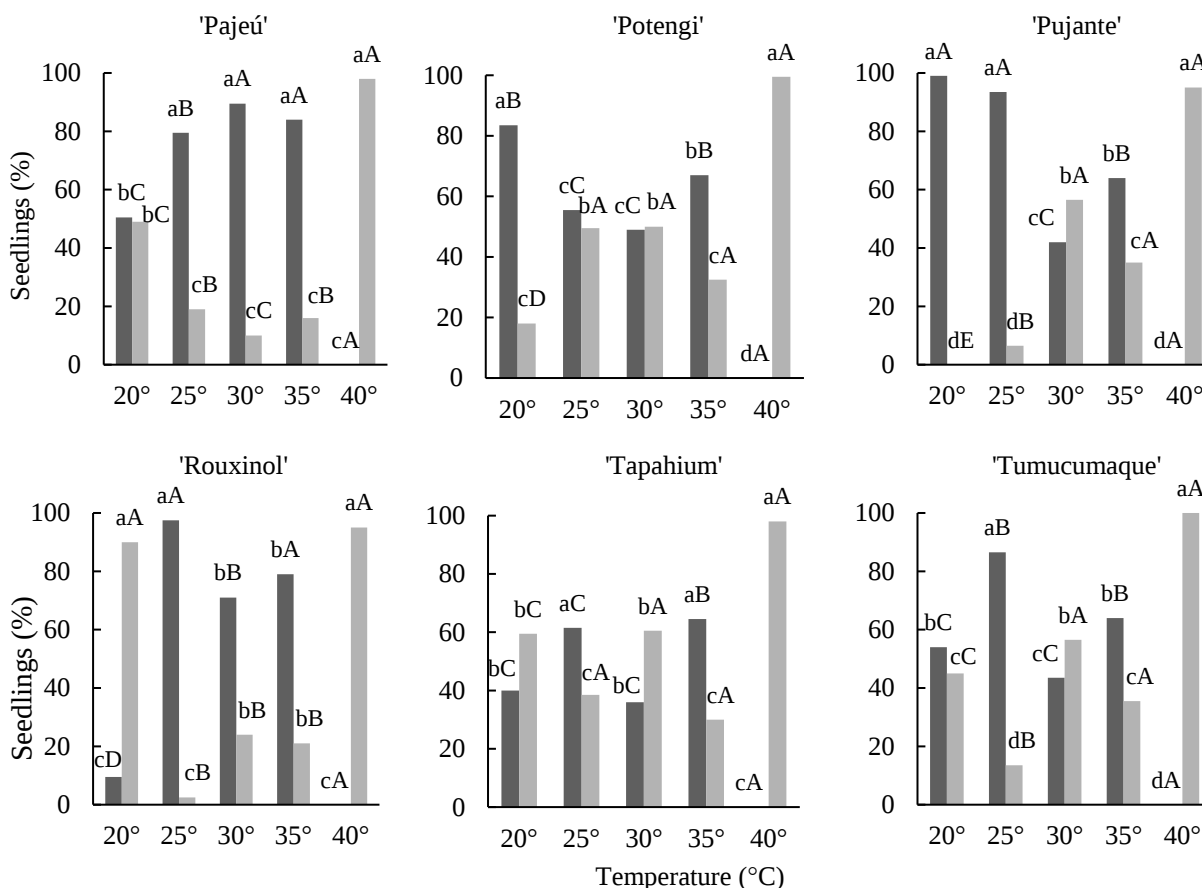


Figure 2. Percentage of normal seedlings (NS) and abnormal seedlings (AS) of cowpea (*Vigna unguiculata* L.) cultivars submitted to increased temperatures. * Lower case letters for temperatures and upper case for cultivars.

Regarding the percentage of normal and abnormal seedlings, the cultivars also responded differently to different temperatures. Temperatures between 25 and 35°C favored the formation of normal seedlings. This feature reinforces the adaptive capacity of the early development of cowpea under conditions of temperature increase. Other crops such as watermelon, for example, have the optimal temperature for seedling development at an average temperature of 25°C (Silva *et al.*, 2018). The well-developed seedlings express the vigor of the seeds that gave rise to them, indicating that they can emerge faster and more uniformly, and establish themselves in adverse field conditions, thus allowing the expected stand to be obtained (Silva *et al.*, 2017).

Seed germination is a critical phase for seedling establishment in the culture medium, as they do not have the ability to withstand adverse environmental conditions, such as high temperatures. In the case of cowpea, the cultivars Acauã, Carijó, Guariba, Gurguéia, Itaim, Juruá, Pajeú, Potengi, Pujante, Rouxinol, Tapahium and Tumucumaque presented a satisfactory performance for the establishment of the plants, being necessary studies regarding the effect of temperature increase in the other phenological stages of the plant.

CONCLUSION

The increase in air temperature between 30-35°C have a positive impact on germination process of cowpea, showing higher percentage of germination within the shortest time and favored the formation of normal seedlings.

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