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Seed thermo-priming accelerates the growth of *Ceiba glaziovii* (Kutze) K.Schum. seedlings

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Abstract

Background: Global climate changes have caused temperature increases that can harm plant development, especially the initial growth and establishment of seedlings for the recovery of degraded areas. However, there are species in which these losses are attenuated when the seeds are thermo-primed to sublethal temperatures. Therefore, the objective of the present study was to evaluate the influence of seed heat conditioning on germination and initial growth of *Ceiba glaziovii* (Kuntze) K. Schum seedlings, an endemic species occurring in seasonally tropical dry forest in Brazil.

Methods: Seeds of *C. glaziovii* were exposed to 70 °C for 15, 30, 45, 60 and 90 minutes as pre-germination treatments. The control did not undergo heat treatment. Germination variables, vegetative growth, survival rate and normal leaves were evaluated.

Results: Thermo-priming negatively affected the germination proportion of *C. glaziovii* seeds with increasing exposure time. However, it significantly enhanced vegetative growth and seedling survival rate of this species after transplanting, without impairing the formation of normal leaves.

Conclusions: Thermo-priming of seeds was found to produce more vigorous seedlings of *Ceiba glaziovii*.

Keywords: High temperature; *barriguda*; vegetative growth; *paineira-branca*; germination; survival.

Introduction

Seed germination and seedling emergence can be promoted or limited by different environmental factors, depending on the heating level and response measured (Kigel 2017; Taiz et al. 2017). In this context, some plants have dormancy in their seeds as a survival strategy until favourable environmental conditions are established (Ferreras et al. 2018; Ferreira et al. 2022). The intensity of this protective mechanism has hampered the production of new seedlings, especially those used for reforestation and the recovery of degraded areas (Oliveira et al. 2019; Ferreira et al. 2022). This is more problematic due to the intensification of drought events caused by global climate change (Güneralp et al. 2015; Xu et al. 2019).

Techniques traditionally used to try to break seed dormancy are not always cheap, effective or safe, as is the case with chemical scarification with sulfuric acid or mechanical scarification with sandpaper or sharp materials (Nascimento 2012; Paixão et al. 2019; Santos et al. 2019). Some of these techniques can harm the applicator, the environment, or the seed embryo, consequently reducing the quantity and quality of the seedlings produced (Santos et al. 2019; Ferreira et al. 2022). Despite these problems, there is evidence that dormant seeds of some species can benefit when they are thermos-primed by promoting germination or increasing stress tolerance in their plants (Romero-Bastidas et al. 2016; Rafael et al. 2018; Pazzaglia et al. 2022; Santos Júnior et al. 2022).

Thermo-priming is prior exposure to different time intervals of elevated temperatures, at a sublethal level, to promote germination metabolic processes, reduce the resistance of rigid integuments by creating microcracks that promote the influx of water to embryonic tissues (Paparella et al. 2015; Rafael et al. 2018; Pazzaglia et al. 2022). Consequently, germination, initial growth and resistance to environmental stresses in plants were increased (Romero-Bastidas et al. 2016; Ling et al. 2018; Pazzaglia et al. 2022; Santos Júnior et al. 2022). Although the temperature range of 20 °C to 30 °C is considered optimal for germination, we know that some seeds need to be exposed to higher temperatures to germinate. However, prolonged exposure can damage the embryo, cause malformation, and reduce seedling vigour (Oliveira et al. 2017; Rafael et al. 2018; Santos Júnior et al. 2022).

Some plant species used for soil enrichment recovery of degraded areas, such as *Ceiba glaziovii* (Kuntze) K.Schum. (Lorenzi, 2009), may present tegumentary dormancy in their seeds, which makes the production of seedlings difficult (Nascimento 2012). Tegumentary dormancy (exogenous) is the impermeability of the tegument imposed by the tissues that surround the seed that prevents entry of water or oxygen because it is thick and rigid, or because it has chemical inhibitors, or because it has high mechanical resistance (Nicolau et al. 2022). *C. glaziovii* is an endemic species from northeastern Brazil, popularly known as "barriguda" or "paineira branca" due to its ability to retain a lot of water in the stem (Du Bocage & Sales 2002) and adjust osmotically under stressful conditions (Santos Júnior et al. 2020).

Studies that show the effects of thermos-conditioning on different aspects of germination and initial growth of *Ceiba glaziovii* are essential, but so far non-existent. This information can allow adequate management and production of *C. glaziovii* and other important plant species for soil enrichment projects and the recovery of degraded areas, production of more vigorous and resistant seedlings to environmental stresses, providing data on the dynamics of survival under high temperature conditions.

This study aimed to evaluate the influence of seed thermo-priming on germination and initial growth of *Ceiba glaziovii* planted as seedlings.

Methods

Study location, origin, and seed morphometric

The experiment was conducted under semi-controlled conditions in an agricultural greenhouse. The average temperature of the agricultural greenhouse ranged from 29.6 °C to 30.6 °C, and the average relative humidity ranged from 64.8% to 70% (Santos Júnior et al. 2020; Santos Júnior et al. 2021). *Ceiba glaziovii* seeds came from the municipality of Petrolina, Pernambuco. Averages of the morphometric measurements of 100 seeds, chosen at random, were: 4.43 mm wide, 6.37 mm long and 5.00 mm thick.

Pre-germination treatments and laboratory conditions

After being manually separated, 600 seeds were weighed, disinfected in 2% sodium hypochlorite (NaOCl) solution for 2 minutes, triple rinsed in distilled water and dried on absorbent paper. The seeds were then distributed in glass Petri dishes containing double filter paper and placed in a drying oven to be thermo-primed at 70 °C for six different exposure times as pre-germination treatments [(0 minutes – control, no exposure), 15, 30, 45, 60 and 90 minutes], with five replications of 20 seeds per treatment. After exposure, the plates were moistened, covered with plastic film and stored under laboratory conditions (25 °C). The temperature chosen was based on the temperature of the ground at a depth of 5-6 centimetres over the course of summer or post-fire events, according to studies (Rizzini 1976; Fichino et al. 2012).

Germination and vegetative growth variables

For 30 days, the following germination variables were evaluated: proportion of germinated seeds, germination speed index (GSI), synchrony index (SI) and time required for 50% of germination (T50%). Germination proportion was determined based on a total count of seeds that either germinated or did not germinate over the course of 30 days. Germination speed index was calculated using GerminatQuant software (Marques et al. 2015), based on the following formula:

$$GSI = SG1/D1 + SG2/D2 .... SGN/DN$$

GSI is the germination speed index, SG is the number of seeds germinated in the day and D are the days for germination to occur. Synchrony index (SI) was calculated according to the equation:

$$SI = \sum C_1,2/N$$

where $C_1,2 = n_i\ (n_i\text{-}1)/2$; and $N = \sum n_i(\sum n_i\text{-}1)/2$

Synchrony indices closer to 1 indicate that germination occurred more simultaneously, but when it is closer to 0 two or more seeds completed germination at different times. The time required for 50% (T50%) of germination to occur was calculated using a formula by Farooq et al. (2005):

$$T50 = t_i + ((N/2 - n_i) * (t_j - t_i)) / (n_j - n_i)$$

where N represents the total number of germinated seeds, n_i is the cumulative number of germinated seeds at time t_i, and n_j is the cumulative number of germinated seeds at time t_j, when $n_i < N/2 < n_j$.

After germination seedlings were transferred to polyethylene bags containing about 4 kg of a mixture of vegetable soil + cattle manure in a 3:1 ratio (v/v) and acclimatised for 30 days. Then, they were distributed according to the heat treatment received on the seeds in which four seedlings of each treatment were selected for growth analysis. Every seven days, for 30 days, vegetative growth was evaluated by counting the number of leaves, measuring plant height (cm) with a ruler and the basal stem diameter (mm) using a digital calliper (Digimess), following methods described by (Benincasa 2003). Furthermore, at the end of the experimental period, the influence of thermo-priming on seedling formation was evaluated, estimating the percentage of normal leaves and proportion of seedling survival.

Data analysis

GerminaQuant software version 1.0 (Marques et al. 2015) was used to calculate germination speed index (GSI) and synchrony index (SI). Data from germinative variables were tested for normality using the Shapiro-Wilk test. When parametric, they underwent analysis of variance (ANOVA), and means were compared using the Tukey test at a significance level of 5%. When non-parametric, they were subjected to Kruskall-Wallis, where means were compared by Dunn's post hoc test. Statistical analyses were conducted using Past software version 3.1. Linear regression was performed for the vegetative growth data of seedlings using SigmaPlot 12.0 software.

Results

Seed germination

Thermo-priming influenced the proportion of germinated seeds with an increase in the exposure time to high temperature (Figure 1), but did not significantly affect other germination variables (Table 1). The likelihood of seed germination tended to decrease as the exposure time at 70 °C approached 90 minutes (Figure 1). Germination ended 13 days after thermal exposure, in which there were no benefits to the germination of the species. Germination ranged from 20 to 41%, with an average time to 50% germination of 13.45 days. Germination was monitored for 30 days.

TABLE 1: Germination variables of *Ceiba glaziovii* seeds thermo-primed at 70 °C at different exposure times. ± standard error (n=100).

Exposure time (min) at 70 °C	T50% (days)[1*]	GSI (days^{-1})[2**]	SI[3**]
0	14.70±0.52 a	1.36±0.33 a	0.24±0.09 a
15	13.50±0.36 a	1.21±0.26 a	0.16±0.01 a
30	10.30±0.31 a	1.15±0.42 a	0.12±0.03 a
45	11.36±0.71 a	1.02±0.22 a	0.25±0.05 a
60	11.53±0.40 a	1.27±0.16 a	0.27±0.03 a
90	19.53±0.85 a	0.63±0.34 a	0.33±0.16 a
Mean	13.45	1.11	0.21

[1] Time to 50% germination (T50%).
[2] Germination speed index (GSI).
[3] Synchrony index (SI).
*Means followed by the same letter do not differ significantly according to the Dunn's post hoc test (P<0.05) or **Tukey (P<0.05).

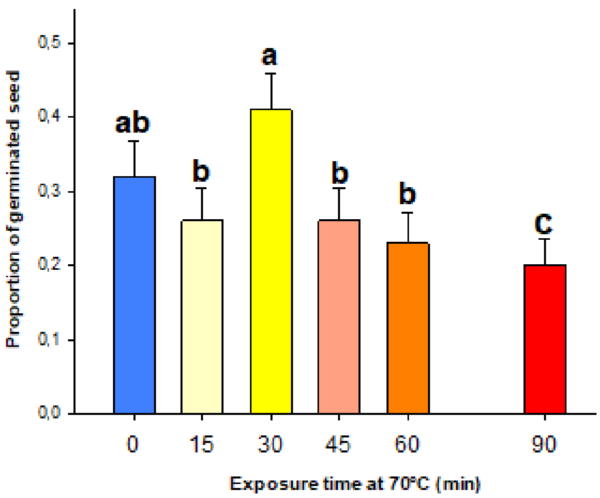

FIGURE 1: Proportion of germinated seeds of *Ceiba glaziovii* seeds thermo-primed at 70 °C at different exposure times (n=100). Means followed by the same letter do not differ significantly according to the Dunn's post hoc test (P<0.05).

Survival and normality of seedling leaves

Thermo-priming did not negatively affect the morphology of the leaves of *C. glaziovii* seedlings. In all treatments, 100% of the leaves were morphologically normal. Additionally, extending the exposure time of seeds to 70°C appeared to enhance the survival rate of seedlings after transplanting (Figure 2).

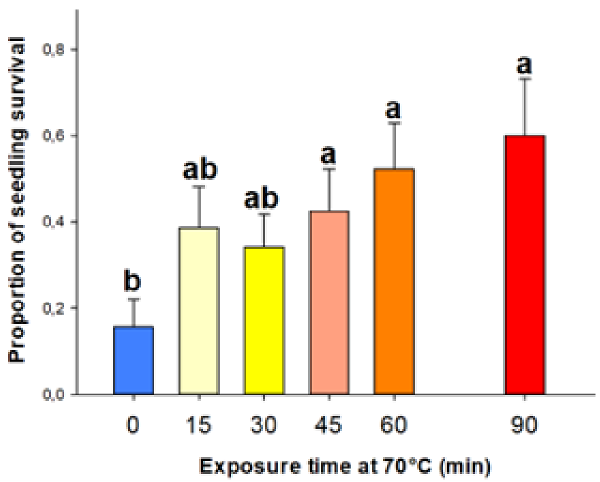

FIGURE 2: Proportion of survival of *Ceiba glaziovii* seedlings, obtained from seeds thermo-primed at 70 °C at different exposure times, after 30 days. Means followed by the same letter do not differ significantly according to the Dunn's post hoc test (P<0.05). ± standard error ($\bar{n}$=28).

Vegetative growth

Thermo-priming of seeds increased the vegetative growth of *Ceiba glaziovii* seedlings, with a linear response (Figure 3). At the end of the experimental period, the increase provided by thermo-priming in height ranged from 24 cm (seedlings from seeds exposed to 70 º for 45 minutes) to 51 cm (exposure to 90 minutes). In stem diameter, this increase ranged from 2.47 cm (60 minutes) to 5.42 cm (90 minutes). Similarly, at the end of the experimental period, there was an increase in the number of leaves, which varied from 4 (45 minutes) to 7.33 and 7.66 (90 and 15 minutes respectively) more than the final number of leaves of the seedlings control (5.5).

Discussion

The results obtained in this study suggest that *Ceiba glaziovii* does not present tegumentary dormancy. Although Nascimento (2012) used 11 pre-germination treatments to break tegumentary dormancy, the maximum germination obtained was less than 40% and in the control seeds about 30%. Our findings are like those observed by Nascimento (2012). We know that forest species generally have tegumentary dormancy in their seeds to increase their chances of survival (Dias et al. 2022). Although this happens and *Ceiba glaziovii* seeds are rigid, their integument is thin and they are not impermeable to water ingress. These

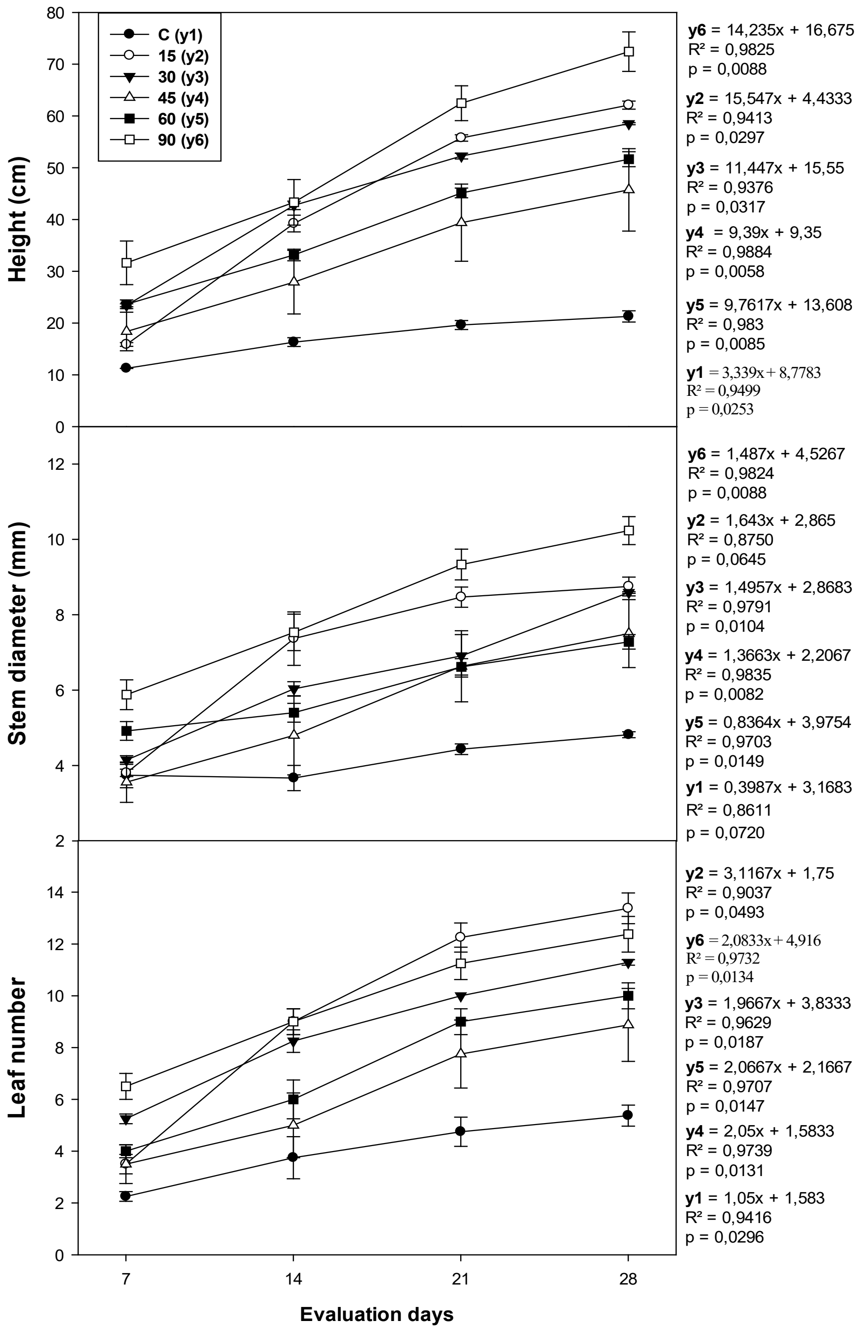

FIGURE 3: Height, stem diameter and number of leaves of *Ceiba glaziovii* seedlings obtained from seeds thermo-primed at 70 ºC at different exposure times. C (0 min as control), 15 min, 30 min, 45 min, 60 min and 90 minutes of exposure. ± standard error (n=4).

findings may indicate that other factors such as the presence of inhibitory substances, immature embryo or morphophysiological dormancy may be hindering germination (Nascimento 2012; Ferreira et al. 2022). Different studies show that tegumentary dormancy can be broken with thermo-priming, as this technique creates micro-cracks in the tegument, allowing to accelerate the entry of water, enzymatic and metabolic activity (Fichino et al. 2012; Romero-Bastidas et al. 2016; Rafael et al. 2018; Santana et al. 2019; Pazzaglia et al. 2022; Santos Júnior et al. 2022).

In addition, the increase in seedling survival after transplanting may be related to increased vigour (Paparella et al. 2015; Barazandeh et al. 2019; Santos Júnior et al. 2022) and resistance to stressful conditions promoted by thermo-priming (Rizzini 1976; Paparella et al. 2015; Ling et al. 2018; Pazzaglia et al. 2022). Evidence shows that thermo-priming activates an alarm period in seeds, allowing a "memory" of a stressful situation, such as exposure to high temperatures, through a stress mark that is stored during germination and is used in the future in environmental periods stressors, hardening the seedlings to respond appropriately to stress (Paparella et al. 2015; Farooq et al. 2019; Serrano et al. 2019; Pazzaglia et al. 2022). In this context, seedlings that have greater vigour, survival and resistance to environmental stresses are essential for places with stressful conditions such as degraded areas or poor soil (Barazandeh et al. 2019; Santos Júnior et al. 2020).

Although germination was not increased by thermo-priming in the present study, the higher vegetative growth of *Ceiba glaziovii* seedlings may be related to an early emergence of the seedling because of the reduced adherence to the seed coat and the creation of microcracks that weakened the seed coat (Rafael et al. 2018; Akhgari & Kaviani 2019; Bueno et al. 2019). In addition, this technique can induce the synthesis of proteins (Ling et al. 2018) and plant growth precursors, such as branched-chain amino acids, raffinose family oligosaccharides (RFOs), lipolysis products and tocopherols (Sani & Jodaeian 2015; Serrano et al. 2019; Pazzaglia et al. 2022). These benefits can be amplified in species adapted to hot climates, such as *Ceiba glaziovii*, as the technique increases its adaptive advantages (Paparella et al. 2015; Oliveira et al. 2021). However, information available in the literature on the role of thermo-priming in plant growth and yield is still scarce (Pazzaglia et al. 2022; Santos Júnior et al. 2022). Similar results were observed by Santos Júnior et al. 2022 in which seedlings of *Enterolobium contortisiliquum* (Vell.) Morong significantly increased vegetative growth when derived from seeds thermo-primed at 70 °C for 45 minutes. Maroufi, Farahani, & Moradi (2011) whose observed an increase in the vigour of *Triticum Aestivum* L. seedlings when exposed to 100 °C for 30 minutes. At the same temperature, for 10 minutes, *Raphanus sativus* L. showed greater seedling length, vigour and dry mass (Maroufi, Farahani & Aghdam 2011). Barazandeh et al.(2019) observed that heat conditioning seeds of *Carthamus tinctorius* L., for 6 h and 10 h at 60 °C, resulted in rapid emergence, establishment, and growth of seedlings, as well as an increase in agronomic characteristics of interest, corroborating the results found in the present study, which suggest the benefit of thermo-priming in seeds with characteristics similar to those of *Ceiba glaziovii*.

In summary, although prolonged exposure to high temperatures reduced the germination of *Ceiba glaziovii* seeds, an endemic species from the Brazilian northeastern semiarid region known as the Caatinga, this same exposure accelerated vegetative growth and appeared to enhance seedling survival (Figure 4).

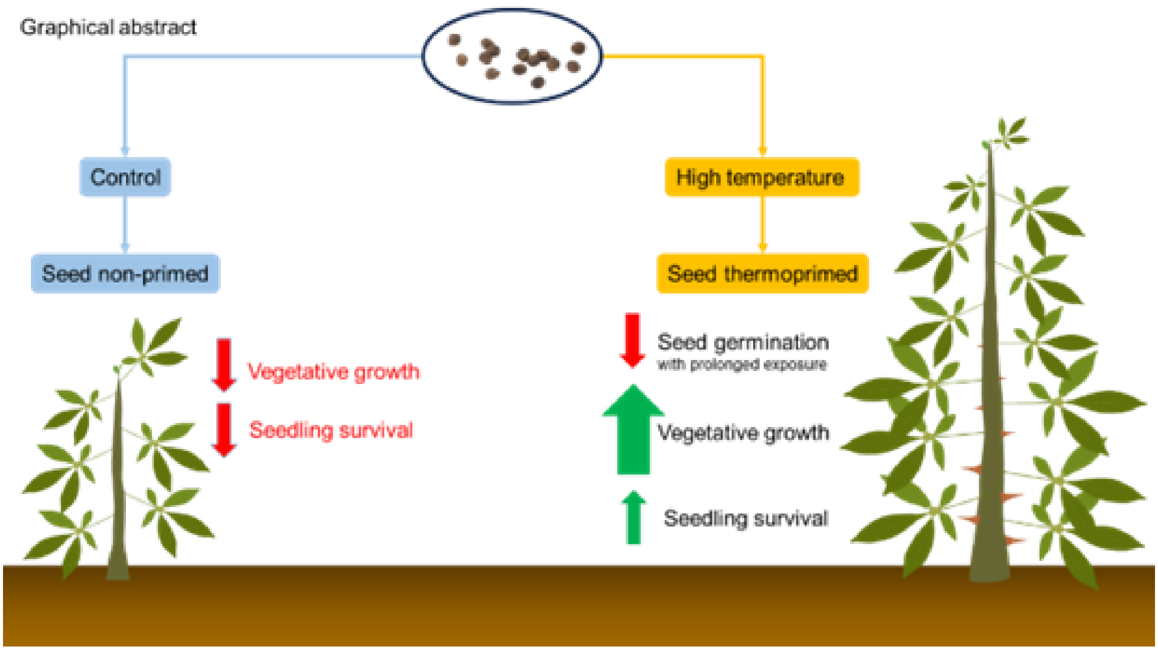

FIGURE 4: Overview of the effects of thermo-priming on the seeds and seedlings of *Ceiba glaziovii*. Red arrows indicate reduction, green arrows indicate increase. Larger arrow indicates the most influenced parameter.

Conclusions

In this study the results showed that *Ceiba glaziovii* seeds did not present tegumentary dormancy. We also saw that *Ceiba glaziovii* seeds thermo-primed at 70 °C produced seedlings with accelerated growth and greater chances of survival after transplanting, favouring the production of more vigorous seedlings within the time limit used in this study. This species is commonly used in programmes of soil enrichment and recovery of degraded areas, and, for these reasons, this cheap, simple and safe technique may enhance production of seedlings of this species.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JL d SJ conducted the experimental work, methodological analyses, writing, and manuscript discussion. EC da S carried out the methodological design, supervision of the experiment, discussion, and review of the manuscript. All authors read and approved the final manuscript.

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