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Hitoshi Hirosse, Edison; Creste, José Eduardo; Castilho Custódio, Ceci; Barbosa Machado-Neto, Nelson
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In vitro growth of sweet potato fed with potassium phosphite

Edison Hitoshi Hirosse, José Eduardo Creste, Ceci Castilho Custódio and Nelson Barbosa Machado-Neto*

*Laboratório de Cultura de Tecidos, Faculdade de Ciências Agrárias, Universidade do Oeste Paulista, Rod. Raposo Tavares, Km 572, 19067-175, Presidente Prudente, São Paulo, Brazil. *Author for correspondence. E-mail: nbmneto@unoeste.br*

ABSTRACT. The main objective of this work was to verify the effect of the substitution of phosphate by its related compound phosphite on the *in vitro* growth of sweet potato nodal segments. Segments from a previous culture were incubated in MS media with the following combinations of phosphate:phosphite: Control (100 / 0%); T1 (87.5 / 12.5%); T2 (75 / 25%); T3 (50 / 50%) and T4 (0 / 100%) in a 5 x 5 factorial arrangement, with five treatments (Control, T1, T2, T3 and T4) and five evaluation periods (7, 14, 21, 28 and 35 days). The design was completely random, with three replicates of two explants per period. The evaluations were made weekly by counting the number of shoots and the length and dry weight of shoots and roots. Increasing the phosphite concentration decreased all attributes measured, including growth velocity, length and dry weight. These results indicate that phosphite cannot be used as substituted of phosphate in sweet potato tissue cultures.

Keywords: *Ipomoea batatas*, nutrition, plantlet, shoot, root.

Crescimento *in vitro* de batata-doce nutrida com fosfito de potássio

RESUMO. O objetivo deste trabalho foi verificar o efeito da substituição do fosfato pelo composto derivado fosfito no crescimento *in vitro* de segmentos nodais de batata doce. Os segmentos obtidos de culturas prévias foram incubados em meio MS com as seguintes combinações de fosfato/fosfito: Controle (100 / 0%); T1 (87.5 /12.5%); T2 (75 / 25%); T3 (50 / 50%) e T4 (0 / 100%) respectivamente, em arranjo fatorial de 5x5, com cinco tratamentos (Controle, T1, T2, T3 e T4) e cinco períodos de avaliação (7, 14, 21, 28 e 35 dias). O desenho experimental foi completamente casualizado com três repetições com dois explantes por período. As avaliações foram feitas semanalmente por contagem do número de brotos, comprimento e peso seco de parte aérea de brotos e raízes. O aumento da dose de fosfito diminuiu todos os atributos analisados, como velocidade de crescimento, comprimento e peso seco, significando que o fosfito não pode ser utilizado em substituição ao fosfato na cultura de tecidos de batata doce.

Palavras-chave: *Ipomoea batatas*, nutrição, plantlete, broto, raiz.

Introduction

Phosphorus (P) is one of the main nutrients for plants. It is a component of the nucleotide triphosphates (NTP) generated during respiration and photosynthesis and is a component of DNA. It is important in flowering, fruiting and root development. When plants lack phosphorus, their leaves acquire a bluish-green colour and then purple tones, followed by yellowing (ABEL et al., 2002; MALAVOLTA et al., 2002). The primary difference between phosphite (designated Phi in this paper) and phosphate (designated Pi in this paper) is that the Phi has a hydrogen atom in place of one oxygen atom (McDONALD et al., 2001). To be metabolised, Pi reacts with the enzyme phosphatase. This enzyme recognises three of the four oxygen atoms and binds to them. The last atom of oxygen becomes available to react with other catalysts.

Phosphite has only three atoms of oxygen. One hydrogen atom takes the place of the other oxygen. For this reason, continuation of the metabolism of Phi is not possible. Thus, Phi cannot participate in the same biochemical reactions as Pi. Phi is discarded by most of the enzymes involved in transfer reactions of phosphorus (PLAXTON, 1998). However, Orbovi et al. (2008) have suggested that Phi could replace Pi for plant nutrition.

Phosphites are liquid products originating from the neutralisation of phosphorous acid (H_3PO_3) with a base. The base used may be sodium, potassium, ammonium hydroxide or other compounds. The base most commonly used for this purpose is potassium hydroxide. This base generates the compound potassium phosphite. Potassium phosphite has excellent plant health facilitation qualities and fungicidal activity. It either acts directly

on the fungi or activates the defence mechanisms of plants to induce the production of phytoalexins (RATJEN; GERENDÁS, 2009).

Phi is quickly absorbed by the roots, leaves and trunk bark and therefore entails a lower energy requirement for absorption by the plant. As an effective chelating agent, it favours the absorption of K, Ca, B, Zn, Mo, Mn and other nutrients. It can be mixed with other products. Some formulations of phosphites can reduce the pH of a solution, thereby improving the efficiency of some herbicides (LOVATT; MIKKELSEN, 2006) and improving plant defences (ORBOVI et al., 2008).

One of the reasons for the ineffectiveness of potassium phosphite for elevating productivity is that products based on phosphorous acid are not considered to be good sources of phosphorus. Generally, these products fail to supply the demand for inorganic phosphate for use in energy transport (STEHMANN; GRANT, 2000).

Phosphites probably do not have sufficient nutritional effects to increase crop productivity. Although Phi compounds are marketed as fertilisers containing phosphorus, an increase in productivity is not expected from application of this nutrient. McDonald et al. (2001), Thao and Yamakawa (2008, 2009), and Thao et al. (2008, 2009) have all argued that Phi cannot be used as a phosphorus source, either by crops or by growing cells. According to Singh et al. (2003), cell cultures grown using Phi as a nutrient have exhibited increased cell death, protein deterioration and DNA damage, perhaps owing to the erroneous incorporation of Phi in place of Pi. In contrast, Orbovi et al. (2008) assert that Phi could be used by plants as a nutrient.

Other effects of Phi application mentioned in the literature include the nutritional balance of plants, better ripening and fruit quality, and superior postharvest quality (BRACKMANN et al., 2004; LOBATO et al., 2008; MOOR et al., 2009; NOJOSA et al., 2005).

The sweet potato (*Ipomoea batatas* L. (Lam)) is native to Central and South America, from the Yucatan Peninsula, Mexico, to Colombia. Its use dates back more than ten thousand years, according to analysis of dried potatoes found in caves located in Chilca Canyon, Peru and evidence found in archaeological writings in the Mayan region of Central America (PEARSALL, 2008).

The sweet potato is grown as an annual crop. It stores food reserves in its roots. These roots have considerable alimentary, industrial and high-calorie (carbohydrate) potential. They contain iron, calcium and phosphorus, and they are rich in vitamins A, B and C (SILVA et al., 2002). The sweet potato responds to Pi provision by increasing the accumulation of starch in its reserve tissues (OLIVEIRA et al., 2005).

Plant tissue culturing involves a set of techniques having broad applications in agriculture. These techniques first isolate small fragments of living tissue called explants. Explants may be a fragment of leaf, root, or stem that can respond to the conditions of the inducing medium. Explants with in vitro regeneration capability are isolated from a plant, disinfected and grown aseptically for indefinite periods in an appropriate culture medium (TORRES et al., 1998). The goal is to obtain a new plant identical to the original, i.e., to make a clone. Cloning is defined as the asexual propagation of cells or organisms to obtain a new individual while maintaining the donor's genotype (TORRES et al., 1998).

The objective of this work was to verify the effects of phosphite on the growth of the sweet potato when phosphite was substituted partially or totally for a phosphate source.

Material and methods

The work was conducted in the Tissue Culture Laboratory of the Faculdade de Ciências Agrárias da Universidade do Oeste Paulista (UNOESTE, Presidente Prudente, São Paulo State).

The study used a cultivar of sweet potato (*Ipomoea batatas* (L.) Lam) clone 1208 donated by the Germplasm Bank of the Agência Paulista de Tecnologia dos Agronegócios (APTA), Pólo Regional da Alta Sorocabana, Presidente Prudente-SP. The potatoes were collected, washed under running tap water with commercial soap, and treated with a disinfecting solution containing 1% Benomyl. The potatoes were placed in plastic trays containing washed sand to allow budding and the formation of nodal segments.

The stems were washed and disinfected with a commercial solution of 20% sodium hypochlorite (NaClO) and 100 μL of Triton X100 for 20 minutes, rinsed with autoclaved distilled water, and then cut into segments having two nodes. The culture medium used was MS (MURASHIGE; SKOOG, 1962), jellified with 2 g L^{-1} Phytagel and 30 g L^{-1} sucrose, pH adjusted to 5.9, aliquoted in 50 mL flasks and autoclaved at 121ºC for 20 min. The plants obtained from this first culture provided the segments for the experiment.

Treatments were conducted using the changes in molar concentrations of phosphorus produced by the exchange of hydrogenous dipotassium phosphate (K_2HPO_4) in equimolar amounts by

potassium phosphite (K_3PO_3). For this purpose, stock solution I of MS medium, which contains KNO_3 and KH_2PO_4, was divided into MSIa, containing 9.5 g of KNO_3 100 mL^{-1} water, and MSIb, containing only phosphate (850 mg KH_2PO_4 100 mL $water^{-1}$). Thus, during the process of exchange, the phosphite was balanced with nitrogen, phosphorus and potassium in the solution with nitric acid, MSIa, MSIb and potassium phosphite, as shown in Table 1.

Table 1. Chemical composition of the culture media used, with corrections to the final concentration of potassium, nitrogen and phosphorus. The initial solution consisted of 0:30:20 (N:P:K) potassium phosphite.

Treatments	MS solutions used		Complements
Control	0% HPO_3^{-2} 100% PO_4^{-3}	Complete MS full strength (I to VII)	None
T1	12.5% HPO_3^{-2} 87.5% PO_4^{-3}	MS (II to VII)	0.25 g Phosphite 19.3 mL MSIa 60.5 μL HNO_3 17.5 mL MSIb
T2	25% HPO_3^{-2} 75% PO_4^{-3}	MS (II to VII)	0.5 g Phosphite 18.6 mL MSIa 121 μL HNO_3 15 mL MSIb
T3	50% HPO_3^{-2} 50% PO_4^{-3}	MS (II to VII)	1.0 g Phosphite 15.9 mL MSIa 242.5 μL HNO_3 10 mL MSIb
T4	100% HPO_3^{-2} 0% PO_4^{-3}	MS (II to VII)	2.0 g Phosphite 485 μL HNO_3 10.4 mL MSIa

The plantlets obtained by micropropagation were removed from the culture bottles under laminar flow and sectioned into nodal segments containing two nodes. These segments were transferred to bottles, each containing two explants and the culture media with different concentrations of Phi. The bottles were sealed with plastic wrap and incubated in a growth room at 25 ± 3ºC with a photoperiod of 16 hours for 35 days. After this period, the bottles were opened and the plantlets were removed and washed in distilled water to remove excess medium. The plantlets were then stretched and measured using a ruler. The measurements taken included the greatest lengths of shoots and roots separately. The number of shoots and roots per explant were counted every seven days.

To assess the dry weight, plant material was placed in paper bags and dried in an oven at 65°C for 72 hours. The material was kept in a desiccator to cool and then weighed on an analytical balance accurate to 0.00001 g (NAKAGAWA, 1999).

A 5 x 5 factorial scheme with 5 treatments (T, T1, T2, T3 and T4) and 5 evaluation periods (7, 14, 21, 28 and 35 days) was used. The experiment used a completely randomised design with three replicates per trial period. Each plot consisted of two explants. Regression equation curves were also calculated, and the statistical significance of the regressions was assessed. Data analysis was performed with the aid of SISVAR software (FERREIRA, 2008).

Results and discussion

One of the main difficulties facing successful establishment of the in vitro culture was disinfection. The branches collected from the mother plant, especially those collected from field material (CHAVES et al., 2005; ERIG; SCHUCH, 2003; PINTO et al., 2002), exhibited high levels of contamination. Such contamination does not occur in new sprouts that grow from potatoes placed in humid sand.

Growth occurred in all treatments with differing concentrations of Phi. The control grew more at all times. T4, with a higher concentration of Phi, had the lowest growth found, either for shoots or for roots (Figure 1A and B).

Different concentrations of Phi altered development. Reduced plantlet growth was observed along with rapid decay. The control and the treatment with the lowest dose of Phi (T1) maintained plant growth until 29 days (maximum point). The other three treatments shortened the time of growth to 22, 23 days, and no growth, respectively, for the higher Phi doses (Figure 1A). Shoot length also decreased from 7.13 cm in the control treatment to 2.7 cm in the treatment with 50% Phi (T3).

The control exhibited maximum root growth until the 30th day. The roots of plantlets treated with the lowest dose of Phi kept growing until the 32nd day. The treatments with higher Phi concentrations altered root growth by shortening the time of growth to 24 days, 20 days and no growth, respectively (Figure 1B). Root length also decreased from 24.08 cm in the control to 9.57 cm in the T3 treatment (50% Phi).

Shoots developed in all treatments (Figure 2), including those with the various Phi concentrations. The control, T1 and T2 exhibited the highest shoot numbers, and they differed very little (1.13 to 1.1). The T3 and T4 treatments, which had the highest Phi concentrations, produced the lowest numbers of shoots (0.78 and 0.53, respectively).

The Phi concentrations affected the shoot numbers of the plantlets. These treatments reduced the shoot numbers and did so rapidly. The control and the T1 treatment (lower Phi dose) maintained the same numbers of shoots on the plants until the 26th and 27th days, respectively. Treatments T2 and T4 shortened this time to 24 days. T3 maintained

growth until the 25th day. T1 exhibited faster initial development, but this was not subsequently maintained. This result may have been obtained because the Phi and the Pi have similar initial uses by the plants, whereas during the subsequent growth the availability of Pi was not sufficient to allow the plantlets to maintain their initial growth rate (Figure 2).

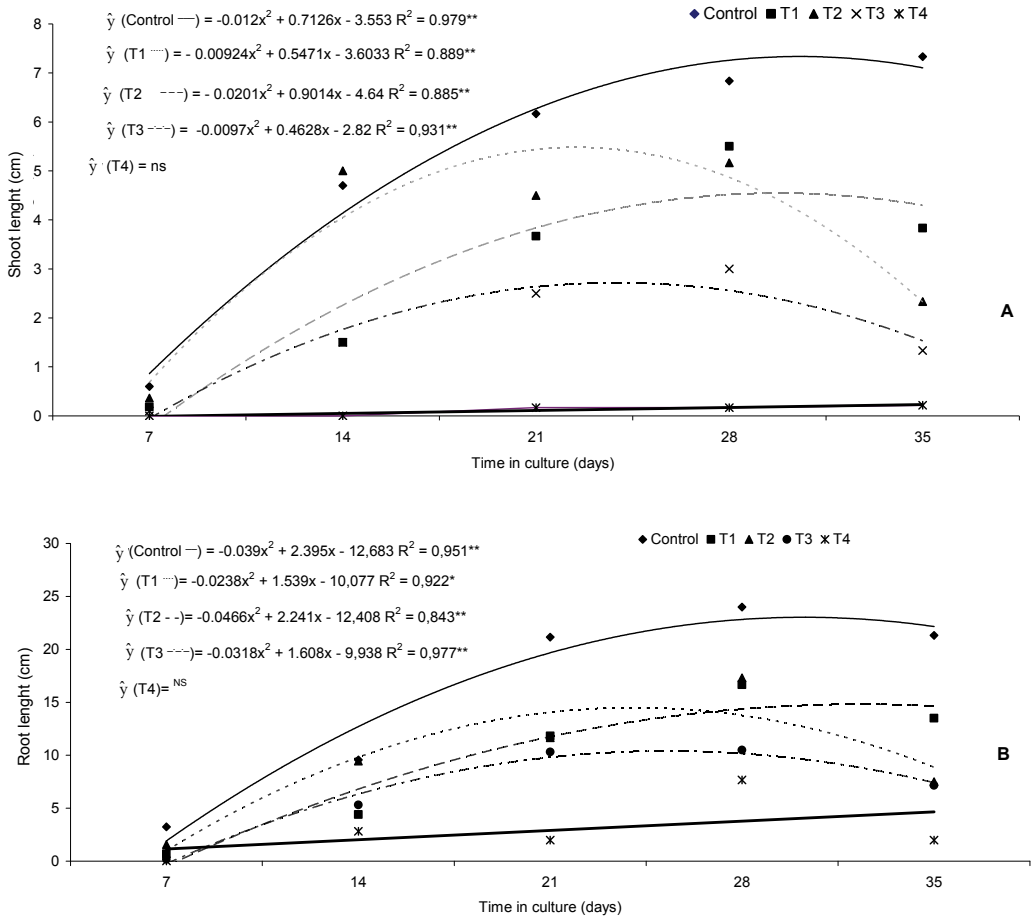

Figure 1. Sweet potato plantlet shoot (A) and root (B) length in media containing different doses of phosphorus (phosphite and phosphate sources) during 35 days of growth.

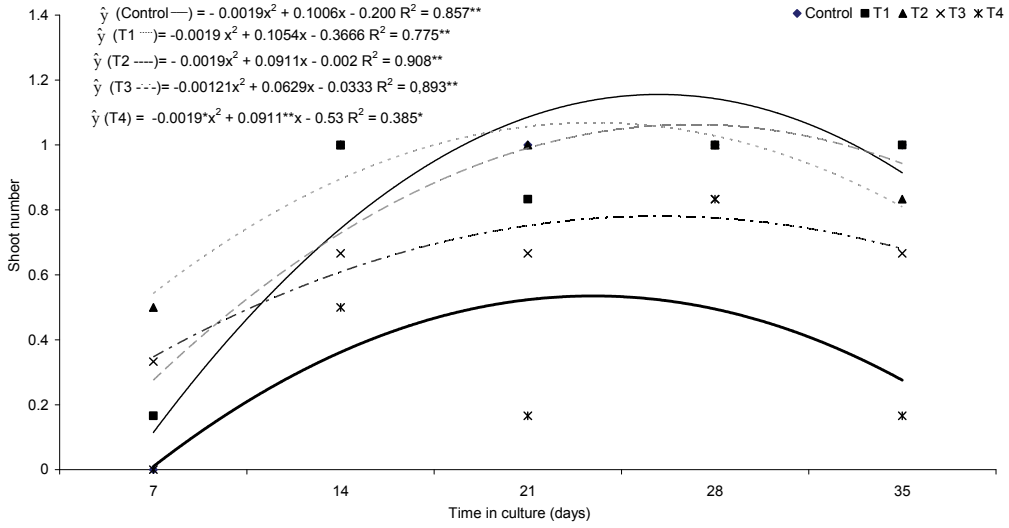

Figure 2. Shoot numbers in sweet potato plantlets in media containing different doses of phosphorus (phosphite and phosphate sources).

Dry mass accumulation (Figure 3A and B) was high for the control treatment, but it decreased as the Phi dose increased. Maximum growth was reached at 28 and 30 days for shoots and roots (Figure 3A and B), respectively, in the control. T1, the lowest Phi dose treatment, showed a maximum shoot dry mass of 0.013 g at 33 days after inoculation. T2 exhibited a short duration of dry mass accumulation (23 days) and 0.015 g of maximum shoot dry mass. T3 exhibited a longer period of dry mass increase (26 days), but the accumulation of dry mass exhibited in this treatment was the lowest observed (0.0057 g) (Figure 3A).

The root dry mass (Figure 3B) showed an increase of 0.00046 g day^{-1} for treatment T1. The root dry mass reached its growth maximum at 21 days after inoculation in treatment T2, 0.007 g. T3 exhibited the lowest dry matter accumulation, 0.00021 g day^{-1} (Figure 3B).

The decreases observed in all measurements show that Phi cannot be used as a phosphorus source. This effect is linked to the high demand for Pi in carbohydrate metabolism. Substitution of Pi by Phi interferes with the capacity for synthesis of substances that function in storage or structure (starch, sucrose and cellulose). The cell maintains the functioning of its basal metabolism as a primary priority, and accordingly, synthesis is limited under these conditions. The mechanism giving rise to this effect is that Phi, unlike Pi, cannot be used as an essential nutrient. Phosphate has important cellular functions (MALAVOLTA et al., 1997). Phi can be used only after conversion to microbial phosphate.

In addition, Phi is a potent inhibitor of the main route of energy production in fungi: the respiration pathway (STEHMANN; GRANT, 2000). In this case, the tissues are very tender, and uptake of Phi is not limited. The plant can absorb Phi but cannot use it for the most basic functions, namely the transport of energy through nucleotide triphosphates (NTP), or the genetic processes (replication, transcription and translation) that require a supply of appropriate NTP.

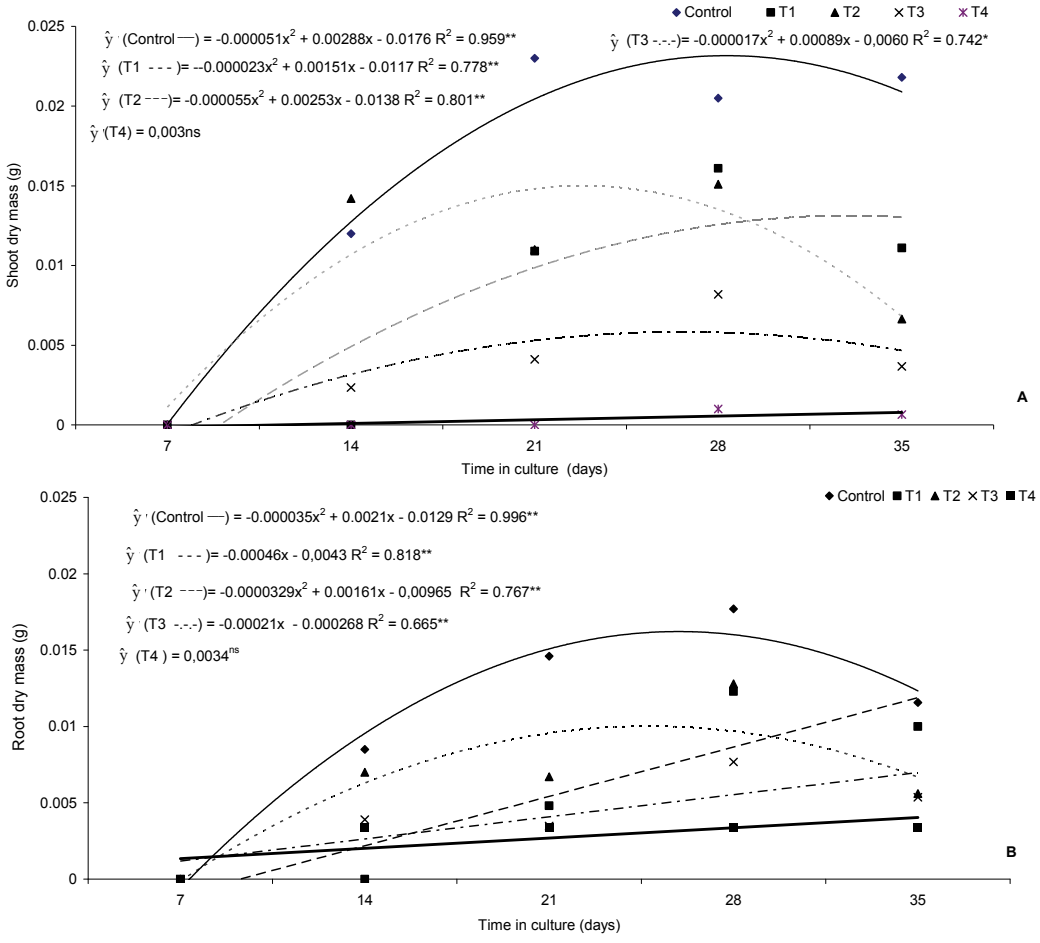

Figure 3. Shoot (A) and root (B) dry mass in sweet potato plantlets in media containing different doses of phosphorus (phosphite and phosphate sources) during 35 days of growth.

Thao et al. (2008, 2009) have investigated different cultures and have shown that Phi is not an appropriate phosphorus source for plant nutrition. Their data dramatically reveal the negative effects of this salt on plant nutrition.

In older tissues, the effects are less drastic than in growing tissues, in which Phi represents the sole source of phosphorus. Less drastic effects are exhibited because the mature tissues can translocate the existing phosphorus and thereby reduce the effects of Phi on developing tissues. In cell cultures of *Brassica napus* subjected to phosphorus deficiency, the older cells accelerate their decay and death, thereby providing phosphate for growing cells (SINGH et al., 2003).

Conclusion

The replacement of phosphate by phosphite in all the doses studied here inhibited the growth of sweet potato explants.

Phosphite cannot be used as a phosphorus source for *in vitro* cultures of the sweet potato.

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