

Controlled-release fertilizer increases growth, chlorophyll content and overall quality of loquat seedlings

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Abstract

Eriobotrya japonica (Thunb.) Lindl. (Rosaceae), commonly known as the loquat tree, is widely cultivated due to production of edible fruits, which can be consumed fresh or processed into different food products. Enhancement of loquat seedlings quality is an important issue for more efficient propagation protocols, including rootstock production. The objective with the present study was to assess the effects of increasing doses of controlled-release fertilizer on loquat seedlings growth, quality and chlorophyll content. Seeds were sowed in containers filled with commercial substrate previously treated with different doses of controlled-release fertilizer (N, P, K, Mg, S and micronutrients): 0.0 (control), 2.5, 5.0, 7.5 and 10.0 kg per cubic meter of substrate (kg m^{-3}). Seedlings were evaluated 182 days after sowing and the data were submitted to polynomial regression analyses. Increasing doses of the fertilizer promoted increasing linear behavior on seedlings height, root collar diameter, leaf area, roots and shoots dry mass and Dickson quality index. Chlorophylls *a*, *b* and total chlorophylls increased up to the dose of 7.5 kg m^{-3} , followed by a decrease at the dose of 10 kg m^{-3} . The dose of 10 kg m^{-3} promoted the highest values of Dickson quality index and growing variables on loquat seedlings.

Keywords: Dickson quality index, *Eriobotrya japonica*, plant nutrition

Introduction

Eriobotrya japonica Lindl (Rosaceae), commonly known as the loquat tree, is a fruit crop native to China and widely cultivated in subtropical regions of Asia and other continents (Lopes et al., 2018). Loquat fruits can be either consumed fresh or processed into jams, marmalades, jellies and chutneys, mainly because of the high pectin content in the pulp (Lopes et al., 2018).

China is the current largest producer of loquat fruits, with about 170.000 ha of cultivated area and an annual output of approximately one million tons (Li et al., 2016). Brazil is estimated to produce about 2400 tons per year, with the cultivation area almost entirely concentrated in the region of Mogi das Cruzes, State of São Paulo (Caballero & Fernández, 2004; Pio et al., 2007).

Grafting is an important agricultural practice for commercial loquat production, since it induces dwarfism in the plants, allowing a denser spacing and giving rise

to a more compact orchard. Quince trees and loquat seedlings are the main rootstocks used for that purpose (Lopes et al., 2018). Therefore, enhancement of loquat germination and seedlings initial growth is an important issue for more efficient propagation protocols and for their use in breeding programs as well as for germplasm testing (El-Dengawy, 2005; Brasileiro et al., 2011).

For most of cultivated forest and fruit tree species, the enrichment of substrates with addition of mineral fertilization represents a well-established practice for seedling formation (Ceconi et al., 2007). Since those species usually require long periods for seedling formation (more than 8 months), substrate fertilization is preferably performed in split applications, which, despite being more adequate for plant development, requires more time, labor and equipment, therefore increasing the production cost (Almeida et al., 2018).

Among the available options for substrate

fertilization, controlled-release fertilizers have the advantage of reducing nutrient leaching (Silva et al., 2015), decreasing labor costs by avoiding split fertilizer applications and reducing seeds and roots injuries caused by excess of soluble nutrients (Shaviv, 2001; Qian & Schoenau, 2010).

The use of controlled-release fertilizers has been studied and is recommended for the production of seedlings of several forest trees and fruit crop species (Mendonça et al., 2007; Serrano et al., 2010; Freitas et al., 2011; Rossa et al., 2011; Almeida et al., 2018; Rosa et al., 2018). However, the use of this technology, to the best of our knowledge, has never been reported on the production of loquat tree seedlings.

Accordingly, the objective with the present study was to assess the effects of substrate supplementation with doses of controlled-release fertilizer (N-P-K + Mg, S and micronutrients) on growth, chlorophyll content and overall quality of loquat seedlings.

Material and Methods

Loquat fruits were collected on September 2016 from a 7 m high loquat tree, with diameter at breast height of 25 cm and approximately 15 years old. The plant is located in the city of Curitiba, Paraná State, South

of Brazil (25°25'40" S, 49°16'23" W, and 934m altitude ASL). The climate of the region is classified as Cfb, temperate humid, according to Köppen's system, with mild summers, cold and dry winters, rain uniformly distributed during the year and frequent occurrence of frosts.

The fruits were pulped and the seeds were shade dried for 72 hours. After drying, seeds were sowed in 120 cm³ polypropylene containers filled with commercial substrate Tropstrato HT® (Vida Verde - Tecnologia em Substratos™, Brazil), previously treated with different doses of controlled-release fertilizer and watered. The sowing was carried out with one seed per container at a depth of 1.0 cm.

Controlled-release fertilizer was added to the commercial substrate to obtain 5 doses/concentrations of the product: 0.0 (control), 2.5, 5.0, 7.5 and 10.0 kg per cubic meter of substrate (kg m⁻³). Subsequently, substrate and fertilizer of each treatment were mixed for 5 minutes in a rotary concrete mixer in order to homogeneously distribute the fertilizer granules in the substrate. The controlled-release fertilizer used in the experiment is a polymer coated fertilizer, which releases nutrients to the substrate for a period of 6 months (Basacote® Mini 6M - Compo Expert Brasil Fertilizantes Ltda.™). The chemical characteristics of the product are presented in table 1.

Table 1. Chemical characteristics of controlled-release fertilizer applied on substrate for loquat seedlings growth.

N (%)	P ₂ O ₅ (%)	K ₂ O (%)	MgO (%)	S (%)	B (%)	Cu (%)	Fe (%)	Mn (%)	Mo (%)
13.0	6.0	16.0	1.4	10.0	0.02	0.05	0.26	0.06	0.015

Source: Compo Expert (2019).

The experimental design was completely randomized, with 5 treatments (doses of controlled-release fertilizer) and 4 repetitions. A total of 20 seeds were sowed for each plot, and, considering a high rate of germination after one month (higher than 80%), it was possible to select the 16 more vigorous seedlings from each treatment to conduct the experiment and for final evaluation. Therefore, each experimental unit consisted of 16 seedlings. After sowing, the containers were kept in a greenhouse room with intermittent misting (20-25° C, > 85% relative humidity) for one month until seedling emergence, and then transferred to a greenhouse (20-30°C) where they were kept until the end of the experiment, with manual irrigation on a daily basis up to substrate saturation.

At 182 days after sowing, the seedlings were evaluated regarding aboveground height (cm) and root collar diameter (mm). Subsequently, one sample composed of fresh leaves from the middle third of 3 seedlings were collected randomly from each plot, immediately frozen in liquid nitrogen and stored in freezer

(-20°C) until chlorophyll analyses were performed. The shoots (stems and leaves) and roots were manually separated and the fresh leaves were analyzed in an optical scanner coupled to the software WinRHIZO Pro v. 2002c (Régent Instruments Inc. 2004) to determine the leaf area. After leaf area analysis, shoots and roots were oven-dried (65°C) until reaching constant mass, thus allowing for the determination of shoot dry mass, roots dry mass and total dry mass. Seedlings quality was assessed by the Dickson quality index- DQI (Dickson et al. 1960), calculated as follows (equation 1):

$$DQI = \frac{\text{Total dry mass}}{\left(\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Roots dry mass (g)}}{\text{Shoots dry mass (g)}} \right)}$$

To determine the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll, samples of 0.3g of fresh leaves (discs from the middle part of the leaf) were macerated in mortar with 10 ml of 80% acetone and placed in test tubes that were then centrifuged (refrigerated centrifuge at 4°C) for 10 minutes at 12000

rpm. An aliquot of 1ml of the supernatants was then transferred to quartz cuvettes and the absorbance of the solutions was determined in spectrophotometer (UV1601-Shimadzu, Kyoto, Japan) at the wavelengths of 645 and 663nm using 80% acetone as the control. The equations described by Arnon (1949) were used to calculate the chlorophyll content in each sample. The results were expressed in milligrams of chlorophyll per gram of fresh leaves (mg g^{-1}).

Averages from the results of each one of the 16 seedlings in a plot were used in the statistical analyses, except from chlorophyll analyses, where plant samples were collected randomly from 3 plants in each of the plots. The results were submitted to the Bartlett test to verify the homogeneity of variances. When homogenous,

data were submitted to polynomial regression analysis (5% probability), using the statistical software Assistat 7.7 (Silva & Azevedo, 2016). The criteria for selecting the model was the significance according to the polynomial regression F- test and, when more than one model was statically valid, the equation with the higher coefficient of determination (R^2) was selected.

Results and Discussion

According to the regression analysis, there was a positive linear relationship between the increasing doses of controlled-release fertilizer and most of the assessed variables (Figures 1, 2 and 3), demonstrating the potential benefits of using this product to improve loquat seedlings production systems.

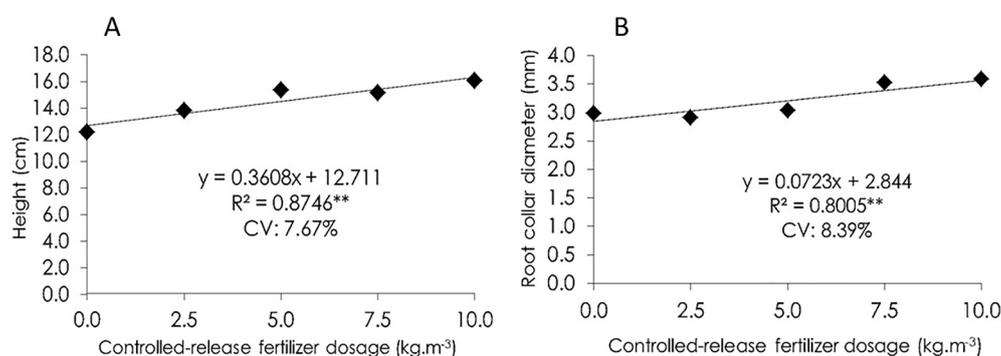


Figure 1. Polynomial regression analyses for aboveground height (a) and root collar diameter (b) of loquat (*Eriobotrya japonica* Lindl.) seedlings treated with increasing dosages of controlled-release fertilizer, at 182 days after sowing. ** Significant at 1% level.

Seedlings aboveground height increased linearly with the controlled-release fertilizer doses. Substrate treatment with 10 kg of the fertilizer per cubic meter resulted in seedlings 22% higher than the control (Figure 1a). Increases in height are a desirable response for nursery grown seedlings because it is an easy-assessment and non-destructive method to estimate seedlings quality (Gomes et al., 2002) and also an important variable to indicate when plants are suited for transplanting into field (Freitas et al., 2011).

The positive effects of the controlled-release fertilizer on seedlings height is, among other nutrients, possibly related to the continuous availability of Nitrogen, phosphorus and potassium to the plants. Availability of Nitrogen is associated with the development of many structural molecules in plant tissues, which will limit their growth (Taiz et al., 2017) and phosphorus is recognized by its role on energy transference in plants, especially on the functioning of ATPase pumps, which, regulated by auxins, will allow for cell expansion and division, and therefore affect stem height (Zhang et al., 2014; Taiz et al., 2017). Potassium is important for a series of biological

processes and has been proposed to play an important role in stem elongation, acting synergistically with auxins and gibberellins in a mechanism to increase osmotic potential and sugar transportation to the growing sites, which ultimately promotes cell expansion and division (Della Guardia & Benlloch, 1980). Potassium from slow- and controlled-release fertilizers is available for a longer period of time and is subjected to a lower leaching rate when compared to other fertilizers (Bley et al., 2017), which can contribute for its effects on plant growth in height.

Seedlings height, however, is not always the best quality indicator and should be considered in combination with other morphological characteristics such as the root collar diameter. *A. angustifolia* seedlings, for example, when treated with high doses of the same controlled-release fertilizer used in this experiment, presented an intense increase in height which was not followed by root collar diameter and, ultimately, resulted in seedlings lodging (Rossa et al., 2011).

The root collar diameter is a quick and easy non-destructive measurement, highly correlated with

future plant performance and survival after transplanting (Bayala et al., 2009). Moreover, it is a primary characteristic to be considered for seedlings used as rootstocks, since the stem diameter is one of the most important variables impacting grafting success (Gomes et al., 2010). In the present study, the root collar diameter of the loquat seedlings increased linearly in response to the doses of the controlled-release fertilizer, with an increment of 16.7% on the seedlings treated with 10 kg m⁻³ in comparison to the control group (Figure 1b).

The controlled-release fertilizer also promoted significant increments on biomass accumulation of loquat seedlings, with positive linear responses to the doses of the product for shoot, roots and total dry biomass (Figure 2a, b and c). Shoots and total dry biomass of seedlings treated with the highest dose of the fertilizer presented

increases of 56% and 52%, respectively, when compared to the control. Increases on seedlings biomass due to the application of controlled- and slow- release fertilizers on substrate have been previously reported for several plant species such as okra (*Abelmoschus esculentus*), eggplant (*Solanum melongena*), açai palm (*Euterpe precatoria*), flooded gum (*Eucalyptus grandis*), Paraná pine (*Araucaria angustifolia*), American cinnamon (*Ocotea odorifera*) and pineapple (*Ananas comosus*), among others (Freitas et al., 2011; Rossa et al., 2011; 2014; Gomes et al., 2017a; 2017b; Almeida et al., 2018). Biomass accumulation is an important feature because it reflects the carbon status of a developing seedling, affecting and being affected by the capacity of capture of light, nutrients and water (Modrzyński et al., 2015).

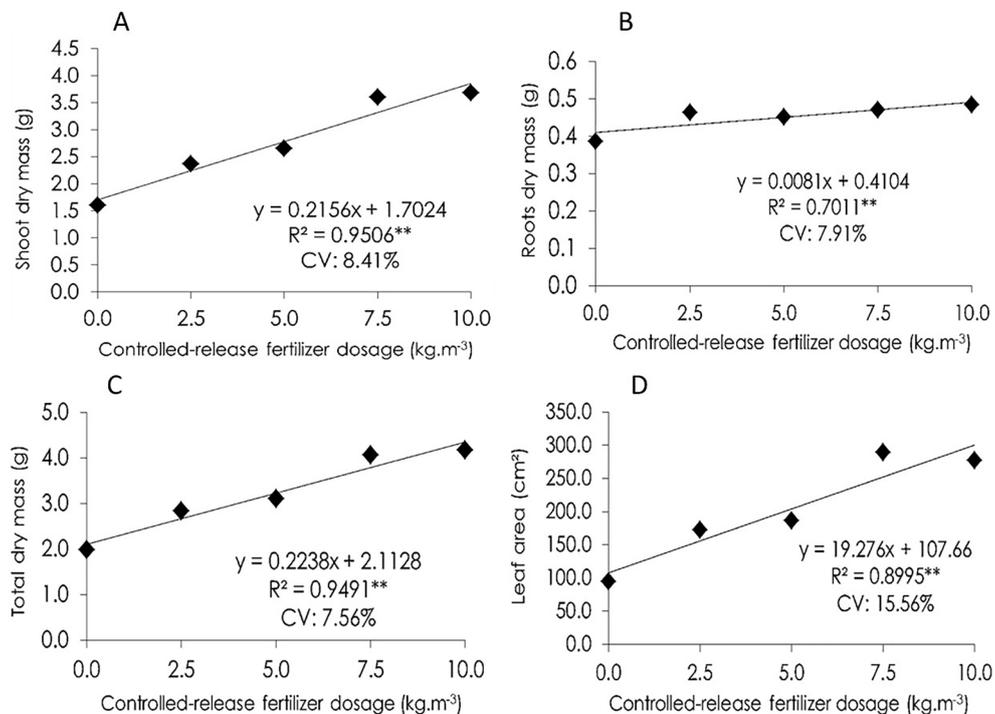


Figure 2. Polynomial regression analyses for shoot dry mass (a) and roots dry mass (b), total dry mass (c) and leaf area (d) of loquat (*Eriobotrya japonica* Lindl.) seedlings treated with increasing dosages of controlled-release fertilizer, at 182 days after sowing. ** Significant at 1% level.

Roots biomass also increased linearly with the doses of the controlled-release fertilizer, with increments up to 20% in comparison to the control treatment (Figure 3b). Higher root biomass is correlated with roots number and volume and is considered a good indicator of seedlings performance after transplanting since it denotes a better capacity of structural support and water and nutrient uptake (Pio et al., 2004; Almeida et al., 2005).

Although there are no previous reports on the effects of controlled-release fertilizers on loquat plants, Pio et al. (2004) reported that seedlings of this species

accumulate more roots and shoots biomass when grown in substrates containing higher levels of organic matter, and, consequently, higher availability of mineral nutrients. These results demonstrate that fertilization of substrate for loquat seedlings cultivation is an effective way to promote more vigorous plants and to optimize the production process.

Biomass increases on loquat seedlings treated with controlled-release fertilizer are probably related to the continuous and balanced distribution of nutrients to the root system, such as nitrogen, phosphorus and the

previously mentioned potassium. Nitrogen serves as a constituent of many plant cell components, including chlorophyll, amino acids and nucleic acids and, therefore, is usually the mineral nutrient required by plants in the greatest quantity, rapidly inhibiting plant growth

when not supplied in enough quantities (Taiz et al., 2017). Phosphorus, in turn, is central to a majority of molecular constituents required for the functioning of plant cells, such as DNA and RNA, cell membranes, ATP, ADP, and NADPH (Zhang et al., 2014).

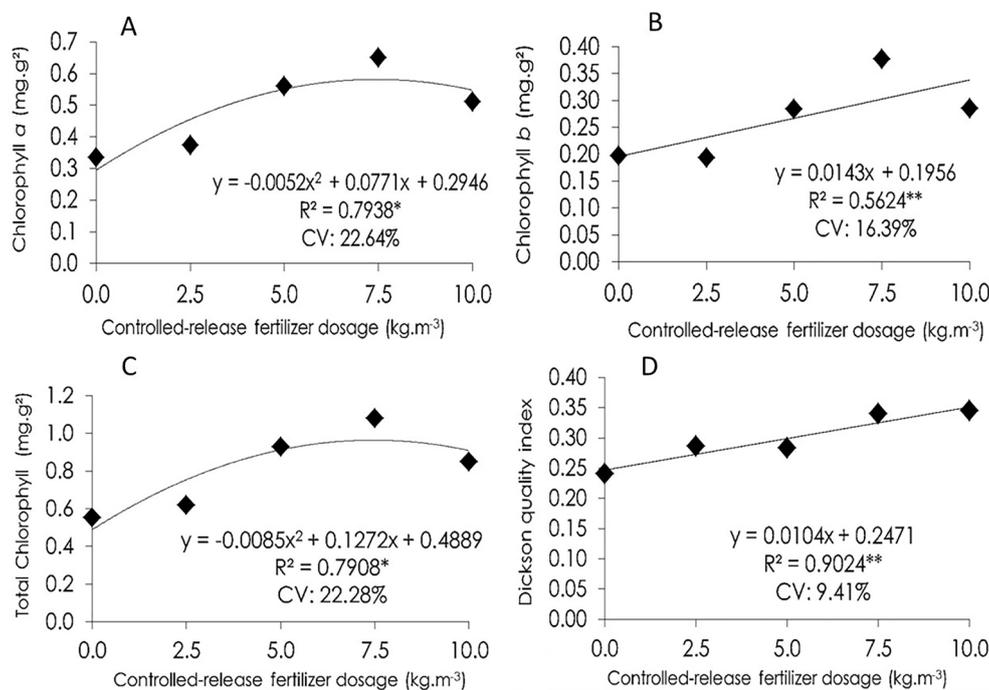


Figure 3. Polynomial regression analyses for the contents of chlorophyll a (a), chlorophyll b (b) and total chlorophylls (c) and Dickson quality index of loquat (*Eriobotrya japonica* Lindl.) seedlings treated with increasing dosages of controlled-release fertilizer, at 182 days after sowing. *Significant at 5% level. **Significant at 1% level.

Higher biomass accumulation on controlled-release fertilizer-treated seedlings is also related to the presence of secondary macronutrients such as magnesium and sulfur and micronutrients such as boron, copper, iron, manganese and molybdenum, present in the formulation of Basacote® Mini 6M (Table 1). Magnesium is involved in the activation of several enzymes and is a component of chlorophyll molecule, while sulfur is a structural component of the plant and the micronutrients participate in several fundamental biochemical processes of plant growth and development (Taiz et al., 2017).

The continuous supply of all essential nutrients is a fundamental feature of substrates for the accumulation of biomass in seedlings, since it contributes to plant health and photosynthesis. One important aspect in this regard is the adequate expansion of leaf area. Similarly to most of the variables, leaf area of loquat seedlings presented and increasing linear behavior according to the doses of controlled-release fertilizer (Figure 3d). A higher leaf area increases the capacity of seedlings to collect light and, therefore, to produce the necessary energy to carbon fixation and, ultimately, biomass accumulation.

The supply of nitric and ammoniacal nitrogen to loquat seedlings through the use of controlled-release fertilizer is possibly related to the responses in leaf area, since there is a direct association between leaf area expansion and N uptake (Ekbladh et al., 2007). Similarly to the observed in the present study, the use of controlled-release fertilizer on tomato plants promoted an expansion of leaf area and was reported to greatly reduce nutrient use without decreasing fruit yield when compared to liquid fertilizers (Kinoshita et al., 2014).

In addition to the leaf area, adequate photosynthesis requires the establishment of a suitable light-harvesting complex, which involves the synthesis of adequate chlorophyll levels. Chlorophyll b contents in loquat seedlings presented statistical significance only in a linear increasing model (Figure 3b), whereas chlorophyll a and total chlorophylls had quadratic responses (Figures 3a and 3c) to the increasing doses of Basacote® Mini 6M. However, what was observed for the levels of all pigments, independently of the statistical model, was a significant increase up to the dose of 7.5 kg m⁻³ followed by a decrease on the 10.0 kg m⁻³ dose, which was still more adequate than the control treatment for chlorophyll

levels.

The increase in chlorophyll levels up to the dose of 7.5 kg m⁻³ demonstrates the positive effects of the controlled-release fertilizer on loquat seedling photosynthetic apparatus. The higher production of pigments in plants treated with controlled-release fertilizer is justified by the continuous supply of important nutrients for chlorophyll formation, for instance nitrogen and magnesium, structural components of the molecule, and micronutrients, such as iron, which is crucial for the formation of protein complexes leading to chlorophyll biosynthesis and cytochrome assembly (Taiz et al., 2017).

In view of the different biometric data used to assess the effects of the controlled-release fertilizer on loquat seedlings, it is necessary to observe the set of responses to estimate their overall quality. One tool widely used for this purpose is the so-called Dickson quality index (Dickson et al., 1960). The Dickson quality index integrates the aspects of total mass, the sturdiness quotient (height: root collar diameter ratio) and shoot: root ratio (Mañas et al., 2009).

In the present study, the controlled-release fertilizer doses promoted a positive linear response of DQI (Figure 2d), with values ranging from 0.24 to 0.34. The highest DQI with the dose of 10 kg m⁻³ of Basacote® Mini 6M was also reported for seedlings of paricá (*Schizolobium parahyba* var. *amazonicum*), Brazilian peppertree (*Schinus terebinthifolius*) and flooded gum (*Eucalyptus grandis*) in studies with similar methods to those used in the present experiment (Rossa et al., 2013; 2014; 2015).

Although Hunt (1990) has established minimum DQI values of 0.20 for coniferous seedlings, several literature reports show that the higher the index, the best are the survival and field performance of plants (Bayala et al., 2009; Mañas et al., 2009). Therefore, the dose of 10 kg m⁻³ of controlled-release fertilizer allowed for the formation of the highest quality loquat seedlings and can be used in commercial production.

In the present study, the dose of 10 kg m⁻³ of the controlled-release fertilizer promoted the best responses for most of the assessed variables. Notwithstanding, in face of the linear increasing models, it is possible to infer that the species can still respond to higher doses of fertilizer and, therefore, further studies should search for maximum technical efficiency doses.

Conclusions

The application of controlled-release fertilizer on loquat seedlings increases their growth, chlorophyll levels and Dickson quality indexes. The dose of 10 kg m⁻³ of the controlled-release fertilizer promotes the best responses

for most of the variables related to loquat seedlings growth and quality. However, in face of the positive linear models to increasing doses of the product, it is possible to infer that the species can still respond to higher doses of fertilizer and, therefore, further studies should search for maximum technical efficiency doses.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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