http://dx.doi.org/10.18512/1980-6477/rbms.v15n1p30-38

PHOSPHATE SOLUBILIZING FUNGI ENHANCE THE GROWTH AND PHOSPHORUS UPTAKE OF SORGHUM PLANTS

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Revista Brasileira de Milho e Sorgo, v.15, n.1, p. 30-38, 2016

ABSTRACT – The beneficial effects of soil inoculation with phosphate solubilizing fungi (PSF) on plant growth have been verified in several plant species. However, the effects of PSF on sorghum [*Sorghum bicolor* (L.) Moench] are still unknown. This study investigated the effect of PSF inoculation associated with the application of reactive phosphate rock (RPR) on growth and phosphorus uptake by sorghum plants. Sorghum plants were grown in 8-L pots filled with a clayey Rhodic Hapludox in a greenhouse. The treatments were: 1) control (non-inoculated and non-fertilized with P); 2) inoculation with PSF (10^8 CFU mL^{-1}); 3) application of RPR (240 mg dm^{-3} of P_2O_5); 4) PSF inoculation (10^8 CFU mL^{-1}) + RPR application (240 mg dm^{-3} of P_2O_5). The plants were inoculated with PSF (*Aspergillus terreus* and *Penicillium pinophilum*) at three days after emergence. The use of RPR and PSF improved the phosphorus-uptake of plants, and resulted in higher dry matter yield of sorghum. Results indicate that the PSF played an important role in sorghum nutrition through the increase in P uptake by the plants, and the use of PSF to stimulate plant growth could be an important contribution to phosphorus fertilizer on sorghum. However, the contribution of these PSF to increasing the P nutrition of crops should be investigated further in field experiments.

Key words: *Sorghum bicolor*, *Aspergillus terreus*, *Penicillium pinophilum*, phosphorus solubilization, reactive phosphate rock.

FUNGOS SOLUBILIZADORES DE FOSFATO AUMENTAM O CRESCIMENTO E A ABSORÇÃO DE FÓSFORO DAS PLANTAS DE SORGO

RESUMO - Efeitos benéficos da inoculação de fungos solubilizadores de fosfato (FSF) no crescimento das plantas têm sido verificados em várias espécies vegetais. No entanto, os efeitos da aplicação de fungos solubilizadores de fosfato em plantas de sorgo [*Sorghum bicolor* (L.) Moench] ainda são desconhecidos. Este estudo avaliou os efeitos da inoculação de FSF e da aplicação de fosfato natural reativo (FNR) no crescimento e na absorção de fósforo (P) em plantas de sorgo. As plantas de sorgo foram cultivadas em casa de vegetação em vasos de 8 L preenchidos com um Latossolo Vermelho argiloso. Os tratamentos testados foram: 1) controle (não inoculado e não fertilizados com P); 2) inoculação de FSF (10^8 UFC mL^{-1}); 3) aplicação de FNR (240 mg dm^{-3} de P_2O_5); e 4) inoculação de FSF (10^8 UFC mL^{-1}) + aplicação de FNR (240 mg dm^{-3} de P_2O_5). Os FSF (*Aspergillus* terreus e Penicillium pinophilum) foram inoculados três dias após a emergência das plantas. A aplicação de FNR e a inoculação de FSF melhoram a absorção de P das plantas, resultando no aumento da produção de matéria seca do sorgo. Os resultados indicam que os FSF desempenharam um importante papel na nutrição do sorgo por aumentar a absorção de P da planta e o uso de FSF para estimular o crescimento das plantas poderá ser uma contribuição importante na fertilização fosfatada da cultura do sorgo. No entanto, a contribuição destes FSF na melhoria da nutrição de P das culturas precisa ser investigada futuramente em experimentos de campo.

Palavras-chave: *Sorghum bicolor* L., *Aspergillus terreus*, *Penicillium pinophilum*, solubilização de fósforo, fosfato natural reativo.

Versão impressa ISSN 1676-689X / Versão on line ISSN 1980-6477
http://rbms.cnpms.embrapa.br/

The bioavailability of nutrients is one of the most relevant factors for crop development. Phosphorus (P) is a vital plant nutrient, available to plant roots only in soluble forms that are in short supply in most soil conditions (El-Azouni, 2008). A wide range of soil microorganisms (bacteria and fungi) are able of mineralizing and solubilizing P from the organic and inorganic soil pools (Gomes et al., 2014; Souchie et al., 2005; Richardson, 2001). Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus*, and fungi of the genera *Penicillium* and *Aspergillus* possess the ability to bring insoluble soil phosphates into soluble forms by secreting acids such as formic, acetic, propionic, lactic, glucolic, fumaric and succinic. These acids reduce the soil pH and bring about the dissolution of bond forms of phosphate (Bolan et al., 1997; Mohammadi & Sohrabi, 2012). Souchie & Abboud (2007) reported P solubilizing capacity of 120 and 15 mg L^{-1} for the fungi and bacteria, respectively.

Phosphorus is added to the soil in the form of phosphate fertilizers, part of which is utilized by plants and the remainder converted into insoluble forms (non-labile soil pools). To circumvent P deficiency, phosphate solubilizing fungi (PSF) play an important role in supplying P to plants in a more environmentally-friendly and sustainable manner (Souchie et al., 2010; El-Azouni, 2008).

Direct application of reactive phosphate rock (RPR) has been used to supply P requirements of crops and grasses in agricultural soils because it is less expensive than triple superphosphate and has similar efficiency as the soluble phosphate sources (Schlindwein et al., 2011). The PSF inoculation is a promising technique to increase P availability in soils fertilized with reactive phosphate rocks (Souchie et al., 2010; Gomes et al., 2014). Several authors reported increases on dry matter yield and P uptake in soybean (El-Azouni, 2008), ground nut (Malviya et al., 2011), wheat (Singh & Reddy, 2011) and maize (Patil et al., 2012), by PSF inoculation. Rodríguez et al. (1999) reported increase in alfalfa growth in soil fertilized with rock phosphate and sugar beet residue through inoculation of *Aspergillus niger* with high P solubilization potential and arbuscular mycorrhizal fungi *Glomus deserticola*. The synergism between these microorganisms also promoted considerable increases in P uptake and growth in clover (*Trifolium pratense*) (Souchie et al., 2010). However, the effects of PSF inoculation in sorghum [*Sorghum bicolor* (L.) Moench] plants are still unknown.

Sorghum is a multipurpose crop and it has a great potential to become one of the most economically important crops in Brazil considering its use for food, both for animals and humans, fodder plant, and for ethanol production. This study investigated the effects of inoculation of phosphate solubilizing fungi associated to application of reactive phosphate rock on plant growth and phosphorus uptake in sorghum plants.

Material and Methods

An experiment was carried out in a greenhouse in Marechal Cândido Rondon, Paraná, Brazil (24º31' S, 54º01' W, and 420 m), where the environmental conditions were: minimum and maximum mean air temperature of 19 and 36 ºC, respectively; mean air relative humidity of 60%. The soil used in the experiment was collected from the plough layer of a Rhodic Hapludox (Eutroferric Red Latosol in the Brazilian classification) with 580 g kg^{-1} of clay, 150 g kg^{-1} of silt, and 270 g kg^{-1} of sand. The soil had the following properties: pH (1:2.5 soil/$CaCl_2$ suspension 0.01M) 5.1, 28 g dm^{-3} of organic matter, 5 mg dm^{-3}

of P (Mehlich-1), 32 $mmol_c$ dm^{-3} of Ca, 11 $mmol_c$ dm^{-3} of Mg, 2 $mmol_c$ dm^{-3} of K, 91 $mmol_c$ dm^{-3} of CEC, and 50% of base saturation. All soil chemical properties were analyzed according to Silva (1999). The soil was fertilized with 150 mg dm^{-3} of N as urea, 100 mg dm^{-3} of K as potassium chloride, 4 mg dm^{-3} of Cu as copper sulfate, 4 mg dm^{-3} of Zn as zinc sulfate, and transferred to 8-L plastic pots.

The experiment was arranged in a completely randomized design with five replicates. The inoculation treatments were: 1) control (non-inoculated with phosphate-solubilizing fungi (PSF) and non-fertilized with P); 2) inoculation with PSF (10^8 CFU mL^{-1}); 3) application of reactive phosphate rock (RPR) (240 mg dm^{-3} of P_2O_5); 4) inoculation with PSF (10^8 CFU mL^{-1}) + RPR application (240 mg dm^{-3} of P_2O_5). The P source used was Gafsa reactive phosphate rock [28% of total P_2O_5, water-soluble fraction less than 1% and 32% of CaO]. The Gafsa phosphate rate was incorporated into the total volume of soil.

Sorghum [*Sorghum bicolor* (L.) Moench] seedlings were thinned to six plants per pot four days after emergence. The plants were irrigated with tap water when required. Three days after emergence, the plants were inoculated with *Aspergillus terreus* and *Penicillium pinophilum*, which are P-solubilizing fungi. The isolates *A. terreus* and *P. pinophilum* belong to the Laboratory of Plant Pathology collection at the State University of Western Paraná and are considered to be isolates with high P-solubilization potential (Oliveira et al., 2009). These PSF isolates were grown in Petri dishes (four days, 30 ºC, darkness) with GL solid medium (Sylvester-Bradley et al., 1982). Spores were suspended in water + Tween 80® (1%) solution, quantified in a Neubauer chamber following the successive dilution until 10^8 CFU mL^{-1} and, subsequently, 1.0 mL of spore suspension was applied to each pot, as previously described by Souchie et al. (2010). Treatments not inoculated with PSF received only the application of GL liquid medium (1.0 mL) to eliminate the possible effect of solution on plant growth.

After 45 days of growth, the crop yield was evaluated in terms of dry matter production of shoots (SDM, g $plant^{-1}$) and roots (RDM, g $plant^{-1}$). Plants of all treatments were harvested separately, dried for four days at 65 ºC, and then weighed. The shoot length was measured (cm $plant^{-1}$) using meter-scale. Root volume (cm^3 $plant^{-1}$) was determined by water displacement using a calibrated cylinder. The plant material from shoots and roots was digested in nitric-perchloric acid and the P concentration (g kg^{-1}) was determined by colorimetry at 725 nm wave length, as previously described (Malavolta et al., 1997). The amount of P accumulated by plants (mg pot^{-1}) was calculated from the dry matter of each pot and its P content in the dry matter.

Original data were analyzed by ANOVA, and means of treatments were compared by the Tukey test at the 0.05 level of confidence. All analyses were performed using Sisvar 5.1 software for Windows (Ferreira, 2011) (Statistical Analysis Software, UFLA, Lavras, MG, BRA).

Results and Discussion

The application of reactive phosphate rock (RPR) improved the shoot and root growth of sorghum plants (Figure 1 and 2). The application of RPR resulted in increase of 58 and 85% in the plant height (Figure 1a) and 74 and 113% in the shoot dry matter (Figure 1b) of sorghum compared to the control, respectively, without and with inoculation of phosphate-solubilizing fungi (PSF). For the root dry matter there was an increase of 147 and 112% (Figure 2a) and for root volume there was an increase of 173 and 139% (Figure 2b) compared to the control, respectively, without and with PSF inoculation. In general, the application of RPR associated to inoculation of PSF resulted in increased shoot growth of sorghum plants. On the other hand, the highest yield of root dry matter only with application of RPR compared to the PSF+RPR treatment may be associated with increase of the root volume of sorghum plants in conditions of low soil phosphorus availability, as reported by Hufnagel et al. (2014). Low-P availability, a nutrient of low mobility on soils, may result in changes in root architecture and morphology to enhance P uptake (Lynch, 2011; Niu et al., 2013). Root structural changes leading to higher P uptake include increased root hair growth (Haling et al., 2013) and length and enhancing lateral root over primary root growth (Wang et al., 2013). These changes can result in a shallow and branched root system, facilitating soil exploration. In turn, when the application of RPR was associated with inoculation of PSF, the improvement in soil P availability resulted in higher P concentration and accumulation in the sorghum shoots compared to treatment that received only application of RPR (Figure 3).

Although the RPR is a low solubility source, but because it was the only source used, the sorghum plant growth was favored by adding fertilizer (Figure 1 and 2). This fact may be explained in part due to the low P level in the soil (5 mg dm^{-3} P Mehlich-1). Characteristics of phosphate fertilizers reactivity are determinant in relation to its agronomic effectiveness. Phosphorus derived of soluble phosphate sources is readily available to plants, especially for short-cycle species. In contrast, the low solubility of RPR gradually increases the P availability in the soil-plant system (Zoz et al., 2010).

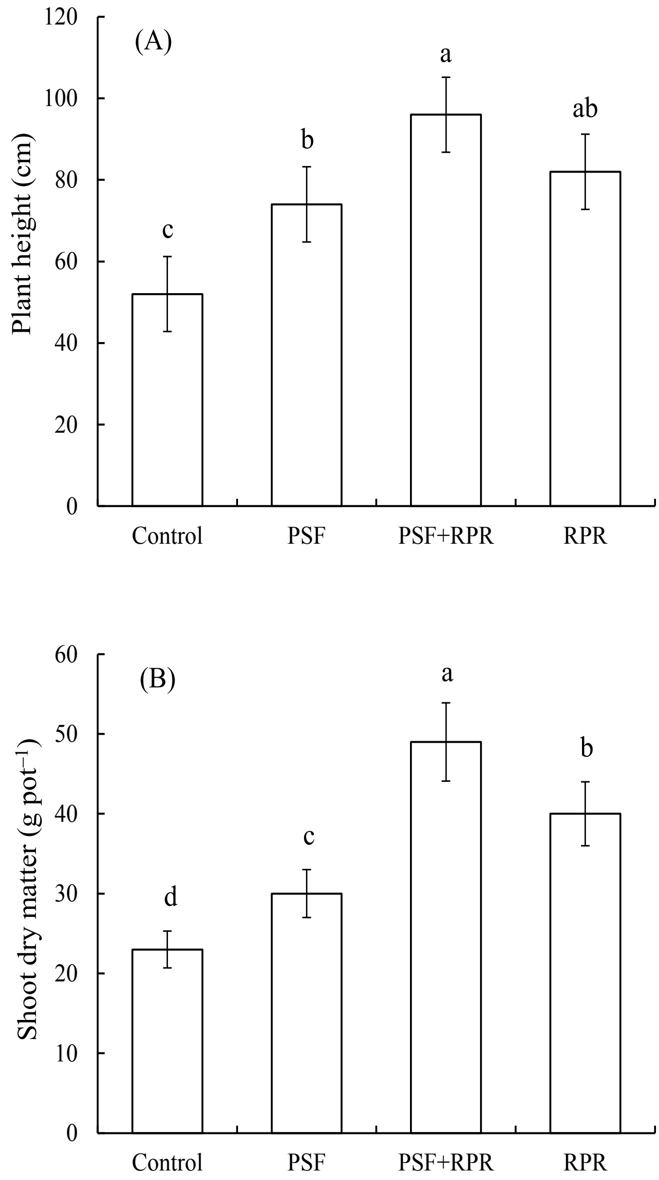

Figure 1. Effects of inoculation with phosphate-solubilizing fungi (PSF) and application of reactive phosphate rock (RPR) on plant height (A) and shoot dry matter (B) of sorghum [*Sorghum bicolor* (L.) Moench]. Bars followed by the same letter are not significantly different according to Tukey test at 5% probability. Data refer to mean values (n = 5) ± standard error.

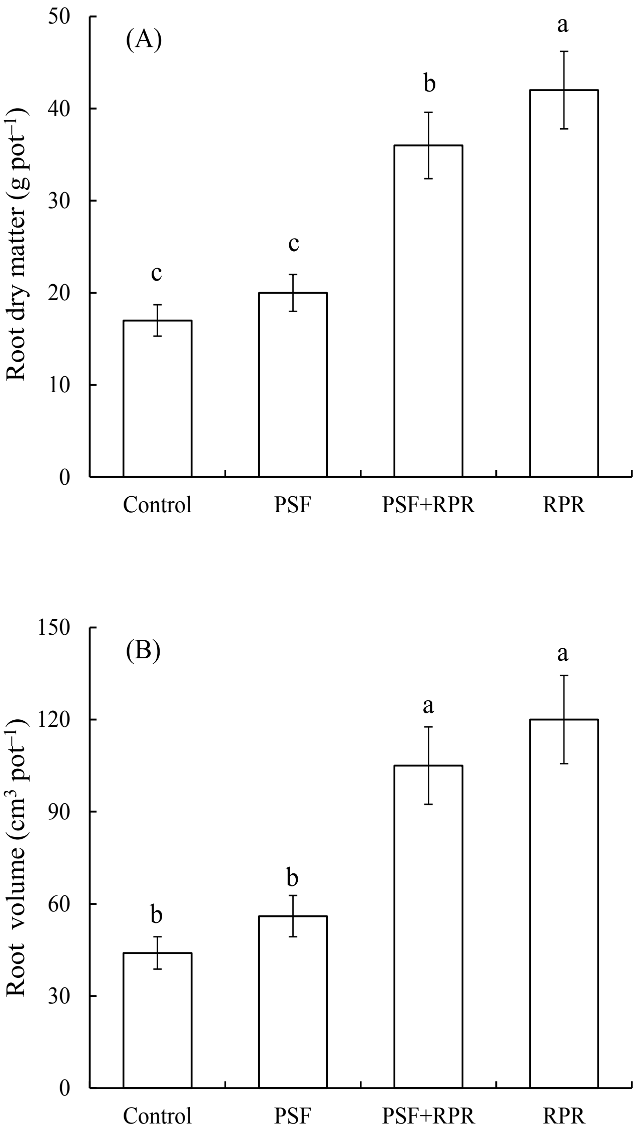

Figure 2. Effects of inoculation with phosphate-solubilizing fungi (PSF) and application of reactive phosphate rock (RPR) on root dry matter (A) and root volume (B) of sorghum [*Sorghum bicolor* (L.) Moench]. Bars followed by the same letter are not significantly different according to Tukey test at 5% probability. Data refer to mean values (n = 5) ± standard error.

Steiner et al. (2009) found that the inoculation of PSF associated to the application of RPR improved the phosphorus uptake and growth of wheat plants. However, these authors reported that inoculation of PSF without the phosphate application had little effect on the wheat development.

Inoculation with P-solubilizing fungi (*Aspergillus terreus* and *Penicillium pinophilum*) showed to be a promising technique at controlled conditions because the sorghum plants inoculated showed better growth compared to the control treatment (Figure 1). However, tests are necessary to evaluate the efficiency of inoculation technique of PSF under field conditions. This is because under field conditions the interaction between plant species and PSF strain may be affected by environmental factors such as temperature, soil humidity, solar radiation and soil type, and influence the successful use of this technique. In this study in greenhouse conditions, there was an increase on shoot dry matter yield (30%) and plant height (42%) compared to the control (Figure 1). However, the PSF inoculation did not affect the root growth of sorghum plants (Figure 2). Under controlled conditions, Wahid & Mehana (2000) found that inoculation with PSF (*Aspergillus niger*, *A. fumigatus* and *Penicillium pinophilum*) increased wheat and common bean grain yields in 30% and around 25%, respectively, using phosphate rock in comparison with superphosphate. Malviya et al. (2011) found a significant increase in growth of ground nut (*Arachis hypogaea*) inoculated with *A. niger* and *P. notatum*. The genera *Aspergillus* and *Penicillium* are the most efficient phosphate-solubilizing fungi (Vassilev & Vassileva, 2003). As reported by Souchie et al. (2006), these microorganisms have potential as inoculants, because they can maximize plant growth. Therefore, the ability of PSF to mobilize P from sparingly soluble sources could be a useful tool in P fertilizer management.

Differences in phosphate-solubilizing ability of fungi cause quantitative or qualitative changes in metabolites produced and suggest the existence of different solubilization mechanisms or processes with variable efficiency (Barroso & Nahas, 2005). Evaluating the Araxá rock phosphate solubilization by *Aspergillus niger*, Mendes et al. (2013) found that the concentration of solubilized P increased rapidly in the first 60 hours, reaching approximately 80 mg L^{-1}. Souchie & Abboud (2007) reported mean P solubilizing capacity of 120 mg L^{-1} for different fungal isolates. The potential of these microorganisms in soil phosphate solubilizing is very important to contribute for P nutrition of crop, especially under low P availability conditions, since often the effect of PSF can only be hormonal. In this study, the increase in the P concentration and accumulation in the sorghum plants inoculated with the PSF demonstrated the phosphate solubilization potential of these fungal isolates. The inoculation of PSF resulted in increase of 61 and 71% in the P concentration (Figure 3a) and 110 and 265% in the P accumulation (Figure 3b) of sorghum compared to the control, respectively, without and with application of reactive phosphate rock (RPR). Similar results were reported by Wahid & Mehana (2000); these authors found that the P uptake of wheat and faba bean crops significantly increased due to inoculation of the soil with different fungal isolates (*Aspergillus* and *Penicillium*). The increase on P uptake of plants with inoculation of PSF have been reported in several crops, such as soybean (El-Azouni, 2008), ground nut (Malviya et al., 2011), wheat (Singh & Reddy, 2011) and maize (Patil et al., 2012).

According to Richardson (2001), microorganisms directly affect the ability of plants to acquire soil P

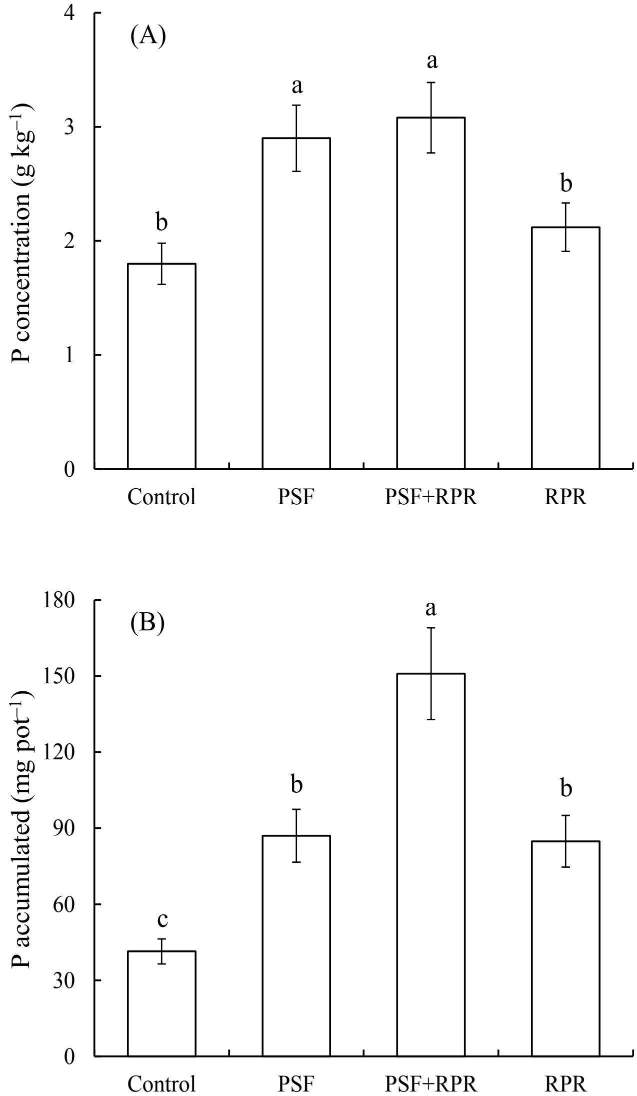

Figure 3. Effects of inoculation with phosphate-solubilizing fungi (PSF) and application of reactive phosphate rock (RPR) on phosphorus concentration (A) and phosphorus accumulation (B) in shoots of sorghum [*Sorghum bicolor* (L.) Moench]. Bars followed by the same letter are not significantly different according to Tukey test at 5% probability. Data refer to mean values (n = 5) ± standard error.

through various mechanisms. These mechanisms include increase growth of lateral roots and root hairs; displacement of the adsorption equilibrium, resulting in transfer of phosphate ions to the soil solution or the increased mobility of organic P forms; and, stimulation of metabolic processes effective in P solubilization and mineralization from scarce available forms of P-inorganic and P-organic.

In general, the effects of PSF were more evident on shoot growth of plants (Figure 1) and sorghum P uptake (Figure 3). These results showed that the fungal isolates used in this study have potential for use as inoculants, and play an important role in managing a sustainable environmental system. However, the effects of PSF to increase the P nutrition and growth of crops should be investigated further in field experiments.

Conclusion

Inoculation with phosphate-solubilizing fungi (*Aspergillus terreus* and *Penicillium pinophilum*) and application of reactive phosphate rock improves the phosphorus uptake of plants resulting in the increased dry matter yield of sorghum at controlled conditions.

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