

Physiological potential of bell pepper seeds under heat and salt stress

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ABSTRACT

Peppers (*Capsicum annuum* L.) are a nutritionally important vegetable grown in Brazil. Abiotic stresses, such as heat and salt, directly affect the germination process of seeds and compromise their yield. This study aimed to evaluate the effects of heat and salt stress on the physiological potential of bell pepper seeds. The experiment was conducted using a completely randomized design arranged in a 4x5 factorial scheme: heat stress with four temperatures (20 °C, 25 °C, 30 °C, and 35 °C) and salt stress with five concentrations of NaCl (0.00, 0.075, 0.150, 0.225, and 0.300 mM). The physiological potential of the seeds was assessed using germination and vigor tests. The interaction between temperature and NaCl concentration significantly influenced all the variables studied. In germination, first germination count, germination speed index, and seedling length, the effect of the interaction was adjusted to a quadratic regression, in which there was a decrease in the variables mentioned until approximately the concentration of 0.150 mM NaCl for the concentrations of 0.225 and 0.300 mM, there was an increase, but it was not superior to the control (0.00 mM). Bell pepper seeds are sensitive to heat and salt stress in which the physiological potential of the seeds was negative at temperatures of 20 °C and 35 °C and with increasing concentrations of NaCl, highlighting the importance of strategies to optimize germination and initial growth of seedlings under such conditions.

Keywords: *Capsicum annuum* L.; Germination; Vegetables; Salinity; Temperature.

Potencial fisiológico de sementes de pimentão sob estresse térmico e salino

RESUMO

O pimentão (*Capsicum annuum* L.) é uma hortaliça de elevada importância nutricional cultivada no Brasil. Estresses abióticos, térmico e salino, afetam diretamente o processo germinativo de sementes e compromete sua produtividade. Este estudo objetivou-se em avaliar os efeitos do estresse térmico e salino no potencial fisiológico das sementes de pimentão. O experimento foi conduzido por delineamento inteiramente casualizado em esquema fatorial de 4x5, estresse térmico com quatro temperaturas (20 °C, 25 °C, 30 °C e 35 °C) e estresse salino com 5 concentrações de NaCl (0,00; 0,075; 0,150; 0,225 e 0,300 mM). O potencial fisiológico das sementes foi avaliado por meio dos testes de germinação e de vigor. Houve interação significativa das temperaturas e concentrações de NaCl para todas as variáveis realizadas no estudo. Na germinação, primeira contagem de germinação, índice de velocidade de germinação e comprimento de plantas observou-se que a interação foi ajustada à regressão quadrática, na qual houve um decréscimo das variáveis citadas, até aproximadamente na concentração de 0,150 mM de NaCl, para as concentrações de 0,225 e 0,300 mM houve um aumento, porém não foi superior a testemunha (0,00 mM). As sementes de pimentão são sensíveis ao estresse térmico e salino no qual o potencial fisiológico das sementes foi negativo às temperaturas de 20 e 35 °C e com o aumento das concentrações de NaCl evidenciando a importância de estratégias para otimizar a germinação e o crescimento inicial de plântulas sob tais condições.

Palavras-chave: *Capsicum annuum* L.; Germinação; Hortaliças; Salinidade; Temperatura.



1. Introduction

The bell pepper (*Capsicum annuum* L.) belongs to the Solanaceae family and is native to southern Mexico and Central America. It is a highly nutritious vegetable whose fruits are a source of bioactive compounds such as flavonoids and carotenoids (Lana and Tavares, 2022). *Capsicum annuum* is grown and consumed throughout Brazil, and the main producing states are Minas Gerais, São Paulo, Ceará, Rio de Janeiro, Espírito Santo, and Pernambuco. These states are responsible for 87% of the country total production, with around 13,000 hectares of planted area, and this vegetable is among the ten most important in the country (Barros et al., 2020).

Bell peppers are grown in Brazil using seedlings, which are produced using high quality seeds. One of the characteristics of bell pepper seeds is their slow germination at room temperature, making direct sowing unfeasible for establishing uniform fields and adequate stands, which is why there is a need to produce seedlings for commercial bell pepper production (Rodrigues et al., 2021). The germination process of bell pepper seeds takes between 8 and 12 days and can reach an extreme of 20 days due to the low temperature of both the environment and the soil (Santos et al., 2020).

Germination is the process by which the seed embryo develops into a seedling. This process is influenced by external and internal factors (Marcos Filho, 2015). External factors include environmental conditions such as temperature, humidity, light, and oxygen. Variations in these elements can significantly impact the germination speed (Rasera and De Castro, 2020). The internal factors are related to the viability and longevity of the seeds, which are related to the genetic characteristics and initial quality of the seeds (Marcos Filho, 2015). The relationship between these factors plays a fundamental role in the seed's ability to germinate, directly influencing the success of the seed's initial performance (Roesler et al., 2023).

The main factor for germination is the availability of water, soils, or substrates with very negative osmotic potential that will delay or decrease germination (Vaz de Melo et al., 2012). The high salt concentration directly influences germination, as water is osmotically retained in saline solutions, so increasing the saline concentration decreases the amount of water available to the plants (Divi et al., 2021).

Seeds are sensitive to salinity, and when exposed to a high salt concentration, water absorption is initially decreased, reducing the speed of physiological and biochemical processes. The inhibition of germination caused by salinity is due to both the osmotic effect and the toxic effect resulting from the concentration of ions in the protoplasm (Muniz and Silva, 2020).

Another important factor in the germination process is temperature, which influences the germination speed and seedling emergence, as well as the biochemical reactions of the germination process (Silva et al., 2019). Each species has its ideal temperature for rapid and uniform germination. For most species, the optimum temperature is between 15 °C and 30 °C, the maximum temperature ranges between 35 °C and 40 °C, while the minimum temperature can reach the freezing point (Rodrigues et al., 2021).

The ideal temperature for bell pepper seeds is between 20 °C and 30 °C (Brasil, 2009). It is worth noting that the interaction of abiotic factors, such as high/low temperatures and water availability, directly affects seed germination and, consequently, the production and quality of vegetable seedlings.

In this context, given the importance of the seed germination process for the production of vegetable seedlings and the need to study the effects of abiotic stresses on this process, this study aimed to evaluate the effects of heat and salt stress on the physiological potential of bell pepper seeds (*Capsicum annuum* L.).

2. Material and Methods

The experiment was conducted at the Seed Laboratory of the Goiás State University UEG, Campus Sul, Unit of Ipameri, located in Ipameri Goiás, from October to November 2023. The experiment used untreated seeds of bell pepper (*Capsicum annuum* L.) cultivar Casca Dura Ikeda (ISLA®).

The experimental design was entirely randomized and arranged in a 5x4 factorial scheme, with five concentrations of salt solution (NaCl 0.00; 0.075; 0.150; 0.225, and 0.300 mM) and four temperatures (20 °C, 25 °C, 30 °C, and 35 °C), with four replications of 50 seeds.

The seeds were subjected to salt stress by soaking the seed test paper (Blotter Paper) with solutions of different salt potentials, and for heat stress, the seeds were placed in a B.O.D. germination chamber at different temperatures. Both the concentrations of the salt solutions and the temperatures have already been mentioned.

Before starting the experiment, the water content of the seeds was determined using the oven method (Brasil, 2009). The samples were placed in an air circulation oven at 105 °C ± 3 for 24 hours, and the result was expressed as a percentage (%).

To assess the physiological potential of the seeds, the following tests were conducted: germination, first germination count, germination speed index, seedling length, dry mass, and fresh mass of seedlings. The analyses were conducted following the RAS (Rules for Seed Analysis - Regras para Análise de Sementes) (Brasil, 2009) and the book Vigor: Conceitos e Testes

(Vigor: Concepts and Tests) (Krzyzanowski et al., 2020).

For the germination test (G) and first germination count (FGC), the seeds were distributed in plastic germination boxes containing seed test paper (Blotter Paper) moistened 2.5 times the weight of the paper with deionized water (control) and the salt concentration solutions predetermined according to the treatments.

The plastic germination boxes were placed in a B.O.D. germination chamber at 20 °C, 25 °C, 30 °C, and 35 °C. The germination of the bell pepper seeds was assessed on the 14th day after the start of the test, and the result was expressed as a percentage of normal seedlings, following the criteria established in the RAS (Brasil, 2009). The FGC test was conducted at the same time as the germination test, but the evaluation was conducted on the 7th day after the start of the germination test.

The germination speed index (GSI) was conducted along with the germination test described above, and daily counts were made of germinated seeds, which presented a 2 mm primary root protrusion. The following calculation was used to determine the GSI: $GSI = (N1/T1) + (N2/T2) + (Nn/Tn)$. N is the number of seeds germinated each day (1, 2, 3, n), and T is the number of days from the start of the test until the day on which seed germination was constant (T1, T2, T3, ..., Tn).

To assess the seedling length (LENG), on the 14th day after the germination test started, the normal seedlings were removed from the plastic boxes, and their length was measured using a graduated ruler, and the result was expressed in centimeters (cm).

After determining the LENG, the normal seedlings were carefully separated to obtain the fresh and dry mass of the seedlings (SFM and SDM). SFM was determined by weighing the normal seedlings from each treatment on a precision scale (0.001 g). After weighing, the normal seedlings were placed in "kraft" paper bags and dried in an oven with forced air circulation set at 65 °C for 72 hours. After this period, SDM was determined by weighing the samples on a precision balance (0.001g), and the results of both SFM and SDM were expressed in mg plant⁻¹ (Krzyzanowski et al., 2020).

The data was subjected to analysis of variance and the means were compared using the Tukey test at 5% probability for temperature and regression analysis for NaCl concentrations. SISVAR software (Ferreira, 2011) was used for statistical analysis.

3. Results and Discussion

The water content of the bell pepper seeds at the beginning was 5.8%. After this, the seeds were

subjected to four temperatures (20, 25, 30, and 35 °C) and five salt concentrations (0.000, 0.075, 0.150, 0.225, and 0.300 mM) to analyze the seeds physiological potential.

However, on the last day of evaluation (14th day), it was found that the treatments conditioned at a temperature of 35 °C did not show a percentage of normal seedlings at all NaCl concentrations, so it was impossible to conduct the statistical analysis for this temperature. High temperatures compromise essential metabolic activities, leading to the denaturation of proteins, deactivation of enzymes, and interference with water absorption, resulting in the inability of seeds to effectively complete the germination stages, showing that bell pepper seeds have an optimum temperature range for germination, between 20 °C and 30 °C, according to the RAS (Brasil, 2009).

Table 1 shows the results obtained from the analysis of variance for germination, first germination count, germination speed index, seedling length, and seedling fresh and dry mass. There was a significant interaction between temperature and saline solution factors for all the variables, which was significant at 1%.

Figure 1 shows the quadratic regression graphs of the interaction between temperature and salt solution for the following variables: germination (A), first germination count (B), germination speed index (GSI), and seedling length (D) of bell pepper seeds subjected to heat and salt stress.

For germination (Figure 1A), the percentage of normal seedlings at 25 °C (R^2 of 0.9755*) started at 88% and decreased until the concentration of 0.150 mM NaCl (74%). Still, for the other concentrations, there was an increase in the percentage of normal seedlings of 76% and 81% at the concentrations of 0.225 and 0.300 mM NaCl, respectively. However, the results were lower than the control.

High concentrations of salts such as NaCl reduce the availability of water and lead to an accumulation of ions in the seed cells, hindering water absorption during germination, affecting the integrity of cell membranes and triggering stress responses that impair cell growth and differentiation, interfering with metabolic processes and compromising the normal development of seedlings (Ferreira and Perez Marin, 2022).

For the first germination count (Figure 1B) at a temperature of 30 °C (R^2 of 0.7729*), it can be seen that the percentage of normal seedlings started at 80% (control) and decreased until the concentration of 0.150 mM NaCl (20%). Still, for the other concentrations, there was an increase in the percentage of normal seedlings. It is worth noting that for the first germination count variable, the seeds conditioned at 20

°C did not produce a percentage of normal seedlings for all the treatments, but there was primary root protrusion since in the germination test, in which the

evaluation was conducted on the 14th day, the percentage of normal seedlings was between 83% and 33%.

Table 1. Summary of the analysis of variance for germination (G), first germination count (FGC), germination speed index (GSI), seedling length (LENG), seedling fresh mass (SFM), and seedling dry mass (SDM) of bell pepper seeds subjected to heat stress (20, 25, 30, and 35 °C) and salt stress (0.0, 0.075, 0.150, 0.225, and 0.300 mM).

Source of Variation	G	FGC	GSI	LENG	SFM	SDM
		%		cm		mg plant ⁻¹
F-value (T)	2.513ns	76.79**	40.317**	51.459**	219.086**	6.95**
F-value (NaCl)	19.367**	9.86**	5.175**	7.957**	24.94**	7.45**
F-value (T x NaCl)	8.981**	5.70**	3.310**	3.183**	29.84**	5.32**
CV (%)	10.39	44.85	16.35	13.63	16.54	38.26

T - Temperature; NaCl - saline solution of different concentrations; CV - Coefficient of variation; ** and * - Significant at 1% and 5% probability, respectively; ns - not significant.

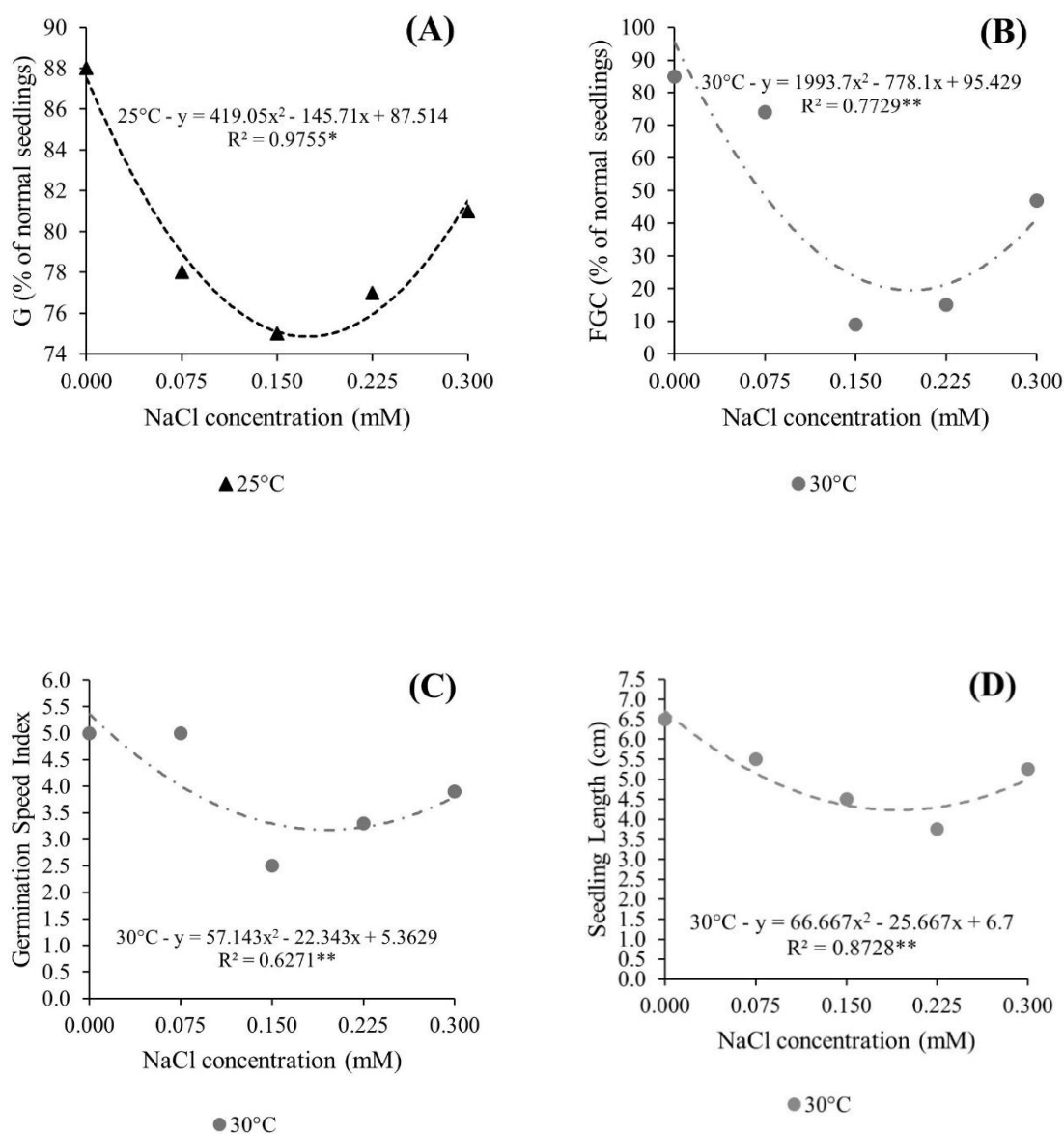


Figure 1. Percentage of normal seedlings from the germination test (A), first germination count (B), germination speed index (GSI), and seedling length (D), originating from bell pepper seeds subjected to heat and salt stress. ** and *Significant at 1% and 5% probability, respectively.

Low temperatures delay seed germination due to decreased enzyme activity, reduced metabolic rate, and slower nutrient transport, resulting in a lower first germination count than higher temperatures (Barros et al., 2020).

In the germination speed index (Figure 1C), the quadratic regression graph shows that the GSI value decreased to 0.150 mM concentration, followed by increased GSI when subjected to 0.225 and 0.300 mM concentrations. The osmotic and ionic stress caused by the salt concentration of 0.150 mM may have reduced the absorption of water by the seeds, delaying metabolic activation and consequently slowing down seed germination. However, the increase in higher concentrations can be attributed to a possible adaptation of the seeds to salt stress, triggering physiological defense responses and allowing a partial recovery of germination speed (Bedin and Neumann, 2022).

When evaluating the seedling length (Figure 1D), it can be seen that the seeds not subjected to salt stress

(control) had seedlings up to 6.5 cm long, but as they were exposed to salt concentrations, there was a decrease in length, with a value of 4 cm at a concentration of 0.225 mM, and an increase in length (5.5 cm) when exposed to concentrations of 0.300 mM.

The results for seedling fresh mass (SFM) and seedling dry mass (SDM) are shown in Figure 2. When evaluating the results for fresh mass (Figure 2A), the seeds conditioned at a temperature of 30 °C showed a decrease in values as the salt concentration increased. However, seeds subjected to a temperature of 25 °C showed higher SFM results as the salt concentration increased, reaching a maximum value of approximately 25 mg plant⁻¹ when exposed to a concentration of 0.225 mM, followed by a decrease at 0.300 mM.

For the seedling dry mass (Figure 2B), there was an increase in dry mass with lower NaCl concentrations, reaching 4 mg plant⁻¹ at 0.150 mM, followed by a decrease with 0.225 and 0.300 mM at 30 °C (R^2 of 0.8991**).

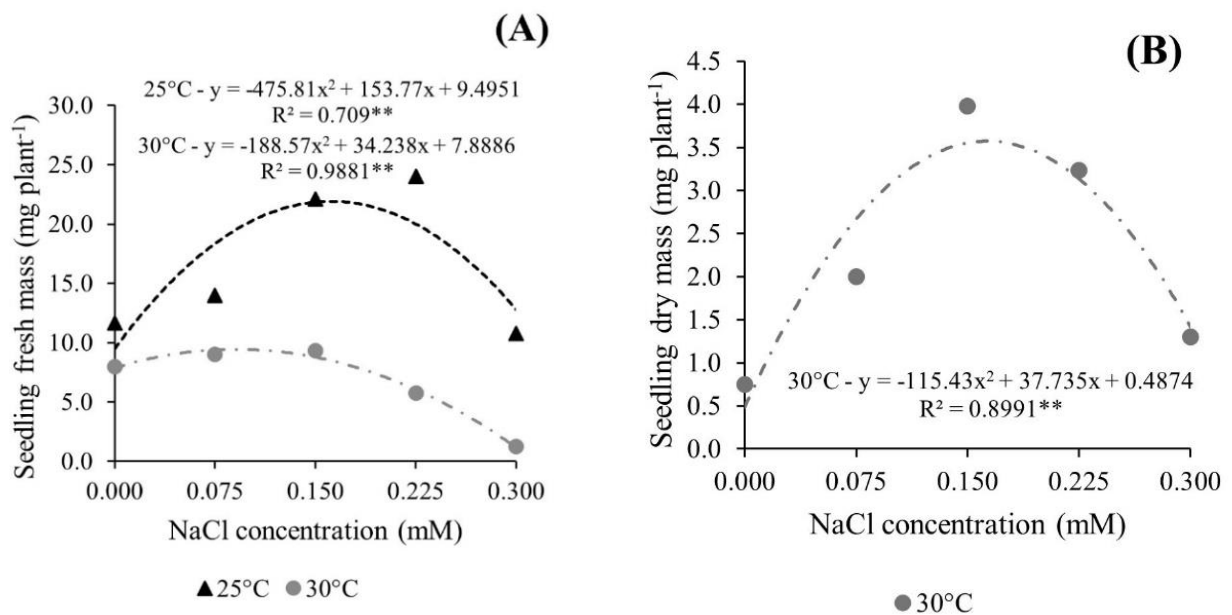


Figure 2. Seedling fresh mass (A) and seedling dry mass (B) originated from bell pepper seeds subjected to heat and salt stress. ** and *Significant at 1% and 5% probability, respectively.

This relationship is because low NaCl concentrations boost initial growth, increasing plant biomass due to increased water absorption to dilute excess salts in the roots. However, higher concentrations generate severe stress, damaging the cell osmotic balance, resulting in excessive salt accumulation, reducing water and nutrient absorption, and decreasing plant mass (Barros et al., 2020).

When evaluating the germination of bell pepper seeds from the cultivar All Big hydroconditioned in a saline medium, Bedin and Neumann (2022) observed that the average germination percentage was higher than

the control, indicating a beneficial effect of this condition on physiological potential. The seeds that initially had low potential benefited from soaking in a saline medium during the test, contributing to the higher germination rates. This is because the saline solution created a condition similar to Halopriming.

Halopriming is described as a conditioning method in which seeds are soaked in low amounts of salts so that enzyme activity is increased and the mobilization of organic substances in the embryo during germination is modified through the regulation of actions inherent to the sodium-potassium pump ($\text{Na}^+/\text{K}^+\text{-ATPase}$) at a

cellular level, contributing to the solubilization of molecules and consequently better performance in the physiological potential of seeds (Ferreira and Perez Marin, 2022).

In a study on the cultivation of bell pepper seedlings under the effect of salinity, Santos et al. (2020) found that saline water up to 1.5 dSm^{-1} did not directly affect the emergence of bell pepper seedlings during transplanting. However, increases in salinity can negatively influence some physiological aspects of plants without compromising the emergence of seedlings or the shoot mass. Previous research has indicated that salinity reduces seed germination in some species compared to non-saline conditions (Qu et al., 2008).

De Lima Nunes et al. (2019), when evaluating the germination and vigor of cowpeas in response to saline and thermal stresses, presented results concluding that temperature variations did not influence germination, while salinity directly interfered with the physiological potential of the seeds. Gorai and Neffati (2007) concluded that the impact of salinity on seed germination can be altered by temperature. Figueiredo et al. (2019), when evaluating different substrates and temperatures on the germination and vigor of tomatoes, concluded that the temperature of $25 \text{ }^\circ\text{C}$ provided the highest percentages of seed germination, while the temperatures of $20 \text{ }^\circ\text{C}$ and $30 \text{ }^\circ\text{C}$ had no significant effect on the germination tests. Theoretically, the germination of seeds of certain species has an optimum temperature under which the seeds could germinate more effectively than under other temperatures (Gu et al., 2018).

Given these results, we can highlight the importance of techniques to deal with heat and salt stress to optimize bell pepper physiological potential under adverse conditions. Investigating the effects of these stresses on seed germination and vigor can help develop coexistence mechanisms, i.e., adaptive strategies that enable the survival of cultivable species under challenging conditions, such as saline and thermal stresses. However, studying these effects under natural conditions is complex since germination conditions such as salinity and temperature are variable, requiring more studies on thermal and saline stress in bell pepper seeds (Hermans et al., 2016).

4. Conclusions

Given the results, it can be concluded that bell pepper seeds are sensitive to heat and salt stress, as the physiological potential of the seeds decreased at temperatures of $20 \text{ }^\circ\text{C}$ and $35 \text{ }^\circ\text{C}$ and with increasing concentrations of NaCl.

Authors' Contribution

Prof. Dr. Katiane Santiago Silva Benett, Natália Arruda and Valeska Cristina Souza Silva de Assis were responsible for study design and revising. Valeska Cristina Souza Silva de Assis, Jaiara Almeida de Oliveira and Natália Arruda were responsible for data collection. Prof. Dr. Katiane Santiago Silva Benett, Natália Arruda, Valeska Cristina Souza Silva de Assis and Jaiara Almeida de Oliveira drafted the manuscript. Prof. Dr. Katiane Santiago Silva Benett, Prof. Dr. Cleiton Gredson Sabin Benett and Natália Arruda revised it. All authors read and approved the final manuscript.

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