

## Thermal amplitude of the air influences the emergence and vigor of seedlings of sour passion fruit

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### Abstract

The sour passion fruit is an allogamous species which presents high genetic variability. It is being propagated commercially via seeds. The propagation of species through seeds can be affected by several factors such as the thermal amplitude throughout the day during the germination and the genetic characteristics of the individual. The objective of this study was to evaluate the germination and vigor of seedlings of genotypes of sour passion fruit under different thermal amplitudes of the air. The experiment was conducted in a germination chamber. The factorial scheme was 5 × 3, referring to the five thermal amplitudes of the air (25.0; 22.5-27.5; 20.0-30.0; 17.5-32.5 and 15.0-35.0 ° C) and three genotypes of sour passion fruit (BRS SC1, BRS GA1 and FB-200) in a completely randomized design with six replicates of 25 seeds per plot. The variables analyzed were percentage of velocity index, mean time of emergence, total length, shoot and root and dry mass of seedlings. The thermal amplitude close to 20.0-30.0 °C favored the emergence and vigor of sour passion fruit genotypes. The germination process and the biometric growth of the passion fruit seedlings are adversely affected in high thermal air amplitudes.

**Keywords:** air temperature; cultivars; germinative process; *Passiflora edulis* L.; propagation.

**Abbreviations:** BRS SC1\_BRS Sol do Cerrado; BRS GA1\_BRS Gigante Amarelo; FB-200\_FB-200 Yellow Master; CV\_coefficient of variation; DAS\_day after sowing; DF\_degree of freedom; ESI\_emergency speed index; Hen\_genotypes; IDM\_individual dry mass; MET\_mean emergency time; RL\_root length; SAR\_Seed Analysis Rule SL\_shoot length; TL\_total length; Temp\_thermal amplitude of the air; VS\_variation sources.

### Introduction

The sour passion fruit (*Passiflora edulis* L.), also called yellow passion fruit, is the main species of the Passifloraceae family grown in Brazil. The species stands out for presenting fruits with appropriate physical-chemical characteristics for consumption, high productivity and great acceptance of juice in the national fruit market (Bernacci et al., 2008; Cavichioli et al., 2011; Silva et al., 2015a). The spread of sour passion fruit can be performed sexually, through seeds, or asexually, through cutting, grafting or tissue culture (Alexandre et al., 2004). The most common form of propagation adopted by passifloricultors is semi-propagation, due to the ease of propagation, less time for the formation of seedlings that will go to the field and the self-incompatibility present in the

species. Therefore, this plant requires genetic diversity for fertilization and production of fruits. In the case of asexual propagation, more than one genotype is needed in the area to enable fertilization, which can be via artificial or natural pollination. Passifloraceous seeds have low germination rates, probably due to the existence of dormancy in the species that must be overcome in order for the germination process to continue (Osipi and Nakagawa, 2005; Zucarelli et al., 2009; Zucarelli et al., 2015). The dormancy process may be associated with genetic factors (Santos et al., 2015), being influenced by the environmental conditions. The seeds are exposed, interfering in the regulation of the water entry mechanisms into the seed, in the degradation reactions and transport of reserves to the embryo and cell biosynthesis

during seedling formation (Marcos Filho, 2015; Welter et al., 2011; Marini et al., 2012). The air temperature has a direct influence on the germination of the sour passion fruit, with optimum temperatures or thermal air amplitudes suitable for the germination process with maximum efficiency. In temperatures below or above the amplitudes, germination does not occur (Pimenta et al., 2010; Alves et al., 2012; Bewley et al., 2014). With regard to genetic factors, the use of seeds with high genetic quality contributes to the success of germination (Andrade and Jasper, 2013). The germination process varies between species of the same family and between genotypes of the same species, as observed in papaya (Andrade and Jasper, 2013) and pitaya (Leone et al., 2014). In this sense, the selection of promising progenies in breeding programs can contribute to reduce the occurrence of dormancy in cultivars of sour passion fruit (Santos et al., 2015). Despite the knowledge that genetic and environmental factors related to variations in air temperature can influence the germination process and seedling development, studies to identify the ideal temperature range for the germination process and early development of sour passion fruit genotypes are scarce. Thus, the work aimed to evaluate the response of seedling germination and vigor in sour passion fruit genotypes when submitted to different air temperature ranges.

## Results and Discussion

### Seedling emergence

According to the analysis of variance, there is a significant influence of the interaction between the thermal amplitude of the air and genotypes in the emergence percentage (14, 21 and 28 DAS), the emergence speed index and the length of the aerial part of the sour passion fruit (Table 1). The isolated factors thermal amplitude and genotypes exerted significant action in the following variables: mean germination time, total length and root length. While the individual dry mass responded only to the genotypes of sour passion fruit. The data regarding the percentage of emergence at seven days after sowing were not presented, due to the absence of seedlings in this period. Similar behavior was found by Andrade and Jasper (2013), when they found that the emergence of seedlings in papaya (*Carica papaya* L.) responded to the temperature  $\times$  variety interaction. Lone et al. (2014) assessing the parameters related to pitaya germination and observed that the genotype  $\times$  temperature interaction significantly influenced the germination percentage, the emergence index and the mean germination time, corroborating the data presented in Table 1. This demonstrates that the difference in thermal demand for germination is not only between species (Zucareli et al., 2009; Zucareli et al., 2015), but can vary between genotypes (Lone et al., 2014), cultivars (Alexandre et al., 2004) and varieties (Andrade and Jasper, 2013) within the same species.

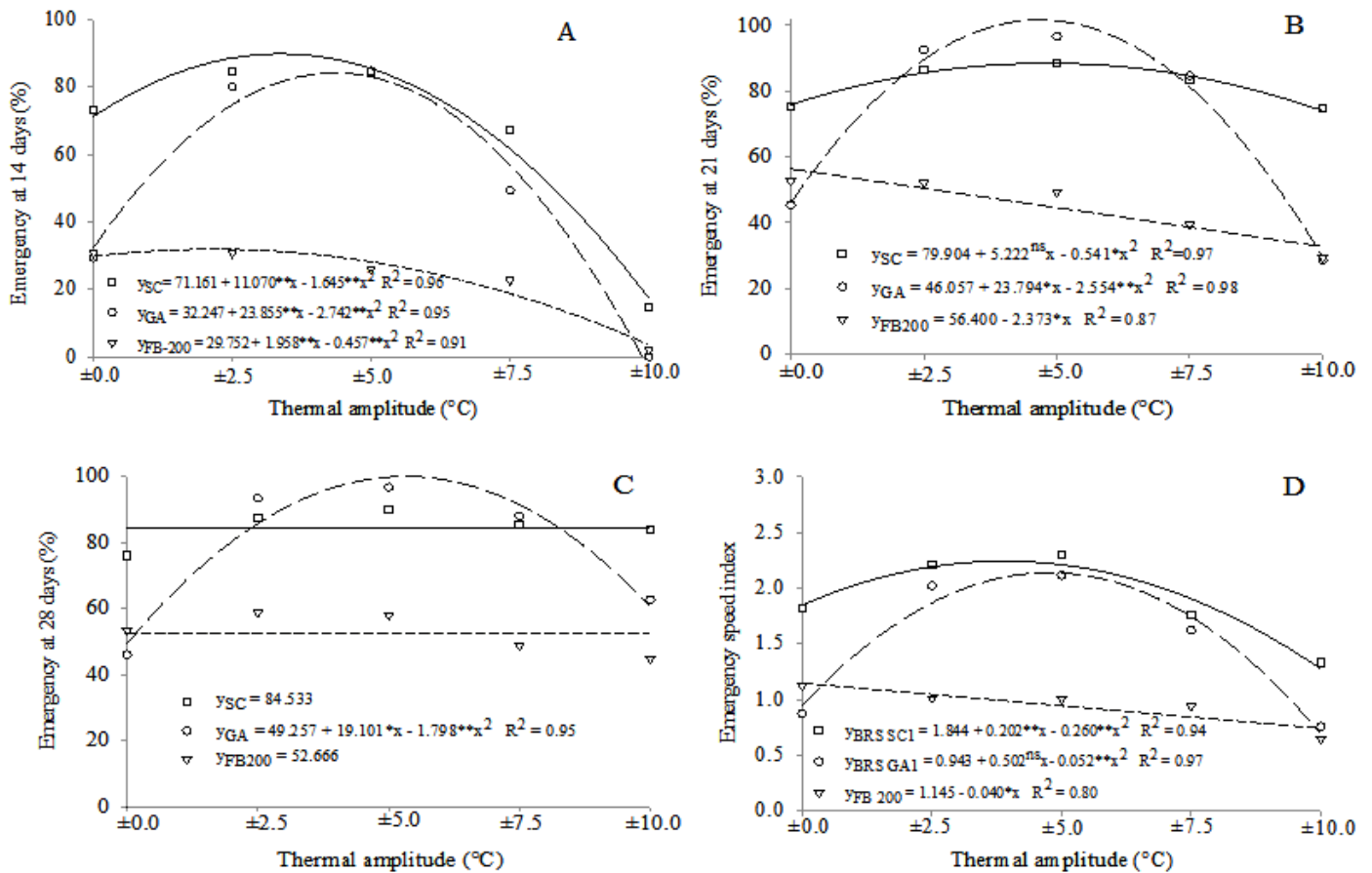
According to emergency results, it appears that in the ranges of  $\pm 2.5$  and  $\pm 5$  °C, the genotypes BRS Sol do Cerrado (Fig 1A) and BRS Gigante Amarelo (Fig 1B) showed emergence above 80%, while under the same conditions, genotypes FB-200 Yellow Master (Fig 1C) showed an emergency close to 60% after 28 days of evaluation. It can be inferred that the genetic makeup interferes with the germination process and presents interaction with the thermal variations of the air throughout

the day. This is confirmed by Lone et al., (2014), when evaluating the germination of seeds in pitaya (*Hylocereus undatus*) genotypes and verified that the germination process is a response to the interaction between genotypes and thermal air conditions. The seedling emergence values at 14, 21, 28 DAS and the emergence speed index of the sour passion fruit genotypes as a function of the thermal amplitude of the air are shown in Fig 1. The seedling emergence at 14 DAS was increased to the amplitudes thermals of  $\pm 3.6$ ;  $\pm 4.3$  and  $\pm 2.1$  °C, respectively, in the genotypes BRS SC1 - 89.7%, BRS GA1 - 84.6% and FB-200 - 31.8% (Fig 1A). At 21 DAS, the maximum emergence percentage was verified in the BRS GA1 genotypes, with 100% of the seedlings emerging in the thermal amplitude of  $\pm 4.6$  °C. Under a constant temperature of 25 °C ( $\pm 0$ ), the highest emergence percentage was also found in the BRS GA1 genotypes, with a value of 56.4% (Fig 1B). At 28 DAS, it was found that 100% of the seedlings of the genotypes BRS GA1 were emerged when submitted to a thermal amplitude of  $\pm 5.3$  °C (Fig 3C). The percentage of emergence in Sol do Cerrado and FB-200 did not fit any regression model, representing a mean value of 84.5 and 52.6%, respectively. The ESI was elevated to the maximum estimated amplitude of  $\pm 3.9$  °C in the genotypes Sol do Cerrado and  $\pm 4.8$  °C in the Gigante Amarelo, with ESI values of 2.24 and 2.15, respectively (Fig 1D). In the FB-200 genotypes, the ESI was reduced by 0.04 per unit increment of the thermal air amplitude, decreasing from 1.15 in the constant temperature from 25 °C to 0.75 in the thermal air amplitude from 15 - 35 °C. The temperature directly influences the germination process, acting on the percentage and speed of seedling emergence, affecting the water absorption by the seed and consequently the enzymatic and biochemical reactions that regulate the metabolism during the germination process (Marcos Filho, 2015; Welter et al., 2011; Bewley et al., 2014). Germination occurs effectively and more quickly at certain air temperature limits or amplitudes, allowing seedling stabilization and adaptation in the field against imposed edaphoclimatic conditions, with differences in temperature requirements and thermal amplitudes of the air between species and genotypes within the same species (Shen et al., 2008; Santos et al., 2015). In several species of passion fruit, the thermal amplitude of the air influences the germination and emergence of seedlings, as verified by Osipi and Nakagawa (2005) in sweet passion fruit (*Passiflora alata* Dryander), by Zucareli et al., (2009) in passion fruit (*Passiflora cincinnata* Mast.) and by Zucareli et al., (2015) in *Passiflora incarnata* L.. The authors describe that the thermal amplitude of 20 - 30 °C proved to be the most efficient in overcoming the dormancy present in the passifloraceae, favoring the germination of seeds and the establishment of seedlings. The increase in the thermal amplitude of the air increased the MET of seedlings of sour passion fruit (Fig 2A). It appears that the seeds submitted to a range of  $\pm 3$  °C took a mean of 11.5 days for seedling emergence, which is the shortest estimated time. Amplitudes above the variation of  $\pm 3$  °C increased the time required for seedling emergence. In the range of 15 - 35 °C, the longest mean time of 17.5 days to emergence was verified. The germination process occurs within certain temperature limits, with adequate thermal amplitudes, while above or below them, there is no germination and consequently emergence of the seedling. In the ranges that is considered optimal, the physiological and biochemical processes are

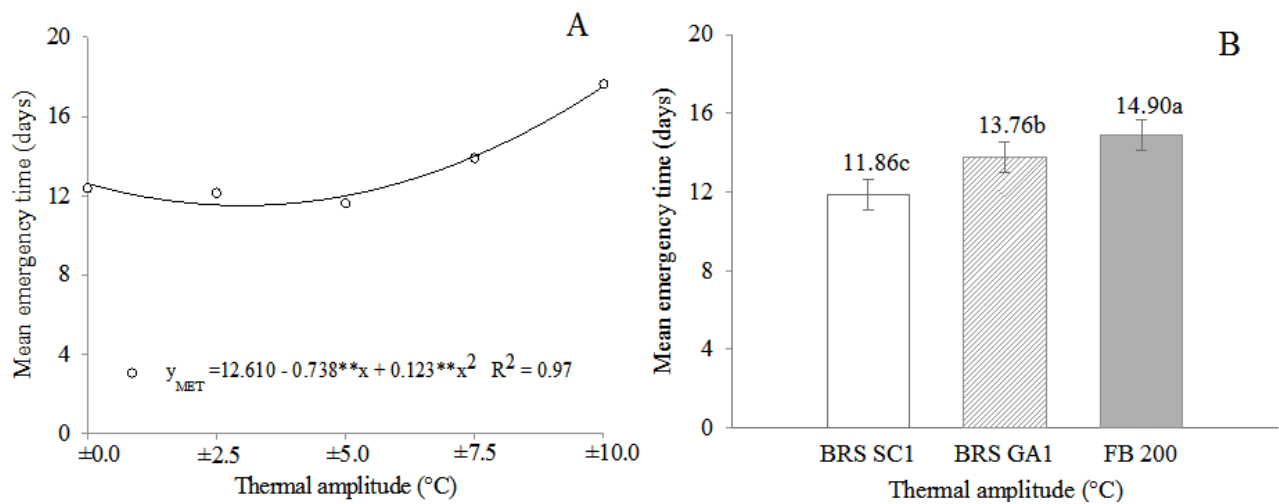
**Table 1.** Summary of the analysis of variance, by the mean square, of the effect of the thermal amplitude of the air (Temp. - T) and of the genotypes (Gen. - G) on the emergence and growth of sour passion fruit seedlings.

VS	Block	Temp.	Gen.	T × G	Residue	CV (%)
DF	5	4	2	8	70	
Emer14 (%)	113.24 <sup>ns</sup>	10,610.40 <sup>**</sup>	13,739.91 <sup>**</sup>	1,401.46 <sup>*</sup>	143.41	26.44
Emer21 (%)	158.47 <sup>ns</sup>	3,694.40 <sup>**</sup>	10,796.97 <sup>**</sup>	1,398.53 <sup>**</sup>	164.61	19.66
Emer28 (%)	168.28 <sup>ns</sup>	1,542.00 <sup>**</sup>	8,378.84 <sup>**</sup>	876.40 <sup>**</sup>	160.43	17.71
MET	1.56 <sup>ns</sup>	108.17 <sup>**</sup>	70.47 <sup>**</sup>	1.14 <sup>ns</sup>	1.54	9.19
ESI	0.11 <sup>ns</sup>	2.34 <sup>**</sup>	6.81 <sup>**</sup>	0.68 <sup>**</sup>	0.13	24.97
TL	0.89 <sup>ns</sup>	3.90 <sup>**</sup>	21.47 <sup>**</sup>	0.88 <sup>ns</sup>	0.61	9.63
SL	0.49 <sup>ns</sup>	32.37 <sup>**</sup>	10.84 <sup>**</sup>	0.87 <sup>**</sup>	0.24	9.63
RL	0.34 <sup>ns</sup>	1.21 <sup>**</sup>	1.63 <sup>**</sup>	0.49 <sup>ns</sup>	0.33	19.38
IDM	30.23 <sup>ns</sup>	19.52 <sup>ns</sup>	77.74 <sup>**</sup>	14.07 <sup>ns</sup>	13.54	28.73

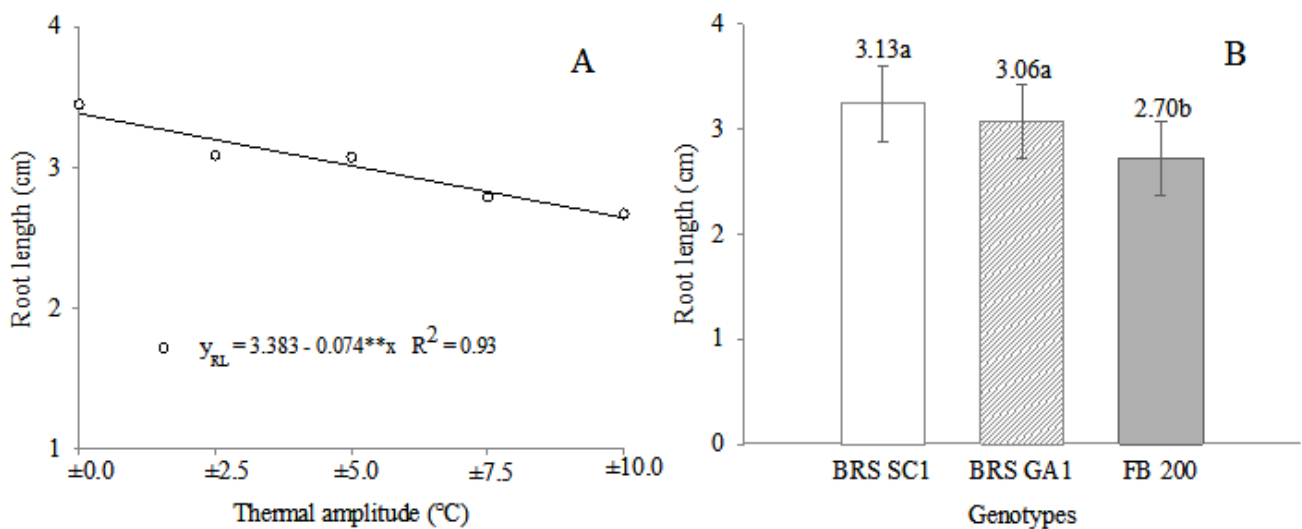
VS= Variation source; DF= Degree of freedom; CV= Coefficient of variation; Emer14 (%) = percentage emergence at 14 days after sowing; Emer21 (%) = percentage emergence at 21 days after sowing; Emer28 (%) = percentage emergence at 28 days after sowing; MET = mean emergency time; ESI = emergency speed index; LT = total length; SL = shoot length; RL = root length; IDM = individual dry mass; \*, \*\*, <sup>ns</sup>= significant at 5%, 1% and non-significant, respectively by the F test.



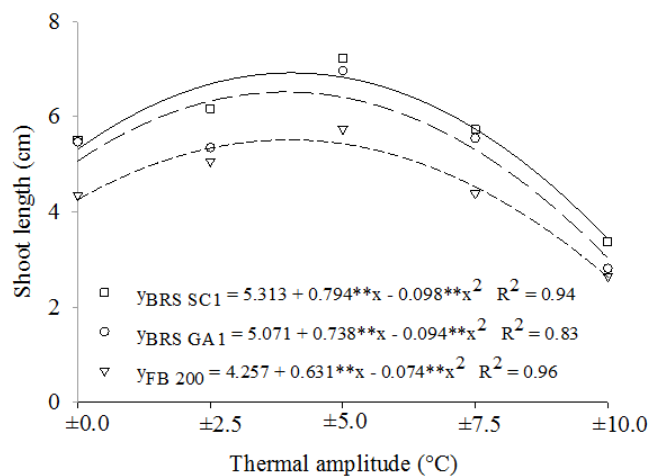
**Figure 1.** Emergence at 14 (A), 21 (B), 28 (C) days after sowing and emergence speed index (D) of the passion fruit genotypes BRS SC1 (—), BRS GA1 (---) and FB-200 (· · ·), depending on the thermal amplitude of the air.



**Figure 2.** Mean emergence time as a function of the thermal amplitude of the air (A) and genotypes (B) of sour passion fruit.



**Figure 3.** Root length of sour passion fruit seedlings as a function of air temperature range (A) and genotypes (B).



**Figure 4.** Shoot length of seedlings in the sour passion fruit genotypes BRS SC1 (—), BRS GA1 (---) and FB-200 (---) as a function of the thermal amplitude of the air.

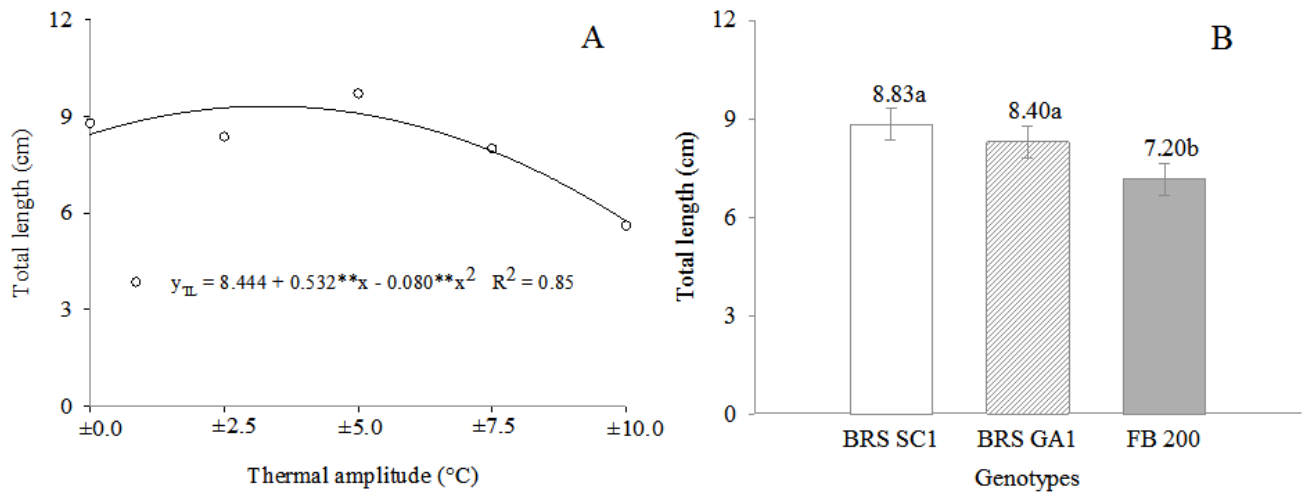


Figure 5. Total length of sour passion fruit seedlings as a function of the thermal amplitude of the air (A) and genotypes (B).

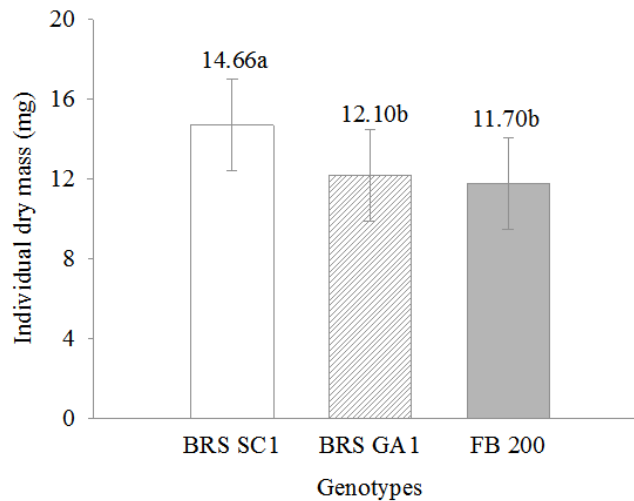


Figure 6. Individual dry mass of seedlings in the sour passion fruit genotypes.

maximized, contributing to the germination in a shorter period of time (Pimenta et al., 2010; Oliveira and Lemes, 2014).

Among the genotypes tested, BRS SC1 was the one with the lowest MET, requiring a mean of 11.86 days, followed by BRS GA1, with a MET of 13.76 days and the FB-200, which on average needed 14.9 days for seedling emergence (Fig 2B). In pitaya, differences between genotypes were also observed in relation to the average time for germination, with variation between genotypes ranging from 4.30 to 13.50 days for germination (Lone et al., 2014). The value of 11.8 days observed in the genotypes BRS SC1 is promising. Silva et al. (2015b) verified the value of 14.1 days in the MET in sour passion fruit seeds submitted to an air temperature range of 20 - 30 °C.

The root length in the sour passion fruit was reduced by 0.07 cm by unitary increase in the thermal amplitude of the air (Fig 3A). Seedlings exposed to a constant temperature of 25 °C had a root length of 3.38 cm, a value 28.03% higher than that

observed for seedlings with a range of 15 - 35 °C. The elevation of the thermal amplitude of the air by ± 10 °C compromised the sour passion fruit RL by 21.9%.

#### Seedling vigor

The reduction in root length with increasing thermal amplitude may be due to the extreme conditions of air temperature that the seedlings were subjected to (range 15-35 °C). In seeds exposed to low temperatures (15 °C) the imbibition process occurs. However, the development of embryo structures, among them, the radicle are limited (Marini et al., 2012). The high temperature (35 °C) allows root development, but the subsequent development is limited by the deterioration of the embryo, followed by the release of exudates in the germinative medium, favoring the development of pathogenic fungi (Steiner et al., 2009).

The root length varied in relation to the evaluated genotypes, as shown in Fig 3B. The genotypes BRS SC1 and GA1 had the longest root lengths with no difference between them, with values of 3.13 and 3.06 cm, respectively. Meanwhile, the FB-200 obtained a value of 2.70 cm in the RL. Steiner et al. (2009) found similar behavior in radish (*Raphanus sativus* L.), who reported that the length of the root is an inherent characteristic of each genotypes, considering that there may be genotypes with short and long roots.

The shoot length the genotypes of sour passion fruit was increased due to the increase in the thermal amplitude of the air (Fig 4). For genotypes BRS SC1, GA1 and FB-200 we verified that LAP increased until the thermal amplitudes of  $\pm 4.0$ ,  $\pm 3.9$  and  $\pm 4.3$  °C, with respective values of 6.9, 6.5 and 5.6 cm. The amplitudes greater than the estimated maximum, reduced the variable under study. The maximum values obtained show that the genotype BRS SC1 was 6.1% higher than the giant yellow and 23.20% higher than the FB-200, demonstrating that there is a genetic difference between the genotypes evaluated in terms of growth and thermal requirement.

The reduction in SL at high thermal amplitudes shows that in extreme temperature conditions, as was the case with the amplitude of 15 - 35 °C, degenerative processes of the seed can occur in the development of the embryo. At low temperature (15 °C), respiration occurs more slowly. In turn, reserves are not become available quickly, affecting the transport of reserves to the embryo and, consequently, seedling growth (Marini et al., 2012). On the other hand, at high temperatures (35 °C) degenerative transformations such as the leakage of substance into the environment are occurred, allowing the development of microorganisms, such as fungi and bacteria, indicating the deterioration and/or death of the seed (Zucarelli et al., 2009; Bewley et al., 2014).

The maximum total length was 9.32 cm observed at the estimated thermal range of  $\pm 3.3$  °C. Greater amplitudes than this inhibited the development of sour passion fruit seedlings (Fig 5). Thus, under an air temperature range of  $\pm 10$  °C, the total seedling length was 63.51% less than the maximum observed (Fig 5A). Extremely low and / or high temperatures (min - 15 °C and max - 35 °C) hinder seedling development. Similar behavior was verified by Alves et al. (2012), when they found that the length of seedlings in *Crataeva tapia* L. was reduced at the mean constant temperatures of 20 and 35°C (lowest and highest temperature tested in the experiment), being favored in the air temperature range of 20 - 30 °C.

The total length of the seedlings varied between the genotypes, and the genotypes BRS SC1 and GA1 did not differ in relation to the variable and presented the highest values, respectively, of 8.83 and 8.40 cm. The lowest value of 7.20 cm was observed in genotype FB-200 (Fig 5B). These results are in line with those presented by Santos et al. (2015), when demonstrating that the initial seedling growth in sour passion fruit genotypes is dependent on the genetic makeup. On the other hand, Alexandre et al. (2004) did not observe significant differences in relation to the progenies of sour passion fruit regarding the total length of the seedlings.

The individual dry mass of the sour passion fruit seedlings differed between the tested genotypes (Fig 6). The sour passion fruit BRS SC1 had an IDM of 14.66 mg planta<sup>-1</sup>, with an accumulation of 21.1 and 25.3% higher than the dry masses of

passion fruit BRS GA1 (12.1 mg) and FB-200 (11.7 mg), respectively.

Similar results were obtained by Santos et al. (2015) in sour passion fruit seedlings from seeds of open pollination and self-pollination, when verifying variation in seedling dry mass between genotypes, suggesting the presence of genetic effects. The determination of dry mass in seedlings is a way to evaluate growth in a precise way and understand how the transfer of seed reserves to the embryonic axis occurs. Therefore, the greater the dry mass of seedlings, the greater the vigor of the seed (Alves et al., 2012; Bewley et al., 2014).

## Materials and Methods

### Experiment set up and conduction

The experiment was carried out in a germination chamber with controlled conditions of photoperiod and air temperature, at the Teaching, Research and Extension Unit/Pomar Campus, belonging to the Phytotechnics Department of the Universidade Federal de Viçosa, in the municipality of Viçosa, Minas Gerais State, Brazil; from August to September 2016.

The experiment was conducted in a 5 × 3 factorial scheme (five air temperature ranges and three commercial genotypes of sour passion fruit), in a completely randomized design, with six replicates per treatment and 25 seeds per experimental unit. The air temperature ranges used were constant 25 °C ( $\pm 0.0$ ), 22.5 - 27.5 °C ( $\pm 2.5$ ), 20.0 - 30.0 °C ( $\pm 5.0$ ), 17.5 - 32.5 °C ( $\pm 7.5$ ) and 15.0 - 35.0 °C ( $\pm 10.0$ ) in incubator of biochemical oxygen demand (BOD) and thermal regulation for the lowest temperature in the period of the lights off and the highest temperatures in the period of luminosity, and the commercial genotypes used were BRS SC1, BRS GA1 and FB-200 Yellow Master. The seeds of the sour passion fruit genotypes used in this experiment were purchased from Viveiro Flora Brasil, Araguari, Minas Gerais, Brazil.

The average temperature of 25 °C was chosen for the application of different thermal amplitudes of the air, due to the fact that this temperature is the most suitable for the seed germination process and initial development of sour passion fruit seedlings, as verified in a pre-evaluation test. The photoperiod was 8 a.m., with the lights on during the day (8 a.m. to 6 p.m.) and 4 p.m. without a photoperiod, with the lights off at night (6 p.m. 01 min to 7:59 a.m.). The lower air temperature in the germination chamber was established in the absence of a photoperiod, while the upper air temperature was established in the presence of a photoperiod. The seeds were selected and standardized in terms of size and weight, later they were sown in an inert substrate (washed sand) at a depth of 2 cm and spaced 4 cm between rows. Irrigation was carried out daily, keeping the substrate moisture sufficient to favor germination and seedling emergence.

### Variables analyzed

The emerged seed count was performed daily, starting at the 7<sup>th</sup> day after sowing (DAS) and ending at the 28<sup>th</sup> DAS, when the germinative process stabilizes, according to the methodology contained in the Seed Analysis Rule (SAR) for the crop of sour passion fruit (Brasil, 2009). Following the methodology proposed by Ferreira and Borghetti (2004), the

percentage of emergency at 7, 14, 21 and 28 DAS, the mean emergency time (MET) and the emergency speed index (ESI) were evaluated. At the end of the experiment with the aid of a ruler graduated in millimeter, shoot length (SL), the root (RL) and the total (TL) were measured. After biometric measurements, the seedlings were packed in paper bags, and taken to an air circulation oven at a constant temperature of 65 ° C over a period of 48 h until reaching constant weight, to determine the individual dry mass of seedlings (IDM), with the aid of a semi-analytical balance.

### Statistical analysis

The germination percentage data showed normal distribution, so there was no need to transform the data for statistical analysis. The data were subjected to analysis of variance by the F test at 5% probability. The mean for the sour passion fruit genotypes were compared using the "Tukey" test ( $p < 0.05$ ). The air temperature amplitude data were submitted to polynomial regression analysis ( $p < 0.05$ ). For data analysis, Sisvar version 5.6 statistical software was used (Ferreira, 2014).

### Conclusions

Thermal air amplitude close to 20.0 - 30.0 C favors the germination process, the emergence speed and the initial growth of seedlings in the genotypes of sour passion fruit. The shortest mean time to emergence was obtained in the temperature variation close to 22.5 - 27.5 °C. The germinative process and biometric growth of seedlings of sour passion fruit are affected in air temperature ranges of 15.0 - 35.0 ° C. Seed germination, seedling growth and biomass accumulation are variable among genotypes, with BRS Sol do Cerrado and BRS Gigante Amarelo genotypes having the best results.

### Acknowledgments

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