Effects of saline stress and temperature on germination and seed vigor of *Luffa operculata* L. Cogn.

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Abstract

Luffa operculata (L.) is a native Brazilian biome plant used for the treatment of respiratory diseases. Although Brazil's flora comprises 67% of the world's plants, only 8% of Brazilian plant species are studied each year. The studies indicate that climate change in tropical biomes intensifies the increase in salinized areas by about 10% per year for several reasons, including high temperatures, low rainfall and high evapotranspiration rates, due to surface water scarcity. Therefore, the aim of the present study was to evaluate the germination behavior and vigor of *L. operculata* seeds submitted to saline stress at different temperatures. To simulate salt stress conditions, sodium chloride was applied at 0.0; 3.0; 6.0; 9.0 and 12.0 dS m^{-1}, at 25, 30, 35, 20-30 °C, with treatments distributed in a 5 x 4 factorial scheme (five levels of osmotic potential and four temperatures), using four replications. The seeds were then evaluated concerning water content, germination tests, first counts, germination speed index and seedling root and shoot lengths. At 30 and 35 °C, the seed germination and vigor of *L. operculata* were less affected up to an osmotic potential of 9.0 dS m^{-1}, where it is considered a moderate salinity tolerance. At 35 ºC, the germination uniformity was reduced to 98 and 93% by applying the 6 and 9 dS m^{-1} solutions, respectively. For the same temperature, the germination velocity remained 6.09 (IVG) until the saline potential of 9.0 dS m^{-1}.

Keywords: salinity, osmotic potential, environmental stress, seed vigor.

Introduction

Luffa operculata L. Cogn., is a native Brazilian plant, albeit not endemic, found along the Gulf of California in Mexico, Panama, the coast of Venezuela, Ecuador and Peru. It has been recorded in the Brazilian Amazon, Caatinga, Cerrado, Mata Atlântica and Pantanal (Filipowicz et al., 2014). Studies estimate that 86% of informal plant traders recommend *L. Operculata* use among the most used herbal medicines in Brazil, especially for the treatment of bacterial infections, such as rhinitis and rhinosinusitis (Silva et al., 2018). Although relevant, and although the Brazilian flora comprises 67% of the world's plants, but only 8% of Brazilian plant species are studied each year (Lima et al., 2016).

In recent years, traditional herbal medicine has been practiced in several countries around the world, such as Germany, Canada, China, the United States, Spain and Russia, given that 25% of industrialized compounds are plant-derived (Palhares et al., 2015). Therefore, the Brazilian flora is of interest for international scientific communities

concerning the research, conservation and rational use of these natural resources. However, environmental changes are occurring simultaneously worldwide, due to environmental pollution and global warming, which may induce rainfall regime changes and threaten the maintenance of plant genetic resources (Pereira et al., 2014; Pinto et al., 2018).

Studies indicate that climate change in tropical biomes intensifies the increase of salinized areas by about 10% annually for several reasons, including high temperatures, low rainfall and high evapotranspiration rates, due to surface water scarcity (Munns and Gilliham, 2015; Melo et al., 2017). It is estimated that 20% of the planet and more than 50% of arable land will be salinized by 2050, including coastal plains, leading to brackish water, due to proximity to the sea (Shrivastava and Kumar, 2015; Santos et al., 2018). During a given period of the year, salinity situations, both for soil and water, may compromise seed germination and

seedling establishment and survival (Martins et al., 2014; Zhang et al., 2019).

Temperature also significantly interferes with seed germination, especially when associated with salinity. The interaction of these factors affects speed and germination, due to influence on water absorption speed and biochemical reactions (Lin et al., 2018). Lower germination rates have been attributed to increased water evaporation at high temperatures, increasing salt concentration by capillary movement (Zhang et al., 2010). Therefore, the ability of certain species to germinate under water stress conditions confers ecological advantages over other species sensitive to saline stress-induced drought (Zhang et al., 2019).

One of the most widespread methods for the determination of plant tolerance to excess salts is the percentage of germination in saline substrates moistened with water (Zhang et al., 2012), as high intracellular Na^+ and Cl^- concentrations can inhibit embryonic cell metabolism during cell division and expansion. This, in turn, leads to delayed germination and may even result in seed death. In this method, the ability to germinate also indicates plant tolerance to salts in subsequent development stages (Lin et al., 2018).

The *L. operculata* germinative responses to environmental variables, particularly to salinity and temperature, have not been elucidated yet. The knowledge in this regard may subsidie management strategies for this species, mainly due to the importance of the plant for traditional medicine (Araújo et al., 2015). In addition, studies on the fibrous structure of the fruit of this species, which has been increasingly applied to energy converters and the immobilization of microbial cells in bioreactors are also paramount (Alves et al., 2018).

In view of the above, the aim of the present study was to evaluate the physiological potential of *L. operculata* seeds under the effect of different osmotic potentials induced by NaCl solutions and temperatures.

Results and Discussion

Seed water content ranged from 10 to 11% (values not shown) was considered uniform, since the maximum variation was of 1%. Seed water content similarity was paramount to avoid physiological potential effects by metabolic activity differences due to seed water content differences (Araújo et al., 2015; Munns and Gilliham, 2015; Pereira et al., 2016).

The maximum percentage of germinated seedlings was verified on the fourth day after sowing at 35 °C and submitted to the control treatment of 0.0 dS m^{-1} in the first germination test (Figure 1). For this temperature, germination uniformity was reduced to 98 and 93%, under 6 and 9 dS m^{-1} solutions, respectively. The osmotic potential required for maximum inhibition of the number of germinated *L. operculata* seedlings was 12 dS m^{-1}. This is an additional indication of the moderate salinity tolerance of this species, suggesting that seed germination may occur under restricted soil moisture conditions.

Germination decreased at all assessed temperatures with increasing osmotic potential, and the highest germination percentages of 97, 95 and 93% were obtained on a substrate moistened with deionized water (0.0 dS m^{-1}) at 25, 30 and 20-30 ºC, respectively (Figure 2). In addition, seed germination rates were higher than 80% even at high temperatures of 30 and 35 ºC until reaching an osmotic potential of 9.0 dS m^{-1}. At higher osmotic potentials, a marked decrease in germination up to 12.0 dS m^{-1} was noted. After this point, germination was occurred only at 25 °C.

Salinity affects seed germination through osmotic effects, ionic toxicity, or a combination of both. Therefore, seed water absorption restriction occurs due to the reduction of the potential gradient between the substrate and its surface, which is caused by the presence of salts, interfering with the water potential of the substrate (Pereira et al., 2016). However, salt tolerant species are referred because of having the ability to acquire a high osmotic potential. They allow the NaCl entry into the cells, using it as the osmolyte to maintain water absorption, which is essential for germination. In these plants, initial toxicity events during the osmotic phase of saline stress can be attenuated by the storage of the Na^+ and Cl^- ions in cell vacuoles (Zhang et al., 2012).

The interaction between temperature and salinity during seed germination may, in part, be the result of sensitivity to high Na^+ content diluted in the substrate, whose toxicity may decrease enzymatic activity, such as α-amylase and proteases and lipases responsible for reserve hydrolysis and consequently seed embryonic axis growth (Farooq et al., 2017). Similar studies carried out by Sohrabikertabad et al. (2013) on *Cucumis melo* L. seeds demonstrated that germination at 35 ºC was reduced from 91 to 7%, when NaCl saline conditions increased to 13 dS m^{-1}. For *L. operculata*, a reduction of 97 to 80% was observed at a NaCl concentration of 9 dS m^{-1}, probably demonstrating the considerable tolerance of this species to the environmental salinity.

Studies have addressed Na^+ concentrations in seedlings stressed with NaCl as a tolerance measure (Sohrabikertabad et al., 2013; Ibrahim et al., 2016; Farooq et al., 2017; Jha et al., 2019). Therefore, little attention is given to the possible individual toxicity of excessive levels of Cl^- ions to saline stress. However, substantial Cl^- concentrations are often found in germinated seedling tissues on substrates moistened with saline solutions. High Cl^- concentrations may be detrimental to cell integrity and may affect cell biochemistry through membrane damage or enzyme inhibition, especially if the vacuole can no longer sequester ions during seed imbibition (Teakle and Tyerman, 2010).

In plant cells, tolerance to salinity at high temperatures is due to plant response mechanisms, such as osmotic adjustment and antioxidant capacity, with salt toxicity attenuated by vacuolar compartmentalization (Farooq et al., 2017). Efficient salt tolerance responses at 35 ºC where observed herein (Figures 1 and 2) concerning germination uniformity and percentages. This suggests that metabolic *L. operculata* seed tolerance to negative salinity effects may be related to osmotic adjustment mechanisms, such as the probable vacuolar compartmentalization of Na^+ and Cl^- ions. Similar results were reported by Sohrabikertabad et al. (2013) after evaluating saline stress in *C. melo* seedlings germinated in NaCl at 35 ºC.

Similar to the results of the first germination count and germination percentage, the germination speed index (IVG) of *L. operculata* seeds was affected by the increased osmotic potentials of the NaCl solutions, evidencing salinity effects
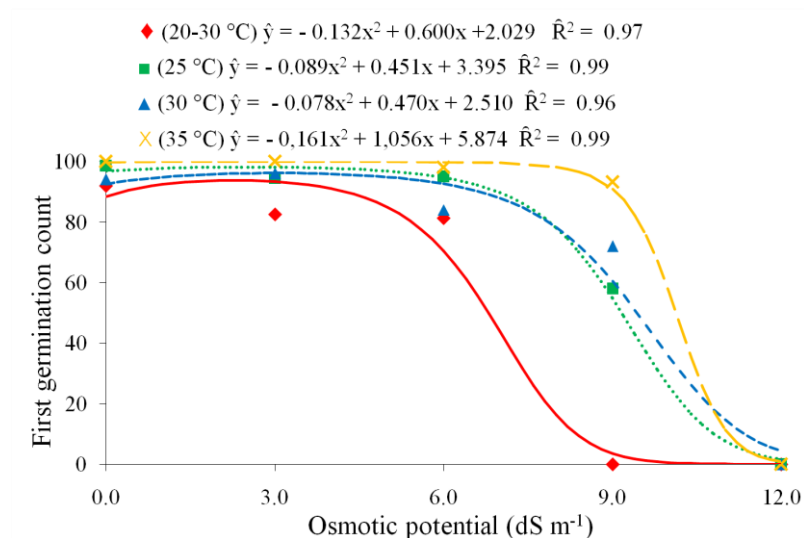
Figure 1. First germination count of *Luffa operculata* seeds subjected to different sodium chloride (NaCl) concentrations and temperatures.
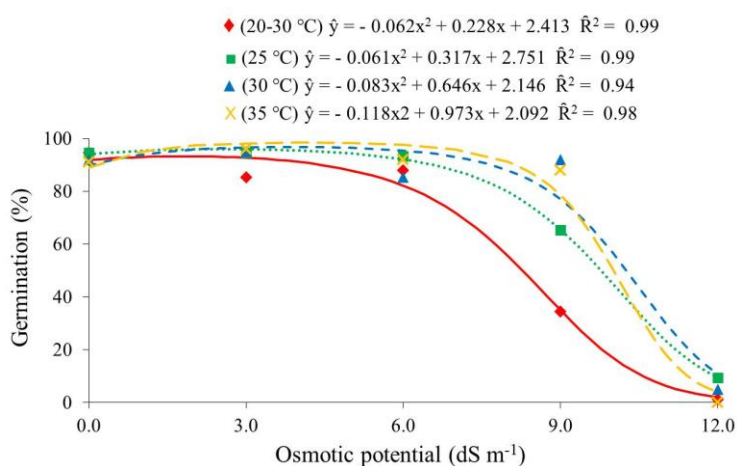

Figure 2. Germination (%) of *Luffa operculata* seeds subjected to different sodium chloride (NaCl) concentrations and temperatures.
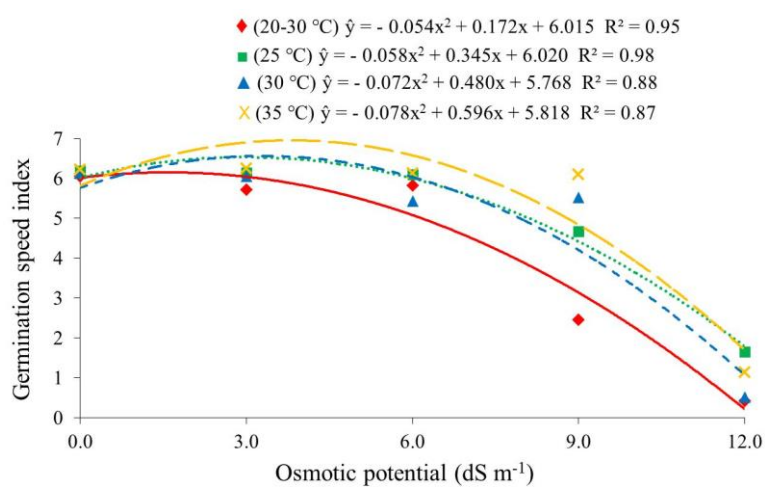

Figure 3. Germination speed index of *Luffa operculata* seeds subjected to different sodium chloride (NaCl) concentrations and temperatures.

Figure 4. Shoot length of *Luffa operculata* seedlings originated from seeds subjected to different sodium chloride (NaCl) concentrations and temperatures.
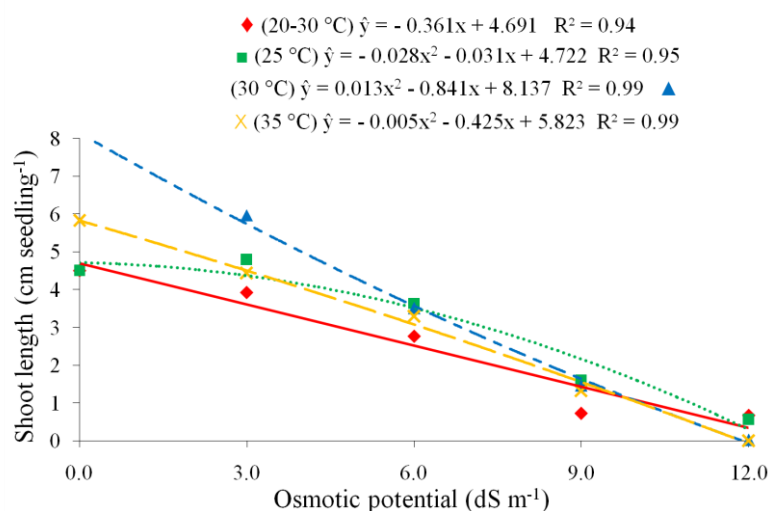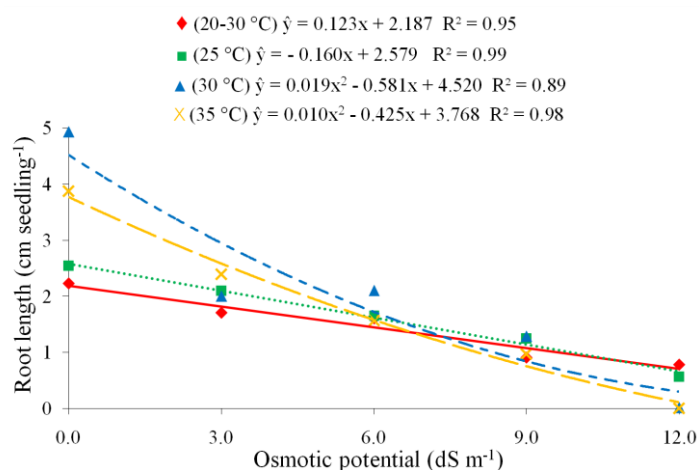

Figure 5. Root length of *Luffa operculata* seedlings originated from seeds subjected to different sodium chloride (NaCl) concentrations and temperatures.

on germination delay. However, water potential was significant, as evaluated by interaction with temperature, with increasing minimum water availability for germination speed even at higher temperatures (Figure 3).

The higher IVGs of 6.96, 6.57 and 6.53 were observed in the absence of salinity (0.0 dS m^{-1}) for seeds evaluated at 35, 30 and 25 ºC, respectively. In addition, significant reductions in IVG values where noted at increased saline concentrations, for all evaluated temperatures. However, decreases in germination speeds were less pronounced for seeds incubated at 35 °C, with IVG values maintained at about 6.09 up to a saline potential of 9.0 dS m^{-1} (Figure 3). These results support the hypothesis that *L. operculata* is tolerant to water stress induced by environmental salinization, mainly because germination speed is considered the first variable affected by decreased water availability (Zhang et al., 2010).

Usually, water stress, caused by salinity affects seeds, delaying water absorption and, consequently, the germination metabolism. Although environmental salinity inhibits germination, the harmful effect of salts is usually reduced at optimal temperatures (Marcos Filho, 2015), as observed in this study, where germination proceeded rapidly at thermal conditions between 25 to 35 ° C. This may be due to the positive interaction between salinity and temperature, which may have attenuated negative osmolarity effects, partially mitigated at high saline concentrations (Figures 2 and 3).

The relationship between environmental factors, such as high temperatures and saline stress, strongly impairs seed germination, probably by accelerating respiration and metabolic events, intensifying toxic salt effects and deterioration (Zhang et al., 2012). However, plant cell toxicity can be attenuated by the action of tolerance

mechanisms, allowing lower ionic cytoplasm concentrations and avoiding metabolic inhibition. Therefore, salinity tolerance may have ecophysiological implications (Farooq et al., 2017), as seeds capable of effectively responding to environmental changes and altering their germination physiology are more likely to survive and establish (Ibrahim et al., 2016; Jha et al., 2019). Regarding shoot and root lengths (Figures 4 and 5), *L. operculata* seedlings presented similar behavior to the IVG at each temperature, with decreasing values with increasing osmotic potentials. Seedlings submitted to germination at 30 °C and water-wetted substrate (0.0 dS m^{-1}), reached the highest shoot and root length values, with means of 8.14 and 4.52 cm/seedling^{-1}, respectively. On the other hand, increase in saline concentrations led to marked decreases of these length parameters at 30 and 35 ºC (Figures 4 and 5).

Saline stress can affect germination by inhibiting water uptake and/or Na$^+$ and Cl$^-$ ion accumulation. These negative effects can be attenuated by the ion binding to seed starch reserves. This mechanism acts in osmotic adjustment control by using organic solutes. Therefore, it probably requires an intensive energy demand, which can compromise seedling growth by leading to metabolic resource unavailability, i.e. sugars. Thus, it can be assumed that metabolic NaCl tolerance would be more important for seeds than other plant life cycle phases, due to their limited carbohydrate reserves (Flowers and Colmer, 2015). Another factor that affects seedling development at the end of the germination process occurs with radicle emission, the first part of the seed to emerge during germination. At this moment, the cellular protoplasm is exposed to excessive Na$^+$ and Cl$^-$ concentrations diluted in the substrate water that surrounds the radicle zone, whose toxic effect favors the reduction of root and seedling elongation rates (Farooq et al., 2017). Studies on seed germination have reported that excess Na$^+$ is often considered to be responsible for seedling growth reduction under salt conditions, while increasing intracellular Cl$^-$ concentrations can restrict cell division metabolism and expansion stages (Keshavarzi, 2011).

There is indirect evidence for some plant species that the ability to accumulate Cl$^-$and Na$^+$ ions in the cytoplasm and cell wall cell vacuole is associated with salt tolerance. However, high concentrations of these ions in the plant cell wall may result in reduced turgor, resulting in atrophied seedlings (Hanin et al., 2016), which is probably the case of the present study. In addition, salinity can promote the accumulation of free amino acids, ammonium and nitrate within seedling cells, reducing the activities of enzymes involved in nitrogen assimilation, necessary for plant tissue formation. For example, in *Curcubita pepo* L., saline stress has been reported as suppressing the action of the enzyme NADH-glutamate synthase and glutamine synthetase (Teakle and Tyerman, 2010; Zhang et al., 2019). The high salinity concentrations induced by NaCl also negatively affect seedling length in other cucurbitaceae, such as *Cucumis anguria* L. (Alves et al., 2014) and *C. pepo* (Harter et al., 2014). Guedes et al., (2011) assessed *Chorisia glaziovii* O. Kuntze seeds and observed that seedling length was less affected by salinity at 25 and 20-30 ° C, similar to the results obtained in the present study. Salinization is a stress factor for plants, as water is osmotically retained in saline solution. Increase of saline concentrations reduces water availability (Munns and Gilliham, 2015).

Materials and Methods

Fruits were collected from ten matrix *L. operculata* plants in the municipality of Areia - PB, Paraíba, Brazil (06° 57' 46" S 35° 41' 31" W). The local climate is classified as hot tropical semi-arid, with an annual average rainfall of 838.1 mm, concentrated from February to April, with an average temperature of 26 to 28 ºC (Ipece, 2005).

The collected material was packed in polyethylene bags and taken to the Seed Analysis Laboratory belonging to the Agricultural Sciences Center of the Federal University of Paraíba, Brazil. The fruits were manually opened for seed removal. The seeds where then placed on paper towels to dry at room temperature (25 ± 3 ºC) for 24 hours. They were then homogenized and the following attributed and tests were performed.

Water content

Water content was determined by the greenhouse method, by maintaining the seeds at 105 ± 3 °C for 24 hours. (Brasil, 2009), using four 25-seed replicates.

Osmotic potential assessments

Saline stress was simulated using sodium chloride (NaCl) as the solute at the following concentrations: 1.76, 3.53, 5.31 and 7.08 g L^{-1}, diluted in distilled and deionized water. The treatments constituted by osmotic potentials were confirmed by the electrical conductivity of the solutions: 3.0, 6.0, 9.0 and 12.0 dS m^{-1}, using a digital conductivity meter. For the control treatment, distilled and deionized water was used to moisten the substrate.

The electrical conductivity values of the NaCl solutions were obtained using the following equation: $CS = \dfrac{0.001(CEs - CEan)Peq}{0.97}$) proposed by Richards (1954), where; CS = 0.001 (CEs - CEan) Peq/0.97, in which CS = concentration (g L^{-1}); CEs = electrical conductivity of the water of the mixture at 25 °C (dS m^{-1}), CEan = electrical conductivity of the water (dS m^{-1}), Peq = equivalent weight of the salt and 0.97 = sodium estimated chloride purity percentage.

Germination test

Four batch of 50-seed replicates were treated with the Captana® fungicide at a concentration of 240g/100 kg^{-1}of seeds. They were then distributed onto two Germitest® paper towels, covered with a third sheet and arranged as a roll. The substrate was moistened with distilled and deionized water or NaCl solutions, using an amount equivalent to 2.5 times the mass of the sheet prior to hydration. The rolls were placed in transparent 0.04 mm thick plastic bags to reduce water loss through evaporation.

The treatments were evaluated by seed germination, conducted in chambers regulated at 25, 30, 35 and 20-30 ± 3 ºC with a12-hour photoperiod. The evaluations were carried out by daily counting from the fourth to the eighth day after the beginning of the tests (Araujo et al., 2015), compared to normal seedling germination (Brasil, 2009). Data were expressed as percentages.

First germination count

This test was conducted alongside the germination test, with normal seedling counts performed on the fourth day after the beginning of the test. Data were expressed as percentages.

Speed germination index

This test was conducted alongside the germination test, from the fourth to the eighth day after sowing, computing the number of germinated seedlings per day and applying the formula proposed by Maguire, (1962).

Evaluation of seedling development

This character was evaluated at the end of the germination test by measuring the length of seedling aerial portions and primary roots, using a centimeter-graduated ruler. The results were expressed as cm/seedling $^{-1}$ (Nakagawa, 1999). The experimental design was completely randomized, with treatments distributed in a 5 x 4 factorial scheme (five levels of osmotic potential and four temperatures), using four replications. The data were submitted to an analysis of variance and polynomial regression. The linear and quadratic models were tested, selecting the significant higher order model (R^2). The variables referring to percentages and first germination counts were submitted to a binomial logistic analysis, using the SAS/STAT® software version 9.3 (Barbosa and Maldonado Júnior, 2015).

Conclusions

L. operculata seed germination and vigor at 30 and 35 °C were less affected up to an osmotic potential of 9.0 dS m^{-1}, at which it developed a moderate tolerance to salinity. *L. operculata* tolerates up to 9.0 dS m^{-1}, about 7.61 g L^{-1} of NaCl salinity, and is relatively tolerant to salt-induced water stress during germination and initial seedling growth stages. *L. operculata* seeds are more adapted to germinate under salt stress moderate, between 6.0 and 9.0 dS m^{-1}. At the temperature of 35 ºC, germination uniformity was reduced to 98 and 93%, when applying the 6 and 9 dS m^{-1} solutions were applied, respectively. For the same temperature, the germination velocity (IVG) remained at about 6.09 until the saline potential of 9.0 dS m^{-1}.

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