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Effect of Irrigation Pattern and Irrigation Level on Growth of *Glycyrrhiza inflata* and the Medicinal Quality of its Root

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ABSTRACT

Background: Although both the root and rhizome of *Glycyrrhiza inflata* have been used as medicinal material, the sales volume and price of its root are much higher than its rhizomes. Limiting the growth of liquorice rhizomes while improving the yield and quality of its root has become an important problem.

Methods: Two irrigation patterns (surface drip irrigation and subsurface drip irrigation, *i.e.* DI and SDI) and three irrigation levels (40%-50%, 60%-70% and 80%-90% of the maximum soil water capacity) were used in this study.

Result: The plant height, crown width, root length, root biomass and concentrations of the five medicinal components (glycyrrhizic acid, glycyrrhetinic acid, glycyrrhizin, liquiritigenin and isoliquiritigenin) of the SDI treatments were significantly higher than those of the DI treatments. The above parameters reached their maximum under the SDI_{90} treatment (SDI with 80%-90% of the maximum soil water capacity). The rhizome growth of the liquorice was promoted with an increase in irrigation amount, but the biomass of the rhizomes under SDI treatment was significantly lower than that under DI treatment. Thus, the regime of SDI with a relatively high water supply can effectively promote the yield and medicinal quality of liquorice roots, while inhibiting the development of its rhizomes.

Key words: *Glycyrrhiza inflata*, Medicinal components, Rhizome, Subsurface drip irrigation, Surface drip irrigation.

INTRODUCTION

Glycyrrhiza inflata is a perennial herb belonging to the genus *Glycyrrhiza* in the Leguminous family. Moreover, it is a medicinal liquorice listed in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2020). In traditional Chinese medicine, its dried roots and rhizomes have been used for its an anti-inflammatory, cough relief, antioxidant, scavenging free radicals and antitumor capabilities (Cao *et al.*, 2020; Wang *et al.*, 2020). Its stems and leaves are also excellent forage for sheep and cattle and are widely used in animal husbandry (Chen *et al.*, 2021). In recent years, the purchase and sale of liquorice has increased tremendously, leading to the decline of its wild resources and in some places, extinction. An imbalance between supply and demand in the liquorice market has become increasingly prominent and has been effectively alleviated by liquorice cultivation. Although both liquorice roots and rhizomes are used as Chinese medicinal materials, the sales volume and price of the roots are much higher than the rhizomes. However, the vigorous growth of rhizomes inevitably reduces nutrient allocation to the roots, thereby reducing the yield and quality of the roots (Wang *et al.*, 2021). Therefore, limiting the growth of the rhizomes while improving the yield and quality of the roots is essential for cost-effective liquorice cultivation.

In farmland ecosystems, soil moisture is one of the main environmental factors affecting plant growth and efficient control of soil moisture is an effective measure for coordinating plant growth and reproduction (Lal *et al.*, 2020). *Glycyrrhiza* plants are typical rhizome clonal plants and there are significant variations in clonal reproductive characteristics under different soil moisture conditions (Ye *et al.*, 2020). In recent years, the cultivation of liquorice in China has been carried out by surface drip irrigation (DI), but it has many shortcomings for the production of liquorice medicinal materials, such as it leads to excessive humidity on the ground, which can further lead to the occurrence of pests and diseases. Moreover, water does not penetrate through deep soil effectively, which reduces the water absorption efficiency of the roots (Githui *et al.*, 2020). Another drawback observed is that the rhizomes of the cultivated liquorice are elongated, root growth is restricted and the medicinal quality of roots is reduced. Moreover, the amount of irrigation was not standardised. This may be one of the reasons for the irregularity in the yield and quality of cultivated liquorice roots. Therefore, exploring high-efficiency irrigation regimes is crucial improving the yield and quality of liquorice medicinal materials.

As a new type of irrigation technology, subsurface drip irrigation (SDI) is a precise irrigation technique in the root

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zone. Compared with traditional DI, SDI can reduce water evaporation from the soil surface, enhance water absorption by the root system and promote root growth. It deepens the root growth of chickpeas (*Cicer arietinum*) and grapes (*Vitis vinfera*) (Kumar *et al*., 2021; Ma *et al*., 2020) and has achieved significant water-saving and yield-increasing effects in crop cultivation. However, the application of SDI to liquorice cultivation has not yet been reported. Therefore, experiments with two irrigation patterns (DI and SDI) and three irrigation levels (40%-50%, 60%-70% and 80%-90% of the maximum soil water capacity) were carried out in this study. We aimed to reveal the influence of different irrigation regimes on the yield of the vegetative organs of *G. inflata* and the medicinal quality of its roots and to explore a water management regime that could significantly promote root growth but inhibit rhizome development.

MATERIALS AND METHODS

Experiment materials

Seeds of the *G. inflata* were obtained from the Institute of Liquorice in Shihezi University. Healthy, full and uniform seeds of liquorice were selected, soaked in 98% sulfuric acid (H_2SO_4) for 30 min and rinsed with clean water.

Experiment methods

Plastic buckets (60×90 cm) were used to perform the pot experiments. River sand was used as the culture medium. In April 2019, 5 seeds of *G. inflata* were uniformly sown in each bucket at a seeding depth of 1 cm. According to the common fertilisation regime for liquorice cultivation in the field, CH_4N_2O (N>46%) (5.33 g), Ca (H_2PO_4) (P_2O_5>46%) (8.53 g) and K_2SO_4 (K_2O>50%) (2.66 g) were spread in each bucket as fertilisers (Fan *et al*., 2016).

Two irrigation patterns (DI and SDI) and three irrigation levels (40%-50%, 60%-70% and 80%-90% of the maximum river sand water capacity) were set. Six treatments were administered: DI with 40%-50% of the maximum river sand water capacity (DI_{50}), DI with 60%-70% of the maximum river sand water capacity (DI_{70}), DI with 80%-90% of the maximum river sand water capacity (DI_{90}), SDI with 40%-50% of the maximum river sand water capacity (SDI_{50}), SDI with 60%-70% of the maximum river sand water capacity (SDI_{70}) and SDI with 80%-90% of the maximum river sand water capacity (SDI_{90}). Each treatment was repeated for five times. Drippers were inserted 20 cm below the surface of the sand layer in the SDI treatments and the flow rate of DI and SDI were set at 2.0 L/ h. Soil moisture was measured and controlled using the time-domain reflectometry (TRIME-PICO IPH, Germany)(Wang *et al*., 2020). Probe tubes were installed to reach a depth of 80 cm at the centre of each bucket. Soil moisture was determined every day at intervals of 10 cm to 60 cm in depth and calibrated using the coring and oven-drying method. Soil irrigation was decided whenever the 40-cm-deep wet layer reached the lower limit. The irrigation amounts were calculated using the following equation:

$$I = 0.1\sum_{i=1}^{n} h_i\, d_i\, (w_i - w_o)$$

Where

I = The irrigation amount (mm).

h_i = The depth of the i layer (cm).

d_i = The soil bulk density of the i layer (g/m^3).

w_i and w_0 = The upper and measured soil moisture contents, respectively (%).

In October 2020, plants were harvested after measuring the plant height and crown width for each treatment. The stems, leaves, roots and rhizomes of each plant were separated and the taproot length of each treatment group was measured. An expression 1100 XL (Epson, Japan) scanner was used to scan the rhizomes and the root analysis system software (WinRHIZO Pro 2013) was used to measure the total rhizome length volume. The stems, leaves, roots and rhizomes were then dried in an oven at 75°C to a constant weight and their biomass was measured. Subsequently, the proportion of rhizome biomass was calculated:

$$\text{The proportion of rhizome biomass} = \frac{\text{Rhizome biomass}}{(\text{Rhizome biomass} + \text{Root biomass})} \times 100\%$$

To determine the content of the five medicinal components (glycyrrhizic acid, glycyrrhetinic acid, glycyrrhizin, liquiritigenin and isoliquiritigenin) in the roots of each treatment group, the ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC - MS/MS) method was applied. The standard curve, UHPLC and MS conditions reference to Fu *et al.* (2013) previous study. The results showed that, within a range of 1-1000 ng/mL, the concentrations of the five standard compounds showed a linear relationship with the peak area. The ion pairs, fragmentation voltages and collision energies used for quantitative analysis are shown in Table 1.

The dried roots were crushed and passed through a 50-mesh sieve, from which 1.00 g of root powder was accurately weighed, 10 mL of methanol was added and an ultrasonic extractor (Shimazu, Japan, power: 300W) was used for ultrasonic extraction at room temperature for 1 h. The supernatant was collected and filtered with a 0.45 μm micro-membrane. UHPLC-MS/MS was used to determine the content of the five compounds in the samples. Each sample was measured thrice and the average value was calculated.

Statistical analysis

SPSS software (version 20.0; IBM Corp., USA) was used to analyse the data. A *t*-test was used to analyse the differences in the significance of each parameter between the two irrigation patterns under the same irrigation level. Duncan's multiple range test was used to analyse the differences in the significance of each parameter among the three irrigation levels under the same irrigation pattern. Origin 2017 (OriginLab, USA) was used to generate charts.

Table 1: Mass spectrometry conditions, regression equations and correlation coefficients of five compounds.

Compounds	Parent ion (m/z)	Daughter ion (m/z)	Ionization mode	Voltage (V)	Collisional energy (V)	Retention time (min)	Regression equation	Correlation coefficients R^2	Linear over (ng/mL)
Glycyrrhizic acid	821.2	350.9*	-	62	42	2.48	Y=87.2 X -3.76	0.9992	1.0 -978.0
		113.0		62	56				
		134.9		2	18				
Glycyrrhetinic acid	469.2	355.1*	-	100	44	3.81	Y=136.8X-122.4	0.9990	1.4-993.2
		409.2		100	46				
Glycyrrhizin	417.0	254.9*	-	52	20	1.00	Y=750.9 X+356.9	0.9999	1.0-992.3
		134.9		52	30				
Liquiritigenin	257.2	137.0*	+	92	24	1.97	Y=509.2X+925.6	0.9980	0.9-962.5
		147.0		92	18				
Isoliquiritigenin	257.0	136.9*	+	2	24	2.47	Y=8775.1X+2617.2	0.9993	1.1- 985.6
		147.0		2	18				

Note: * stands for quota ion.

RESULTS AND DISCUSSION

Effects of irrigation patterns and irrigation levels on the morphological characteristics and biomass of *G. inflata*

Plant growth is not only controlled by their own genetic material but also by biological or non-biological environmental factors, among which soil moisture has a significant impact on plant morphogenesis and photosynthetic product accumulation (Alshameri *et al*., 2020; Ram *et al*., 2016; Al-Shareef *et al*., 2018). The results showed that, under both irrigation patterns, the growth of *G. inflata* was promoted by an increase in water supply (Fig 1). Irrigation levels had relatively smaller effects on the plant height, crown width and taproot length of liquorice, but they had a significant effect on stem and leaf and root biomass. Compared with the DI_{50} treatment, the plant height, crown width and taproot length of the DI_{90} treated samples increased by 15%-37% and the stem and leaf biomass and root biomass were promoted by 176% and 166%, respectively. Similarly, the plant height, crown width and taproot length of SDI_{90} treated samples were higher than those of the SDI_{50} treatment group by approximately 12%-14%. However, its stems and leaves biomass and roots biomass were 2.67 and 1.89 times as high as those of the SDI_{50} treatment. This means that *G. inflata* prefers to invest more photosynthetic products in the growth of stem height, branching and taproot length under low soil moisture conditions to ensure the ability to capture more light and absorb more water in the deep soil layer and more resources accumulate in the biomass of the stems, leaves and roots under higher soil water conditions. Therefore, a sufficient water supply should be provided to promote biomass accumulation in stems, leaves and roots during liquorice cultivation.

The plant height, crown width, taproot length, stem and leaf biomass and roots biomass of *G. inflata* under the SDI treatment were all higher than those observed in the DI treatment group under the three irrigation levels. Thus, the result obtain was the same as that of studies on watermelon (*Citrullus lanatus*), corn (*Zea mays*) and alfalfa (*Medicago sativa*) (Alam *et al*., 2002; Xu *et al*., 2015; Reddy *et al*., 2020). This may be because the ground surface under SDI conditions was drier than that under DI treatment and the aeration of the soil improved. The evaporation at the surface soil was reduced, which was more conducive to the absorption and utilisation of water by the roots (Kang *et al*., 2004). Furthermore, SDI directly supplies water to the root zone of plants, thereby reducing the supply of ineffective water and waste by water evaporation from the ground (Umair *et al*., 2019). Thus, SDI is more suitable than DI as it prevents water loss by evaporation and provides better soil moisture distribution near the active roots of *G. inflata*.

Effects of irrigation patterns and irrigation levels on the development of rhizomes

In addition to being a clonal reproduction organ, the rhizome of liquorice also has functions in nutrient storage. Under the two types of irrigation patterns, the irrigation amount had a positive effect on the growth of the rhizomes; that is, the higher the irrigation amount, the more vigorous the growth of the rhizomes (Fig 2), which was similar to the results reported by Xiao *et al*. (2011). The reason may be that cloned plants would store part of their photosynthetic products in the rhizomes under higher water conditions to buffer the adverse effects of environmental changes and ensure the normal growth of the plants.

We found that compared with DI treatment, the SDI treatment significantly inhibited the development of rhizomes (Fig 2). In this study, the total length, total volume, biomass of rhizomes and proportion of rhizome biomass of the SDI_{90}

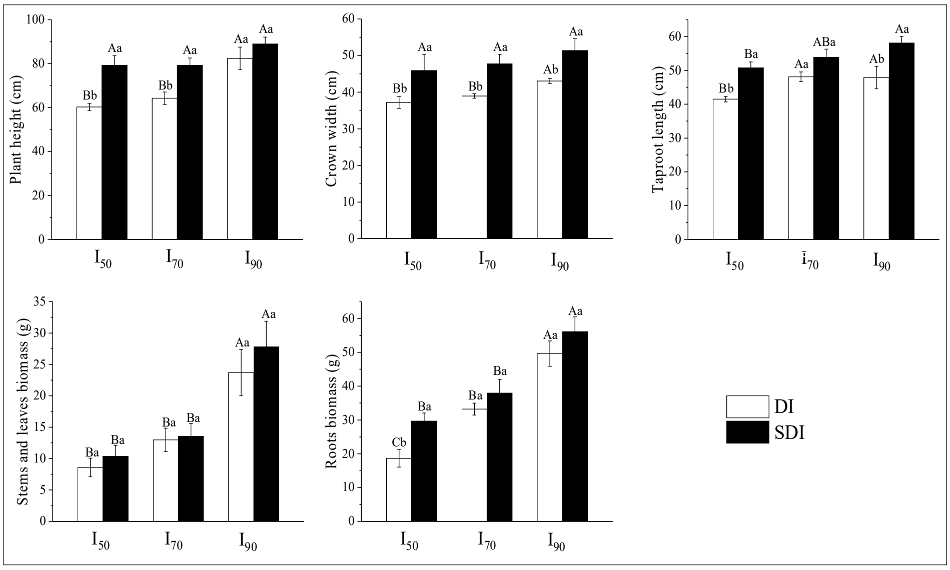

Fig 1: The morphological characteristics and yield of *G. inflata* under different irrigation methods and irrigation levels treatments (Mean ± SE). Different capital letters indicate significant differences between the data of irrigation levels treatment (P<0.05) and different lowercase letters indicate significant differences between the data of irrigation methods treatment (P<0.05). I_{50}: Keep river sand water content in 40%-50% of the maximum river sand water capacity, I_{70}: Keep river sand water content in 60%-70% of the maximum river sand water capacity, I_{90}: Keep river sand water content in 80%-90% of the maximum river sand water capacity.

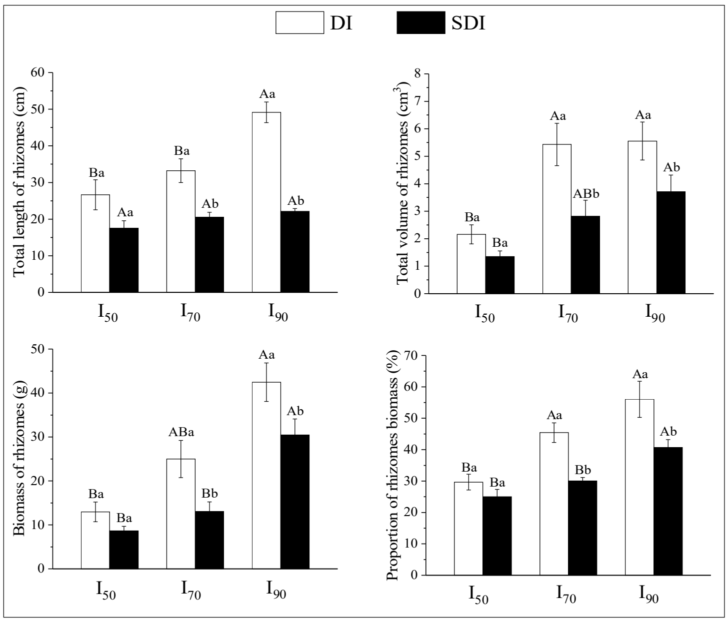

Fig 2: The clonal reproduction traits of *G. inflata* under different irrigation methods and irrigation levels (Mean ± SE). Different capital letters indicate significant differences between the data of irrigation levels treatment (P<0.05) and different lowercase letters indicate significant differences between the data of irrigation methods treatment (P<0.05). I_{50}: Keep river sand water content in 40%-50% of the maximum river sand water capacity, I_{70}: Keep river sand water content in 60%-70% of the maximum river sand water capacity, I_{90}: Keep river sand water content in 80%-90% of the maximum river sand water capacity.

treated samples were reduced by 54.0%, 33.3%, 28.2% and 27.4%, respectively, compared with the DI_{90} treated samples. The total length, volume, biomass of rhizomes and rhizome biomass of the SDI_{70} treated samples were reduced by 38.1%, 48.1%, 47.6% and 33.8%, respectively, compared with the DI_{70} treatment group. The total length, total volume, biomass of rhizomes and proportion of rhizome biomass of the SDI_{50} treatment group were reduced by 34.0%, 37.5%, 33.2% and 15.7%, respectively, compared with the DI_{50} treatment group. This may be because SDI treatment reduced the water evaporation level from the ground and increased the water content in the soil around the roots. The rhizome growth pattern tended to be "aggregated," which was characterized by its small number, short length and low biomass of the rhizomes. This is conducive to the occupation and utilization of environmental resources as soon as possible (Nie *et al*., 2018). However, under the DI treatment, the amount of water evaporated from the soil surface increased, but the water content in the soil decreased. Under this condition, the rhizome's growth of the liquorice tended to be in a "guerrilla" pattern. By increasing the length and biomass of the rhizomes, the rhizomes would extend to resource-rich areas to obtain necessary water, which is usually considered an escape strategy for plants (Colmer and Voesenek, 2009). Therefore, compared with the DI treatment, the SDI treatment significantly reduced the nutrient investment in the rhizomes, thereby increasing root yield.

Effects of irrigation patterns and irrigation levels on the contents of the medicinal components present in the root of *G. inflata*

The medicinal components in the roots of *G. inflata* are secondary metabolites with specific activities and the formation and accumulation of the secondary metabolites are closely related to soil moisture (Albergaria *et al*., 2020). Moreover, the contents of medicinal components are the basis of the quality and sales price of liquorice medicinal materials. Glycyrrhizic acid present in *G. inflata* roots is the main bioactive component with anti-viral, anti-inflammatory, anti-tumour and other major pharmacological activities (Zhang and Ye, 2009). Glycyrrhetinic acid, a triterpenoid aglycone component of the natural product glycyrrhizic acid, has been found to possess remarkable anti-proliferative and apoptosis-inducing activities in various cancer cell lines (Xu *et al*., 2017). As another major effective component in *G. inflata* root, flavonoids have significant anti-tumour and antioxidant activities and the most represented flavonoids are glycyrrhizin, liquiritigenin and isoliquiritigenin (Farag *et al*., 2015).

In this study, with an increase in irrigation amount, the contents of the five medicinal components in the root showed an increasing trend (Fig 3). The concentrations of glycyrrhizic, glycyrrhetinic, glycyrrhizin, liquiritigenin and isoliquiritigenin during the DI_{90} treatment were higher than that observed during DI_{70} and DI_{50} treatment. Similarly, the concentrations of these components in the SDI_{90} treatment group were higher than those in the SDI_{70} and SDI_{50} treatment groups. Under the same irrigation levels, the concentrations of the five medicinal components in the SDI treatment group were all higher than those in the DI treatment group. Compared with DI_{90} treatment, the concentrations of glycyrrhizic acid, glycyrrhetinic acid, glycyrrhizin, liquiritigenin and isoliquiritigenin of SDI_{90} treatment increased by 29.3%, 33.7%, 36.5%, 75.6% and

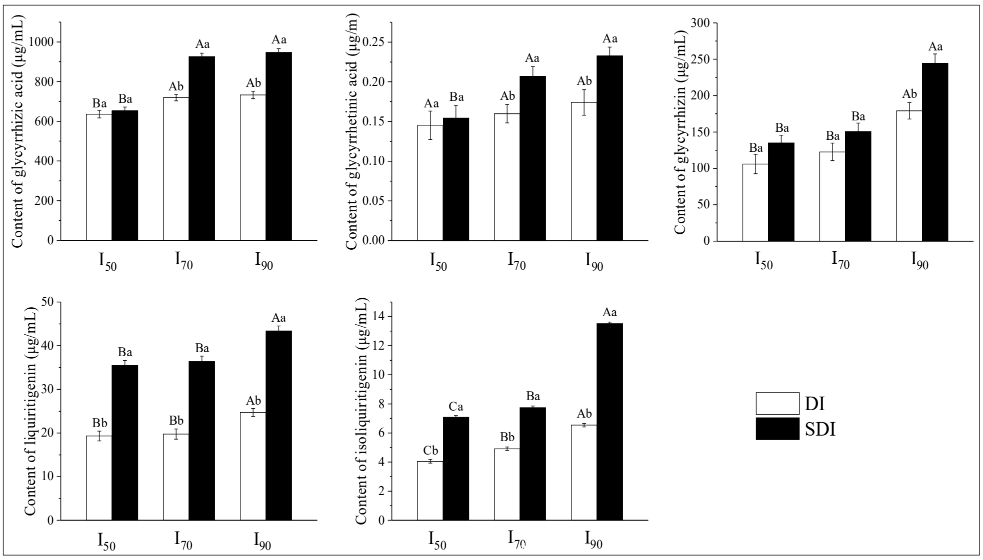

Fig 3: Contents of five medicinal components in the taproot of *G. inflata* under different irrigation methods and irrigation levels treatments (Mean ± SE). Different capital letters indicate significant differences between the data of irrigation levels treatment (*P*<0.05) and different lowercase letters indicate significant differences between the data of irrigation methods treatment (*P*<0.05). I_{50}: Keep river sand water content in 40%-50% of the maximum river sand water capacity, I_{70}: Keep river sand water content in 60%-70% of the maximum river sand water capacity, I_{90}: Keep river sand water content in 80%-90% of the maximum river sand water capacity.

106.6%, respectively. Compared with the DI_{70} treatment, the concentrations of these components in the SDI_{70} treatment increased by 28.7%, 29.6%, 23.0%, 84.1% and 57.5%, respectively. Compared with the DI_{50} treatment, the concentrations of these components in the SDI_{50} treatment increased by 2.8%, 6.3%, 27.4%, 84.0% and 74.6%, respectively. Thus, the data suggest that SDI treatments were more conducive to the accumulation of the five medicinal components in the root than DI treatments. Therefore, using the SDI with a higher water supply can significantly improve the quality of the liquorice medicinal materials.

CONCLUSION

In summary, although DI treatment with a high level of irrigation may improve the growth of roots of *G. inflata*, it may spur rhizome development. However, SDI treatment can not only increase the growth of the roots and the content of its medicinal components but can also significantly inhibit the development of rhizomes. It is worth noting that although SDI shows excellent yield-increasing potential, its drippers are more easily blocked than those of DI. Because the plugged drippers of SDI are more difficult to position and their replacement is more difficult, the repair and replacement costs of SDI systems are more expensive than DI. The drippers in SDI are expected to operate satisfactorily for a longer duration and filtration techniques that reduce accumulated effects on dripper plugging may be of relatively greater importance than in DI. These advantages and disadvantages should be carefully defined in future studies.

Conflict of interest: None.

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