ISSN 0104-7760
v.25 n.1 2019

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DORMANCY BREAKING, GERMINATION, AND PRODUCTION OF *Mimosa bimucronata* (DC.) KUNTZE SEEDLINGS

GIASSON, C.; BARETTA, C. R. D. M; SOBRAL, L. S.; BALDISSERA, R. Dormancy breaking, germination, and production of *Mimosa bimucronata* (DC.) kuntze seedlings. **CERNE**, v. 25, n. 1, p. 68-75, 2019.

HIGHLIGHTS

Higher germination of seeds was obtained with water treatments.

This finding is not supported by present Brazilian Rules for Seed Analysis.

Germination must preferably be conducted in germitest paper at 25 °C.

Emergent seeds must preferably be sown in bigger containers under shaded conditions.

Germination and seedling attributes indicate the species can be used as an intermediate successional plant.

Keywords:
Container
Scarification
Substrate
Temperature

ABSTRACT

Mimosa bimucronata is a pioneer, easily adapted, fast-growing species used in restoration programs. The objectives of this study were to experimentally compare (1) methods for dormancy breaking; (2) suitable temperature and substrate for germination; and (3) suitable light intensity and substrate volume for seedling production of *M. bimucronata*. In the first two experiments, the variables percentage of germination, speed of germination and germination mean time were analyzed. In dormancy test, seeds were submitted to ten methods divided into three groups: hot water, sulfuric acid, and control. In the second experiment, effects of five different temperatures and four substrates were evaluated. In the third experiment, morphometric and biomass variables of seedlings were evaluated in two substrate volumes and two light intensities. The most effective method for breaking dormancy was hot water at 80 °C for one minute with post-treatment immersion in water at room temperature for 24 hours. This finding contrasted with the recommended method for dormancy breaking in the Brazilian Rules for Seed Analysis. The most suitable substrates and temperatures for germination were germitest paper at alternating temperatures of 25-35 °C and sand at constant temperature of 30 °C. The most adequate volume of substrate for seedling production was 280 cm^3 in both shaded and full sunlight conditions.

Historic:
Received 07/11/2018
Accepted 04/01/2019

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DOI:
10.1590/01047760201925012612

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INTRODUCTION

Natural resources exploitation has been causing intense degradation of forest ecosystems worldwide, stimulating the practice of restoration programs. Climate and soil are potentially limiting factors for forest restoration and presence of early successional species often changes the environment, facilitating the colonization of later-successional species (Marcuzzo et al., 2015). Consequently, production of viable forest seedlings has grown as an academic and professional activity aimed at helping restoration projects (Oliveira et al., 2014).

Mimosa bimucronata (DC.) Kuntze (Fabaceae) is distributed along the Atlantic forest domain in Brazil. It is a selective hygrophilous, invasive species able to colonize poorly drained and rocky soils, hence being a suitable species for environmental restoration programs. *Mimosa bimucronata* seeds show physical dormancy caused by seed coat impermeability (Ribas et al., 1986; Brito et al., 2014) resulting from the hardness due to the presence of superficial waxy layer and great quantity of suberin, deposition of lignin on cell base, and presence of fatty acids in intercellular spaces of palisade layer (Fowler and Bianchetti, 2000; Marcos Filho, 2015), which hamper oxygen and water absorption necessary for seed germination.

Ecologically, dormancy ensures spatial-temporal distribution of germination, but for seedling production the attribute is not desirable since there may be low percentage of germination (Abreu et al., 2017). Therefore, pre-germination treatments reduce time between sowing and seedling emergence, providing higher quality seedlings able to better adapt to harsh environmental conditions (Abreu et al., 2017).

Seed treatment with chemical scarification and hot water are efficient pre-germinative methods generally used to overcome seed dormancy in *Mimosa* species (Sperandio et al., 2013; Brito et al., 2014; Decezare et al., 2015). Ribas et al. (1996) showed that immersion of seeds in water at 80 °C followed by 24 hours of natural cooling, as well as sulfuric acid immersion for five minutes, were efficient methods to promote dormancy breaking in *Mimosa bimucronata*. For *Mimosa setosa*, Sperandio et al. (2013) showed that sulfuric acid and hot water (70 and 80 °C) treatments were efficient. On the other hand, sulfuric acid treatment for 5 and 10 minutes was the best dormancy-breaking method for *Mimosa ophthalmocentra* (Brito et al., 2014). Acid scarifications can enhance seed germination (Baskin and Baskin, 2014). Corrosion produced by the solution leads to seed coat cracking, while immersion in hot water promotes greater permeability (Floss, 2011) by promoting seed coat softening, which together with heat can stimulate germination.

Temperature and substrate can affect the proportion and uniformity of germination (Popinigis, 1985). The two factors act either alone or interactively and different germination responses can be obtained when seeds are submitted to the same temperature (Figliolia and Piña Rodrigues, 1995). Constant temperatures can start germination in some species of *Mimosa* genus, while some others need alternating temperatures, and others can germinate with both methods (Nogueira et al., 2013; Holanda et al., 2015; Shibata et al., 2016).

Besides pre-germinative methods, the quality of seedlings produced in a nursery can be influenced by several factors, such as water and nutrients availability, as well as light intensity and container size. Use of inadequate containers can influence both root and shoot systems, affecting the whole plant growth (Vargas et al., 2011). Light is directly associated with plant development via many physiological processes (Floss, 2011), and different Fabaceae species respond differently to light exposure. Câmara and Endres (2008) studied the optimal shading level to produce *Mimosa caesalpiniifolia* and *Sterculia foetida* seedlings and found that both species developed greater stem diameter and root growth at 50% shade. Same pattern was found for *Swietenia macrophylla* in different substrates and shaded conditions produced in vegetal compounds (Roweder et al., 2015). On the other hand, *Copaifera langsdorffii* seedling height and number of leaves were higher at full sun (Lopes et al., 2013); and *Hymenaea courbaril* showed higher emergence in that condition (Carvalho Filho et al., 2003). For *Sclerolobium paniculatum*, the best conditions were 50% shade followed by full sunlight exposure (Felfili et al., 1999).

Therefore, our study aimed to experimentally test (1) water and acid treatments to overcome dormancy, (2) substrate and light treatments to germinate seeds, and (3) light and container volume treatments to grow seedlings of *Mimosa bimucronata*.

MATERIAL AND METHODS

Seeds of *M. bimucronata* were bought from Bentec Seeds (Rio do Sul municipality, state of Santa Catarina, Brazil) and, according to the company, propagules were collected in May 2016 in a forest fragment in Ijuí municipality (28°23'21.15" S - 53°55'14.66" O, 319 m), southern Brazil. Seeds were stored in a climatized environment (17 °C) and were processed before starting the study. Experiments were performed in the Laboratory of Seed Analysis at the Chapecó Region Community University from May to December 2017.

Dormancy-breaking and Germination Experiments

For dormancy breaking (Experiment 1), we used 4,000 seeds randomly distributed in four replicates of

four sets of categorical variables, totaling 10 treatments: (1) 96% sulfuric acid scarification for 1, 5 and 10 minutes; (2) 80 °C hot water immersion for 1, 5 and 10 minutes; (3) 80 °C hot water immersion for 1, 5 and 10 minutes with post-treatment immersion in room temperature water for 24 hours; and (4) control treatment. Seeds were distributed on three blotters moistened with 2.5 times their weight. The blotters were placed in seed germination boxes (11 × 11 × 3 cm), which were placed in a Mangelsdorf germinator with constant temperature of 25 °C. Seeds were counted for 12 consecutive days.

For germination tests (Experiment 2), we used 8,000 seeds distributed in a factorial design (4 substrates × 5 temperatures) with four replicates of 100 seeds per treatment. Dormancy was broken by submitting seeds to 80 °C hot water for 1 minute with post-treatment immersion in water for 24 hours. The choice of dormancy overcoming method was based on the results obtained in Experiment 1. Seeds were buried (1 cm depth) in substrates in germination boxes with (1) 150 g of sterilized (105 °C/24 hours) fine sand moistened with 60% of its water retention capacity; (2) 250 g of vermiculite moistened with 60% of its water retention capacity using distilled water; (3) germitest paper moistened with two times its weight (Brasil, 2009); and (4) three blotters moistened with 2.5 times their weight using distilled water. The treatments were randomly placed in Mangelsdorf germinators with white light and daily photoperiod of 12/12 hours at constant temperatures of 20, 25, and 30 °C; and in biochemical oxygen demand (BOD) germinator with alternating temperatures of 20-30 and 25-35 °C. Germination was monitored for 13 consecutive days.

For dormancy and germination experiments we analyzed the variables (1) germination percentage (G) as the number of seeds protruding 2-mm-long primary roots; number of abnormal and inviable seeds were also counted following the Rules for Seed Analysis (Brasil, 2009); (2) speed of germination (SG) (Maguire, 1962) as, where G_i = number of normal plants counted from the first to the last day, and N_i = number of monitoring days; and (3) germination mean time (in days) (Labouriau, 1983). Where n_i = number of normal plants in each count interval and t_i = running time from start to the last monitoring day.

$$SG = \sum (Gi / Ni) \quad [1]$$

$$GMT = \left(\sum ni.ti / \sum ni\right) \quad [2]$$

Seedling Growth Experiment

A completely randomized experiment was performed in the Unochapecó Forest Vivarium. Before the experiment, we broke the dormancy of the seeds as described in *Dormancy-breaking and germination experiments* section (Experiment 1) using hot water at 80°C for one minute with post-treatment immersion in water at room temperature for 24 hours.

Emergent seeds were sown directly in conical containers inserted in plastic trays 80 cm above ground filled with organic compost produced in the University's compost bin. We performed a bifactorial experiment with seedlings submitted to (1) the factor light intensity, full sunlight and 50% shade; and (2) the factor container volume, 100 cm^3 and 280 cm^3. The shaded condition was achieved by using a plastic screen over the plastic trays in the shaded treatment. Each treatment contained three replicates (plastic trays) of 30 seedlings. Organic compost basic composition was moisture content: 34.49% (65 °C); pH: 6.8; P_2O_5: 0.20%; K_2O: 0.20%; Ca: 2.72%; Mg: 0.43%; N: 1.61%. Analyses were performed at the Agricultural Research and Rural Extension Company of Santa Catarina (Epagri).

After 90 days, we measured seven plant attributes in the 12 central seedlings: collar diameter (CD), plant height (H), number of leaves, total dry weight (TDW), shoot dry weight (SDW), root dry weight (RDW), and Dickson quality index (Dickson et al., 1960).

$$DQI = TDW / (H / CD + SDW / RDW) \quad [3]$$

Morphometric variables were measured with a caliper rule. Root and shoot parts were packed in Kraft paper sacks, dried (65 °C) in muffle until constant weight and measured with precision analytical scale. Seedlings of the three central rows of the plastic trays were used to measure morphometric variables. The border seedlings were disregarded. The mean of the 12 central seedlings were used as treatment replicates.

Data analysis

Dormancy breaking

ANOVAs were performed to test G, SG and GMT mean differences among treatments with Tukey's *a posteriori* test. Normal distribution assumption was tested with Shapiro-Wilk's test and homoscedasticity with Levene's test. SG data showed heteroscedasticity ($p = 0.02$), so means were compared with the Welch's F test (Logan, 2010).

Germination

Two-way ANOVAs were performed to test G, SG and GMT mean differences among treatments

with Tukey's *a posteriori* test. We log-transformed G non-normal data as *logit* = *log* (*p*/1 - *p*), where *p* is the proportion of germination in each experimental unit. *Logit* transformation is suitable for percentage data because it maps monotonically the proportions and possesses a natural interpretation of data, contrasted to the familiar arcsine transformation (Warton and Hui, 2011).

Seedling growth

Two-way ANOVAs were performed to test differences among treatment means of the response variables collar diameter, plant height, shoot dry weight, root dry weight, and DQI. We used a permutational two-way ANOVA (Bonnini et al., 2014), a nonparametric analysis that used sum of squares as test criterion, to test differences among treatment means of number of leaves, since this variable did not follow parametric assumptions even after transformations.

RESULTS

Dormancy breaking

Germination started at day 2 and the experiment ended at day 12 when germination stabilized. Mean percentage of germination (G) was significantly lower in the control treatment ($p < 0.001$), which also showed lower speed of germination and larger number of hard seeds and dead seeds percentage. Seeds in control germinated three to five times slower compared to the other treatments. Percentage of germination was consistently high in the hot water treatments during the experiment, which also led to higher values of speed of germination and lower germination mean time. Treatments of hot water for 1 and 5 minutes with post-treatment immersion in water for 24 hours germinated faster and showed the lowest germination mean times. Acid treatments showed lower germination speed index and longer germination mean time when compared to the hot water treatment (Table 1).

Germination

Germination started at day 3 and the largest number of abnormal seedlings was found in the blotter under alternating temperatures. There was a significant interaction between substrate and temperature effects on the means of G, SG, and GMT ($p < 0.001$). In general, 25 °C temperature promoted higher germination in all substrates, but in germitest substrate germination showed the best performance. For substrates sand and vermiculite, higher percentages of germination occurred at constant temperatures of 20, 25, 30 °C and alternating temperatures of 20-30 °C. Blotter substrate showed higher germination at 25 °C and germitest at 25 °C. Alternating temperatures of 20-30 °C and 25-35 °C promoted higher germination in germitest paper (Table 2).

TABLE 1 Means ± standard errors of germination (G), speed of germination (SG), and germination mean time (GMT) of *Mimosa bimucronata* seeds submitted to dormancy-breaking treatments.

Treatment	G (%)	SG	GMT (days)
Control	25.00±0.58 d	5.61±0.23 d	5.97±0.09 d
H_2SO_4,1 min.	65.00±0.67 c	14.85±0.22 c	4.62±0.23 a
H_2SO_4,5 min.	65.00±0.53 c	13.59±0.18 c	5.42±0.19 b
H_2SO_4,10 min.	75.00±0.39 b	16.17±0.16 c	5.03±0.14 b
80°C, 1 min.	92.00±0.32 a	19.05±0.13 b	5.35±0.41 b
80°C, 5 min.	89.00±0.38 a	21.32±0.38 b	4.45±0.12 a
80°C, 10 min.	86.00±0.40 a	19.06±0.13 b	5.07±0.20 b
80°C, 1 min., 24 h	89.00±0.15 a	28.01±0.45 a	3.47±0.37 a
80°C, 5 min., 24 h	86.00±1.17 a	26.77±0.50 a	3.51±0.31 a
80°C, 10 min., 24 h	86.00±0.22 a	19.88±0.50 b	4.51±0.20 a

Means followed by same letter do not differ at 5% significance level (*a posteriori* Tukey's test).

Speed of germination mean was higher at temperatures of 20 and 25 °C in the vermiculite and blotter substrates. Alternating temperatures of 20-30 °C and 25-35 °C in the germitest paper and at temperature of 30 °C and alternating temperatures of 20-30 °C in sand resulted in lower seed germination mean times (Table 3).

GMT means were lower in sand at temperature of 30 °C and in germitest paper at alternating temperatures of 25-35 °C. At the temperature of 25 °C, GMT means in all substrates were also low and did not differ. Higher GMT mean was found in blotter at alternating temperatures of 25-35 °C (Table 4).

TABLE 2 Means ± standard errors of percentage of germination (G) of *Mimosa bimucronata* seeds submitted to different temperature and substrate treatments.

	Temperature				
Substrate	20 °C	25 °C	30 °C	20-30 °C	25-35 °C
Sand	94.00 ±1.80 Aa	95.50 ±1.00 Aa	94.00 ±2.90 Aa	95.00 ±1.50 Aa	90.00 ±2.90 Bb
Vermiculite	95.00 ±1.50 Aa	95.00 ±1.70 Aa	94.00 ±2.20 Aa	94.00 ±1.80 Aa	86.50 ±6.90 Bb
Blotter	89.50 ±3.80 Bb	96.50 ±1.90 Aa	89.00 ±0.80 Bb	86.00 ±3.50 Bb	75.00 ±7.80 Cc
Germitest	93.50 ±1.20 Ba	94.00 ±0.50 Aa	92.00 ±1.20 Ba	95.00 ±1.26 Aa	96.00 ±0.80 Aa

Means followed by the same uppercase letter in the row and lowercase letter in the column show temperatures or substrates without significant differences, respectively (*a posteriori* Tukey's test).

TABLE 3 Means ± standard errors of speed of germination (SG) of *Mimosa bimucronata* seeds submitted to different temperatures and substrates.

	Temperature				
Substrate	20 °C	25 °C	30 °C	20-30 °C	25-35 °C
Sand	14.57 ±1.40 Bb	19.45 ±1.10 Ba	22.66 ±1.00 Aa	21.70 ±1.50 Aa	15.89 ±1.70 Bb
Vermiculite	15.99 ±0.30 Aa	18.06 ±0.40 Aa	18.23 ±1.10 Ab	16.28 ±2.00 Ab	14.86 ±2.30 Bc
Blotter	14.72 ±0.90 Bb	19.4 ±1.10 Aa	17.85 ±1.00 Ab	15.65 ±0.50 Bb	10.90 ±1.10 Cc
Germitest	17.22 ±0.50 Ba	20.05 ±1.00 Aa	18.36 ±2.10 Bb	21.79 ±2.50 Aa	24.17 ±3.40 Aa

Means followed by the same uppercase letter in the row and same lowercase letter in the column show temperatures or substrates without significant differences, respectively (*a posteriori* Tukey's test).

TABLE 4 Means ± standard errors of the germination mean time (GMT), in days, of *Mimosa bimucronata* seeds submitted to different temperatures and substrates.

	Temperature				
Substrates	20 °C	25 °C	30 °C	20-30 °C	25-35 °C
Sand	6.56 ±0.10 Bb	5.38 ±0.20 Aa	4.65 ±0.20 Aa	5.00 ±0.30 Aa	6.63 ±0.50 Bb
Vermiculite	6.49 ±0.10 Bb	5.59 ±0.20 Aa	5.84 ±0.20 Bb	6.21 ±0.60 Bb	6.43 ±0.40 Bc
Blotter	6.79 ±0.10 Cb	5.37 ±0.30 Aa	5.61 ±0.30 Ab	6.06 ±0.10 Bb	7.09 ±0.10 Cc
Germitest	5.92 ±0.20 Ba	5.09 ±0.10 Aa	5.44 ±0.70 Ab	5.12 ±0.60 Aa	4.55 ±0.70 Aa

Means followed by the same uppercase letter in the row and lowercase letter in the column show temperatures or substrates without significant differences, respectively (*a posteriori* Tukey's test).

Seedling growth experiment

There was a significant interaction between light intensity and substrate volume on the number of leaves, but other variables responded only to substrate volume (Table 5). There were 26 more leaves in seedlings grown in larger volumes and under shaded conditions compared to full sunlight and smaller volumes. Larger substrate volume increased the seedling height by 10 cm, and collar diameter by 0.62 mm. Root and shoot dry weights increased by 2.61 g and 5.9 g in larger volume, respectively. Finally, DQI mean was 44% larger in that substrate volume.

TABLE 5 Means ± standard errors of seven attributes of *Mimosa bimucronata* seedlings submitted to different light intensities and substrate volumes.

	Light intensity (%)		Substrate volume (cm^3)	
Variables	Full sunlight	Shaded	100	280
Collar diameter (mm)*	1.98±0.43	1.90±0.46	1.64±0.24	2.24±0.40
Dickson quality*	0.76±0.27	0.71±0.30	0.51±0.09	0.96±0.18
Number of leaves#	39.50±11.07	41.98±16.46	29.8±6.42	51.50±10.73
Plant height (cm)*	28.38±6.37	29.79±7.20	24.18±4.40	33.9±5.12
Root dry weight (g)*	4.05±1.46	4.18±1.46	2.82±0.50	5.37±0.52
Shoot dry weight (g)*	9.40±3.49	8.67±3.51	6.04±1.00	12.01±1.58
Total dry weight (g)*	13.44±4.90	12.84±4.87	8.90±1.35	17.38±1.75

* Significant effect of substrate volume. # Significant interaction between light intensity and substrate volume.

DISCUSSION

Percentage of germination of *M. bimucronata* in hot water in our study was higher than those of other species of *Mimosa* (Santos et al., 2011). Times of acid exposure in the present study negatively affected germination (Pacheco and Matos, 2009). Different responses to acid could be found in other leguminous tree seeds, and for *Mimosa artemisiana* five minutes of immersion yielded 20% of germination (Santos et al., 2011). In our study, the time of exposure in acid treatments was insufficient to fully remove fat layers in the seed coat, causing lower percentages of germination compared to water treatments.

Water temperature and time of immersion could promote germination and all water treatments in our study showed better germination performances. This technique, in general, is indicated for other forest species (Floriano, 2004). However, considering all three attributes – percentage, speed and germination mean time – only hot water with post-treatment in water for 24 hours rendered better performances. The Instructions for Forest Species Seed Analysis (Brasil, 2013) recommend acid treatments for dormancy breaking in *M. bimucronata*. However, based on our results, this rule should be revised.

Ribas et al. (1996) found 97% germination in *M. bimucronata* seeds when submitted to sulfuric acid for five minutes. The acid treatment did not differ from those with hot water in their study. On the other hand, our results point to a safe and effective method to promote dormancy breaking using hot water. It is important mainly for forest vivariums because chemical scarification with sulfuric acid demands care to avoid accidents and a proper disposal of residues (Brancalion et al., 2011; Padilha et al., 2018).

Considering the three germination attributes, *Mimosa bimucronata* performed better at alternating temperatures of 25-35 °C in the germitest substrate, a pattern also found for other Fabaceae species (Shibata et al., 2016; Silva et al., 2017). Reasons for better responses of germination at alternating temperatures are not fully known, but thermal variation may change the inhibitors and promoters of germination. Concentration of the former, e.g. abscisic acid (ABA), are diminished during low temperature phase, while promoters, e.g. gibberellins, increase at higher temperatures (Marcos Filho, 2015).

Some alternating temperatures can simulate natural fluctuations occurring near ground and may be regarded as a censoring mechanism for gaps in the vegetation, favoring some pioneer species with small seed (Pons, 2000). Enzymatic mechanisms act at different temperatures and this response is probably linked to ecological adaptations to the environment (Baskin and Baskin, 2014). At temperature of 25 °C there was no difference in the behavior of seed attributes (Alves et al., 2002) and only speed of germination was markedly different from the alternating temperatures in the germitest paper.

All morphometric and biomass variables of the seedlings were affected by substrate volume. It was expected that bigger containers would positively influence seedling development because of higher nutrient content, a pattern also found for other *Mimosa* species (Stüpp et al., 2015). Larger containers also favor root growth and spatial distribution (Ferraz and Engel, 2011), which in turn stimulate shoot growth. Unlike other species (Ferreira et al., 2005), the height of *M. bimucronata* seedlings did not respond to light conditions.

The number of leaves was larger under shaded conditions with no effect on root growth and distribution. That is interesting since this species is considered as an early successional species in Brazil (Carvalho, 2004). Seedlings exposed to shaded conditions would allocate more biomass to stems and leaves, increasing their shoot/root ratio (Santelices et al., 2015). Higher mean of *Mimosa bimucronata* root biomass in larger containers resulted in more leaves only under shading, suggesting full sunlight inhibits biomass allocation to the shoot parts. This species seeds are barochoric, resulting in a spatial distribution of seeds mainly underneath the mother-plant. Thus, *M. bimucronata* seedlings response to shading can be an adaptive attribute evolved from the selection of shade-tolerating individuals during early development stages.

Overall, *M. bimucronata* seeds originated from different geographic locations seem to behave in diverse ways regarding dormancy breaking. Our seeds came from lower altitudes and higher latitudes and must be treated with hot water for better results. Given the barochoric dispersal syndrome, it is not surprising that germination attributes of seeds performed better under alternating temperatures simulating the intermittently shaded habitat found underneath the mother-plant. The same factor can also explain larger number of leaves in shaded containers. This attribute can justify the use of this species as an early secondary species in restoration. We suggest that further studies of *M. bimucronata* should focus on evaluating the apparent seed phenotypic plasticity linked to geographical distribution, which can influence the production of seedlings for restoration programs developed in different geographical locations.

CONCLUSIONS

Our findings show that *Mimosa bimucronata* dormancy-breaking official recommendations should be revised. Water treatments always outperformed acid ones, guaranteeing better performance. Germination of the species must preferably be conducted in germitest paper at 25 °C. For seedling production, plants must be sown in bigger containers, but light intensity did not influence *Mimosa bimucronata* attributes, except for number of leaves. More leaves were produced in bigger containers under shaded conditions.

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