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Transfer of clonal coffee seedlings from the conventional system to the modified hydroponic system

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ABSTRACT: Cloning plants by cuttings, mineral nutrition via modified hydroponics and the use of alternative substrates emerges as technological innovations for seedling production. The objective in this study was to evaluate the transfer of cuttings from the conventional system to the modified hydroponic system. The following variables were analyzed: seedling lenght; number of shoots; shoot lengnt; total leaf number; number of remaining leaves; leaf area; root area; stomatal conductance, density, and functionality; stomatal opening; root volume, area, length and diameter; leaf area ratio; specific leaf area and weight of specific leaf matter. For statistical analysis, a completely randomized design (CRD) with 5 treatments, six replications and ten plants per plot was used. From the results obtained, it is possible to conclude that: the rooting of *Coffea arabica* cuttings is not necessary in a conventional system, and it can be performed directly in a modified hydroponic system; an adaptation in the proposed modified hydroponics system with nebulization in the environment is necessary for rooting, for the obtention of a higher quality seedling.

Key words: cloning; coffee tree; greenhouse; vermiculite

Transferência de mudas clonais de café do sistema convencional para o sistema hidropônico modificado

RESUMO: A clonagem de plantas por estaquia, a nutrição mineral via hidroponia modificada e o uso de substratos alternativos surgem como inovações tecnológicas para a produção de mudas. Objetivou-se com o presente trabalho avaliar a transferência das estacas do sistema convencional para o sistema de hidroponia modificada. Foram analisadas as seguintes variáveis: comprimento das mudas; número de brotos; comprimento do broto; número total de folhas; número de folhas remanescentes; área foliar; área radicular; condutância, densidade e funcionalidade estomática; abertura dos estômatos; volume, área, comprimento e diâmetro das raízes; razão de área foliar; área foliar específica e o peso da matéria foliar específica. O delineamento foi o inteiramente casualizado (DIC) com 5 tratamentos, seis repetições e dez plantas por parcela. A partir dos resultados obtidos é possível concluir: não é necessário o enraizamento de estacas de *Coffea arabica* em sistema convencional, sendo que o mesmo pode ser feito diretamente em sistema hidropônico modificado e é preciso uma adaptação no sistema proposto de hidroponia modificada com nebulização no ambiente no enraizamento das estacas, a fim de obter uma muda de maior qualidade.

Palavras-chave: clonagem; cafeeiro; casa de vegetação; vermiculita

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Introduction

The plant cloning method by cuttings is a technology that has been useful in coffee improvement, as it allows the selection and multiplication of superior plants at any development stage of a breeding program, thus reducing the time for the obtention of a new cultivar with greater productive potential (Bazoni et al., 2020).

Therefore, the use of cuttings is of great interest, as it provides a reduction in seedling production time, besides anticipating planting, consequently taking advantage of the rainy season and increasing field set (Baliza et al., 2013).

However, seedling production through cuttings requires care and several factors can influence growth and development. One of these factors is the mineral nutrition of these seedlings, which must be judicious, since the root system is poorly developed at the beginning of the production process, making it necessary to use methods that can optimize nutrient absorption.

As an alternative for a sufficient, balanced nutrition and with greater control of nutrient supply, the hydroponic system has been used in several agricultural crops, but there are still few studies in relation to coffee (Lima et al., 2021). This system has several advantages such as less water used (considering a "closed" supply system) and inert substrate (reducing the incidence of pests and diseases), being able to provide the nutrients that the plant needs in a more available way through nutrient solution (Savvas & Gruda, 2018).

The objective of this study is to seek a protocol for the production of seedlings by cuttings that uses the traditional method of cloning and is completed in a modified hydroponic system in order to promote greater rooting. The modified hydroponics system proposed in this work can be more economical, in view of greater water savings, greater sanitation and the opportunity to reuse the structure built for several years, being feasible even for even small nurseries. In addition to the advantages described, the proposed system can produce more vigorous seedlings that will be available for planting in the field at the beginning of the rainy season.

Materials and Methods

The experiment was carried out in the nursery of the Coffee Sector, and in the Horticulture Sector, located in the Department of Agriculture of Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil.

For the obtention of the cuttings, healthy matrix *Coffea arabica* L. plants ('Mundo Novo IAC 379-19') were selected from an experimental field on Universidade Federal de Lavras.

It was carried out in the cultivation system for the production of seedlings in modified hydroponics, proposed by Faquin & Chalfun (2008). The changes in relation to the conventional hydroponic system are related to the fact that it is a closed system for the supply of the nutrient solution, which eliminates the need for aeration. In this system, the seedlings absorb nutrients from a nutrient solution proposed by the above-mentioned authors, which is another modification, since most solutions are adapted for plant species.

In the system, an inert substrate is used to support the root system, and only the bottom of the container with the substrate is in contact with the solution in the "pools". Therefore, it is possible to maintain the hydroponic characteristics and, at the same time, obtain a consistent root system supported by a substrate in order to preserve the structure of the rhizosphere, avoiding defects impacting on the coffee crop as the curving of the main root. This system was found inside a greenhouse also with a metallic structure with a transparent plastic cover and a 50% shade layer under the cover, but it did not have a spray irrigation system or temperature control with fans.

The tubettes containing vermiculite were placed in crates and the cubes of phenolic foam, in their own polystyrene plates, previously adjusted on the edges of boxes of synthetic material, with dimensions of 3.20 × 0.60 × 0.30 m, called "pools", and leveled on masonry benches, inside a greenhouse covered with double-sided polyethylene film. The "pools" were covered with white polyethylene film, with an opening for positioning the tubettes and foam, thus avoiding the entry of light and the consequent growth of algae in the nutrient solution.

The "pools" had a closed system for circulating the nutrient solution, where the tank received the solution proposed by Faquin & Chalfun (2008), coming from a 1,000 L reservoir. The solution circulated in the pools through the engine-pump assembly by activating the timer with an interval of 15 minutes of circulation and 45 minutes off. The excess of the nutrient solution in the "pool" returned to the reservoir by gravity, through its own pipe. The nutrient solution was exchanged every 30 days.

After harvest, cutting preparation and planting in 120 cm^3 tubettes containing vermiculite, these tubettes were kept in an air-conditioned greenhouse and, after 25, 50, and 75 days, they were transferred to the hydroponic system. In the planted cuttings, a pair of leaves cut in half was kept, in order to maintain a photosynthetic area for obtaining carbohydrates for them; in addition, the base of the cuttings were treated with the growth regulator and rooting stimulant - AIB (indolebutyric acid). As a control treatment, seedlings were kept in the conventional system until the end (final evaluation time) and also placed directly in the hydroponic system right after cutting. Thus, the experiment aimed to determine the optimal time for transfer from the conventional system to the modified hydroponic system from cutting.

Thus, five treatments were considered, taking into account the number of days after cutting in which the seedlings were transferred from the traditional system to the modified hydroponic system; they are: control (seedlings kept in a conventional air-conditioned greenhouse until the end of the evaluations), 75, 50, 25, and 0 days after cutting (DAC).

In the control that was kept in the air-conditioned greenhouse, the nutrient supply was through the application of the slow-release fertilizer Osmocote Plus®, at the following mineral concentrations: 15% N; 9% K_2O; 12% P_2O_5; 0.06% Mg; 2.3% S; 0.05% Cu; 0.45% Fe; 0.06% Mn; and, 0.02% Mo; at a dose of 12.5 g L^{-1} substrate, according to the methodology of Rezende et al. (2017). In the other treatments, the nutrients were supplied using a nutrient solution from the modified hydroponic system.

After 150 days, the following evaluations were carried out: seedling lenght (cm), shoot number (mm), shoot lenght (cm), total leaf number (TLN), number of remaining leaves from the cuttings (RL).

From the leaf area and dry matter weight data, the following characteristics were estimated: leaf area ratio (LAR, $cm^2\ g^{-1}$), obtained through the ratio between total leaf area (TLA) and TDMW; specific leaf area (SLA, $cm^2\ g^{-1}$), which relates the surface to the dry matter weight of the leaf itself, obtained through the ratio of TLA by DMWL and specific leaf matter (SLM, mg cm^{-2}), which is the ratio of DMWL and TLA.

The roots were analyzed by the SAFIRA software, 'Fiber and Root Analysis System', developed by Embrapa Instrumentação, where the roots were removed from the containers, carefully washed in water, positioned next to the scale (cm), on a black surface for contrast, and photographed with the aid of a professional camera. Subsequently, data on volume (mm^3), area (mm^2), length (cm), and root diameter (mm) were analyzed.

After 150 days it was measured stomatal conductance (SC, µmol $m^{-2}\ s^{-1}$) was obtained from the leaf vapor flow through the stomata to the external environment using a porometer (SC-1 Decagon Devices); readings were performed in the median region of the limbus completely extended, between 8 a.m. and 10 a.m.

The images obtained by scanning electron microscopy were analyzed using the UTHSCSA-Imagetool imaging software. The number of stomata was counted and the polar and equatorial diameters (µm) were measured to analyze the following variables:

- Stomatal density (DEN = ne mm^{-2});
- Relationship between polar diameter and equatorial diameter, functionality (FUN= dp/de).

From these images, stomatal opening (µm) was measured.

The collected data were tabulated, submitted to normality and homogeneity tests and analyzed by SISVAR® (Ferreira, 2019). To compare the means, the Scott-Knott test was used at 5% significance.

Results and Discussion

The development of *C. arabica* clonal seedlings was significantly affected by the transfer time after cutting, from the air-conditioned greenhouse system to the modified hydroponic system.

It was observed that seedling survival and, consequently, the number of remaining leaves was higher in treatments that spent a longer time period in the air-conditioned greenhouse environment, before transfer to the modified hydroponic environment (control, 75, and 50 DAC). Plant survival of these three treatments was 83.33, 91.66, and 95.00%, while seedlings (0 DAC) kept in the hydroponic system had only 58.33% immediately after cutting (Table 1). The number of remaining leaves was higher in the control treatments (seedlings kept in a conventional air-conditioned greenhouse until the end of the evaluations) and in those at 75 DAC, being higher than those at 50 DAC, which were also in the group with the greatest survival.

However, even with higher seedling mortality, at 0 DAC, a greater plant development in total lenght (10.970 cm), number of leaves (9.390) and shoot lenght (5.924 cm) was observed. Thus, the immediate transfer of cuttings (before rooting) to the modified hydroponics environment does not seem to be indicated as proposed due to the high mortality, but future adaptations may increase plant survival in this treatment, taking advantage of the greater growth observed in plants that have survived.

As for the number of shoots, the transfer from the conventional system to the modified hydroponic system did not change this characteristic significantly.

The relative humidity of the air in the seedling formation environment is important for survival, acting to maintain the water status, since the cuttings do not have roots to absorb water and nutrients (Peloso et al., 2017). This explains the survival results in this study since, in the climate-controlled greenhouse where the seedlings that showed better results were kept for longer, an average relative humidity of 84.9% was observed during the experimental period, while in the modified hydroponics environment, the observed humidity was only 41.5%.

The lower seedling survival cut directly in the modified hydroponic system (0 DAC), may also be related to the

Table 1. Survival, total lenght (TL), leaf number (LN), remaining leaves (RL), shoot number (SN) and shoot lenght (SL) in clonal coffee seedlings started in a conventional cultivation system and transferred to the modified hydroponics system.

Treatments	Survival (%)	TL (cm)	LN	RL	SN	SL (cm)
Control	91.666 a	5.570 b	4.814 c	1.625 a	1.815 a	2.520 b
75 DAC	95.000 a	7.098 b	5.662 c	1.612 a	1.656 a	3.665 b
50 DAC	83.333 a	6.366 b	7.174 b	1.190 b	1.775 a	3.096 b
25 DAC	75.000 b	6.952 b	7.480 b	1.243 b	1.736 a	3.665 b
0 DAC	58.333 c	10.970 a	9.390 a	0.583 c	1.688 a	5.924 a

* Means followed by the same letter in the column do not differ by the Scott Knott test at 5% probability.

salinity of the nutrient solution, which causes the osmotic pressure to increase and, consequently, even the solution with available water can become an environment of difficult rooting of cuttings (Andrade Júnior et al., 2013).

The highest values observed for total plant lenght, number of leaves and shoot lenght at 0 DAC occur as, according to Dominghetti et al. (2016), seedlings produced by cuttings show greater growth, the more water available the environment presents, as is the case with the modified hydroponic system, and in this case the seedlings of this treatment (0 DAC) spent more time in this system with continuous supply of nutrient solution.

The number of remaining leaves is an important characteristic to be evaluated, once it is a way to maintain a transpiration surface in the plant material, and thus maintain a photosynthetic rate to ensure the production of carbohydrates and hormones that will be necessary for rooting, since these cuttings do not have roots formed to absorb water and nutrients. However, they are affected by high temperatures, as they are more prone to water loss through transpiration, which is why it is necessary to use mist irrigation (Guimarães et al., 2019). These facts explain why the remaining leaves are more persistent in the control and at 75 DAC since, in these treatments, they spent more time under mist irrigation; and less persistent at 0 DAC, not in an environment with this type of irrigation. Thus, an adaptation in the proposed modified hydroponic system with nebulization in the environment until cutting rooting could be an interesting solution.

The results of total height, number of leaves and shoot height are contrasting to those of remaining leaves. This shows that, although the seedlings taken to the hydroponic system immediately after cutting had a smaller number of remaining leaves and, consequently, lower production of carbohydrates, the greater availability of water and nutrients during the entire production time, possibly caused the plants to have a good growth (Souza et al., 2013).

With these results, it is clear that, in the way it was proposed in this experiment, seedling rooting in a greenhouse system and then transfer to the modified hydroponic system is more indicated, unless adaptations are made in order to achieve greater seedling growth during the production process as observed in this experiment with seedlings with greater total height, number of leaves and shoot height.

The surface area and root volume were higher in the seedlings cut directly in a modified hydroponic system compared to the other times, which presented similar results to each other (Table 2). The surface area of the roots of seedlings cut directly in a hydroponic system is up to 353.20% more than that of seedlings produced with the other treatments and, as for root volume, it can reach 215.78%.

At 0 DAC, the longest root length was observed (29.940 cm), followed by 25 DAC (25.320 cm); 50 and 75 DAC had similar results (22.247 and 20.906 cm, respectively) and finally, with lower performance for this characteristic, the control (11,690 cm). Thus, the root length of seedlings taken to the hydroponic system immediately after cutting is up to 156.12%, higher than that of the seedlings in the control treatment (seedlings kept in a conventional air-conditioned greenhouse until the end of the evaluations) (Table 2).

Root diameter was not affected by the transfer from the conventional cultivation system to the modified hydroponic system (Table 2).

The better performance of the root system, in terms of surface area, volume and length at 0 DAC, is possibly related to the fact that this treatment spent longer in the modified hydroponic system; therefore, in longer contact with the nutrient solution. This greater growth is possibly due to the greater availability of water and nutrients that this system provides (Souza et al., 2013).

In this study, the seedlings formed at 0 DAC presented greater development of the root system, which is a desirable characteristic in seedling formation. Seedlings with more developed root systems, when transferred to field conditions, will have more chances of success in their establishment (Matos et al., 2020; Menezes et al., 2020).

The difference in contact time with the 0 DAC nutrient solution for the other treatments was at least 25 days and, in this period, the cuttings had not yet been rooted. However, the best results of the characteristics of roots at 0 DAC, possibly occur as the formation of callus in the cuttings starts before rooting itself, and the longer contact time with the solution likely accelerated this process. Furthermore, the use of the AIB hormone in the cuttings in this study is shown to be beneficial and capable of inducing callus in the cuttings (Jesus et al., 2013).

When working with the formation of *C. arabica* seedlings by cutting, Jesus et al. (2013) observed that, 45 DAC, the cuttings had high percentages of calluses; however, they still did not have roots. Thus, the idea that rooting in the modified hydroponics system has been accelerated is reinforced.

Table 2. Surface area (RSA), volume (RV), length (RL) and diameter (RD) of roots in clonal coffee seedlings started in a conventional cultivation system and transferred to a modified hydroponic system.

Treatments	RSA (mm^2)	RV (mm^3)	RL (cm)	RD (mm)
Control	7287.094 b	2404.437 b	11.690 d	0.815 a
75 DAC	4783.262 b	1597.739 b	20.906 c	0.820 a
50 DAC	4438.302 b	1255.276 b	22.427 c	0.638 a
25 DAC	6291.435 b	1997.384 b	25.320 b	0.698 a
0 DAC	20114.645 a	3963.881 a	29.940 a	0.783 a

* Means followed by the same letter in the column do not differ by the Scott Knott test at 5% probability.

The worst performance of the control regarding RL was due to the so-called “natural pruning”, which occurs when the roots reach the lower hole of the tubettes and come into contact with oxygen. As a result, new root sprouting occurs inside the tubette, thus increasing its volume. Such facts explain the reason for the control, even with the worst performance in relation to length, to be not different in relation to the volume and area of roots of treatments 75, 50, and 25 DAC, which did not undergo “natural pruning”.

There were no significant differences in mean root diameter, and seedlings from all treatments had values below 3 mm. According to Rena & Guimarães (2000), in adult coffee trees, roots with diameters smaller than 3 mm are classified as fine or absorbent roots.

Fine roots are considered the most important in the root system, as they are strongly related to the processes of absorption, transport of substances and varied biosynthesis (Jesus et al., 2013). The results of this study corroborate the results of these authors who, when evaluating the roots of coffee seedlings from cuttings and sowing, concluded that 98% of the root system is formed by fine roots, with a diameter lower than 2mm.

The results obtained in this experiment reinforce the idea of Jesus et al. (2013), that shoot growth depends on root growth since, at 0 DAC, the best shoot and root performance was observed. Thus, it is possible to reinforce the idea that there is no need to start rooting coffee cuttings in a conventional system and then transfer them to the modified hydroponic system, provided that the latter is adapted to increase seedling survival.

Leaf area and growth characteristics in seedlings started in a conventional cultivation system and transferred to a modified hydroponic system were affected. It was observed that the total leaf area of the seedlings at 75 DAC was higher, followed by 0 DAC (Table 3). In both treatments, there was no water restriction although, at 75 DAC, which showed the largest total leaf area, it spent a period in the air-conditioned greenhouse, when it was transferred to the modified hydroponic system, receiving continuous water supply.

Although no treatment has undergone severe water restrictions, the superior results at 75 DAC may be related to the fact that, when this treatment was transferred to hydroponics, with full water availability, the cuttings had already rooted; thus, they were more effective in absorbing water and nutrients. Meanwhile, the other treatments were not yet completely rooted when transferred from the system.

Due to the greater leaf area, Taiz & Zeiger (2017) stated that the greater growth in height and greater accumulation of leaf and total dry matter are an adaptive strategy to give plants greater photosynthetic efficiency, since it has a greater area for light absorption. Therefore, the results are complementary and in accordance with this statement as, at 0 and 75 DAC, the best results were observed for the characteristics mentioned (total height, total and leaf dry matter) and, as a result, they presented greater total leaf area.

Some growth characteristics were also influenced by the transfer of seedlings from the conventional system to the modified hydroponic system. Seedlings of treatment 0 DAC had lower values for leaf area ratio (15.08 cm^2 mg^{-1}); on the other hand, it showed the best result for specific leaf mass (0.045 mg cm^{-2}). Specific leaf area did not show significant differences among treatments (Table 3).

According to Dutra et al. (2012), a higher specific leaf mass is desirable, once it indicates greater leaf thickness; therefore, there is a greater reduction in water loss from the seedlings. The superiority of the seedlings of treatment 0 DAC in relation to the others regarding specific leaf mass was due to the greater leaf dry matter accumulation of this treatment. This suggests that, when spending more time in the hydroponic system, under constant water supply, these seedlings presented greater leaf thickness, enabling greater water balance by reducing water losses.

Under water restriction conditions, photosynthetic rates are reduced, as there is a lower CO_2 influx into the substomatal chamber. This is possibly due to the lower stomatal conductance, which prevents water loss through transpiration. Under these conditions, stomatal control was considered the most limiting factor for photosynthesis.

Stomatal closure causes a reduction in stomatal conductance, and it is the plant first strategy to prevent leaf dehydration, thus maintaining the water status, which reflects on its survival, even after going through periods of lack of stomata. water and high temperatures (Taiz & Zeiger, 2017).

Such facts contribute to this study since, in the control, there was possibly greater water restriction than in the hydroponic system, combined with high temperatures; in

Table 3. Total leaf area (TLA), leaf area ratio (LAR), specific leaf area (SLA) and specific leaf mass (SLM) in clonal coffee seedlings started in a conventional cultivation system and transferred to a modified hydroponic system.

Treatments	TLA (cm^2)	LAR (cm^2 mg^{-1})	SLA (cm^2 mg^{-1})	SLM (mg cm^{-2})
Control	13697.19 c	27.22 a	42.92 a	0.025 b
75 DAC	26249.42 a	25.33 a	38.92 a	0.026 b
50 DAC	17522.11 c	32.31 a	50.08 a	0.023 b
25 DAC	16111.20 c	25.97 a	37.88 a	0.027 b
0 DAC	21474.66 b	15.08 b	27.91 a	0.045 a

* Means followed by the same letter in the column do not differ by the Scott Knott test at 5% probability.

Table 4. Stomatal conductance (SC), density (DEN) and functionality (FUN) and stomatal opening (SO) in clonal coffee seedlings started in a conventional cultivation system and transferred to a modified hydroponic system.

Treatments	SC (μmol m^{-2} s^{-1})	DEN (mm^2)	FUN	SO (μm)
Control	82.023 b	160.404 c	2.236 a	3.144 a
75 DAC	176.200 a	197.831 a	1.867 b	3.289 a
50 DAC	161.381 a	184.936 b	1.853 b	3.289 a
25 DAC	200.650 a	197.832 a	1.809 b	3.520 a
0 DAC	185.391 a	180.219 b	1.804 b	2.576 a

* Means followed by the same letter in the column do not differ by the Scott Knott test at 5% probability.

this treatment, lower values of stomatal conductance were observed and, consequently, lower levels of chlorophyll, which negatively affected the photosynthetic process (Table 4).

Although the possible water restriction in the control is not so accentuated, drying on the substrate surface was observed, due to the intermittent irrigation. According to Peloso et al. (2017), stomata can quickly respond to variations in air or soil moisture, or the interaction of both. Thus, the smallest values of stomatal conductance in the control are explained.

The stomatal density of plants from treatments 75 and 25 DAC was higher by 23.33%, compared to the control, which presented the worst performance for this characteristic. The greater stomatal density favors the input of CO_2 into the leaves and, since it is an essential gas in the photosynthetic process, the increase in the uptake of this gas favors photosynthesis, which indicates that the physiological characteristics of the plant were favored, verifying the adaptation of plants to the environment they are subjected to (Taiz & Zeiger, 2017; Fana et al., 2020).

Contrary to what was observed for stomatal conductance, stomatal functionality was superior in seedlings formed exclusively in the conventional system (control); there was an increase of about 19.7% in relation to the other treatments (Table 4).

Stomatal functionality is given by the relationship between the polar diameter and the equatorial diameter of the stomata. According to Peloso et al. (2017), this relationship indicates stomatal shape: the higher this relationship, the more ellipsoid the stomata and the more functional, and the smaller the relationship, the less ellipsoid and the lower the functionality.

The same authors reported that, under water restrictions, plants are able to change the size of the stomata to aid tolerance to this condition; stomatal decrease reduces water loss to the environment through transpiration. Thus, the results of this study can be explained, since the water supply in the control was more limited in relation to the other treatments, which possibly led the plants to change the size of their stomata, making them more functional to support the conditions under which they found themselves.

According to Table 4, at stomatal opening, there were no differences between treatments.

Conclusions

The rooting of *Coffea arabica* cuttings is not necessary in a conventional system, and it can be performed directly in a modified hydroponic system.

An adaptation in the proposed modified hydroponics system with nebulization in the rooting environment is necessary, for the obtention of a higher quality seedling.

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Compliance with Ethical Standards

Author contributions: Conceptualization: SHBC; Data curation: ETT; Formal analysis: RJG; Funding acquisition: AMC; Investigation: SHBC; Methodology: SHBC, AEL; Project administration: RJG; Resources: AMC; Software: ETT; Supervision: SHBC; Validation: RJG; Visualization: SHBC; Writing – original draft: AFF, SHBC, AMC, ETT; Writing – review & editing: AFF, AEL, AMC, RJG, ETT.

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