Biodiversity and Microorganisms Variation of Endemic Duku (Lancium domesticum Corr.) Rhizosphere in the Wet and Dry Main Growing Site in Jambi, Indonesia

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Abstract

Duku are endemic plants of Jambi Province, Indonesia. They inhabit and used to grow well in alluvial along riverbanks, but lately have experienced sudden death disease outbreaks for the last decades. This situation has been prevalent since the frequent flooding due to the depletion of forest areas upstream. Since it is caused by water mold Phytophthora palmivora, it was supposed that sudden death disease should only occur in wet areas, but factually, the disease is also found in dry areas. This inspired us to investigate other soil biology factors of its rhizosphere in wet and dry habitats. Samples came from the rhizosphere of diseased and healthy duku in several alluvial areas of the Batang Hari River representing wet and dry habitats. This study found that the soil microbial community in dry and wet habitats was inhabited by mycorrhizal communities, bacteria, and fungi. The relative abundance of microbes in dry and wet habitats in healthy and diseased plants showed varied species. In the wet habitat-healthy plants, the relative abundance of Sclerocytis was 33.33%, Gigaspora 33.34%, and Glomus 33.33%. In the wet habitat-diseased plants, the relative abundance of mycorrhizae was Sclerocytis 66.67%, Glomus 33.33%, and no Gigaspora. The bacteria group was found to be the largest at 70.49% in dry habitats and 72.13% in wet habitats. The fungal group was 14.08% in dry and 16.39% in wet habitats. Mycorrhiza showed the smallest percentage in the rhizosphere of duku plants. We found that VAM correlated negatively with N, and P in the rhizosphere, and Glomus sp. existence correlated positively with soil K.

Keywords: AMF, Glomeraceae, Mycorrhizae, Sudden death, Water mold.

Introduction

Duku (Lansium domesticum Corr.) “Fig. 1”, a typical Indonesian fruit-producing plant, a tropical fruit tree that belongs to the family Meliaceae, grows well in the alluvial Batanghari River and stretches across several districts in Jambi Province. It was a high economic plant of the farmer as well as superior fruit of Jambi Province. The tree has a sweet berry, freshly eaten table fruit. The seed of the duku fruit is toxic to the malaria parasite. The leaves of duku have a chemical ingredient that suppresses human skin tumor formation, a hair restoration agent with lansic acid as the active ingredient.

Nowadays it has experienced a decline in production. The decline is partly due to sudden death disease on duku trees which has been widespread in Jambi Province since the 2000s. This disease has caused high economic losses and caused up to 100% mortality in most of the main growing areas. This soil-borne disease is known to be caused by Phytophthora palmivora, a water mold pathogen.
in favor of wet areas, making it suitable to inhabit the alluvial of the frequently flooded Batanghari River.

Recurrent flooding has been prevalent for several years. The overflow of the Batang Hari River has resulted in the inundation of several duku growing areas. Global climate change influences rainfall and temperature patterns, leading to more extreme weather events such as floods, and droughts. It can create ideal conditions for fungi and other organisms that cause diseases in plants and may also lead to an increase in soil-borne plant diseases. Global climate change might have affected the existence of biological agents that have the potential to be used to control sudden death disease. Duku plants are well-adapted to different habitats, including both dry and flooded/wet areas, making it an interesting case for studying microbial diversity in the rhizosphere.

The sudden death disease outbreak in Jambi duku appears to be closely related to the natural resistance traits of the parent trees and microbial diversity in the rhizosphere. Duku mother trees along the Batanghari River consisted of 2, 40% similarity groups, independent of alluvial location. However, the presence of diseased and healthy duku plants in both dry and wet ecosystems suggested that some of these parent tree groups were susceptible to the disease. Agronomic traits that indicated resistance to sudden death disease were leaf size and number of fruits per stalk. However, resistant trees were also characterized by relatively few fruits per stalk. We need to prevent these endangered populations from becoming extinct. Such phenomena attracted attention to explore and determine other predisposing aspects of this disease. Therefore, this study aims to determine the rhizosphere biodiversity in duku centers that are often and not affected by flooding.

**Materials and Methods**

**Soil sampling**

Soil samples were collected from 3 villages in Batanghari and Muaro Jambi districts. The soil around the roots was taken in a configuration from 4 opposite points. Samples were taken in areas that frequently flooded and never flooded representing wet and dry areas. Samples were taken from diseased and healthy plant roots.

The topsoil (0-20 cm depth) was collected from the site +/- (06°55'18.1" S and 106°47'10.8" E) “Fig. 2”. The soil was then transported to Laboratory, air-dried, and sieved (2 mm). The biology and chemical properties of soil were analyzed following standard methods in the Microbiology and Soil Laboratory, Department of Soil Science, Faculty of the Agriculture University of Bengkulu.
Mycorrhizal Isolation

Isolation of mycorrhizal spores from soil samples followed the protocol of a moist sieving method and centrifugation⁶. The soil suspension was filtered with multilevel filter sizes of 500, 125, and 63 µm arranged from top to bottom. The filtered soil suspension in 125 and 63 µm sieves were poured into a test tube before being added with a 60% sucrose solution into 1/3 part of the test tube and centrifuged at 2,500 rpm for 3 minutes. The liquid transition between sucrose solution and water was aspirated using a pipette and washed before being poured through a 63 µm size filter. The supernatant was examined for mycorrhizal identification.

Bacterial and Fungal Isolation

The dilution plates and soil plate methods were used for bacterial and fungal isolation from the soil³. As much as 10 g of soil sample was added to 90 ml of sterile distilled water in Erlenmeyer, shaken for 30 minutes to form a suspension. The suspension was then serially diluted up to 10⁶ dilution. A total of 100ml was spread to Petri dish on Martin Agar (MA) media for fungi, and Nutrient agar (NA) for bacteria for further checking. Collected species have been checked with a standard light microscope (Olympus, BX50, Hamburg, Germany).

Results and Discussion

Analysis of the Major Community Composition of Soil Microbes in the Rhizosphere of Duku Roots Along Dry and Wet Habitat

The microbial communities composition of the soil in dry and wet habitats was mycorrhizal, bacteria, and fungi. Of the three types of microbes found in the duku rhizosphere, mycorrhiza showed the smallest percentage in both habitats. The abundance of mycorrhiza in dry environments/habitats tended to be greater (15.49%) than in wet conditions/wet habitats (11.48%). While bacteria were found to be more abundant; in wet, 72.13%, and 70.49% in dry habitats. Furthermore,
the fungal group was 14.08% in dry and 16.39% in wet habitats “Fig. 3”.

Figure 3. Microbial community composition in duku root rhizosphere in dry and wet habitats

Species composition in mycorrhiza, bacteria, and fungi in dry and wet habitats

Taxonomic composition analysis showed that the mycorrhizal species found were *Sclerocytis*, *Gigaspora*, and *Glomus*, both in dry and wet habitats “Fig. 4A”. There were no differences in the number and species of bacteria in dry and wet habitats, namely: *Erwinia* sp, *Rhizoid* sp, *Agrobacterium* sp, *Achromobacter* sp, *Bacillus* spp, *Rhizobium* sp, *Pseudomonas* sp, *Azotobacter*, and *Streptomyces* sp, “Fig. 4B”. The fungal community in the dry habitat consisted of 6 species, while in the wet habitat, only 5 species were found: *Fusarium*, *Aspergillus*, *Glomisporium* sp, *Gliocladium* sp, and *Rhizopus* “Fig. 4C”.
Relative abundance of mycorrhizal, bacterial, and fungal species based on habitat in healthy and diseased plants

The relative abundance of microbes in dry and wet habitats in healthy and diseased plants also showed varied species. Mycorrhizal species found in the dry habitat of healthy duku plants (KS+): Glomus 42.86%, Gigaspora 28.57%, and Sclerocytis 28.57%. In the dry habitat with sudden death duku (KS-), the relative abundance of Glomus species was found to be 50% higher than in the dry habitat of healthy plants, while Sclerocytis and Gigaspora were relatively lower, at 25% each. Furthermore, in the wet habitat-healthy plants (BS+), the relative abundance of Sclerocytis was 33.33%, Gigaspora 33.34%, and Glomus 33.33%. While in the wet habitat-diseased plants (BS-), Sclerocytis 66.67% and Glomus 33.33%, and no Gigaspora was found “Fig. 5A”.

Figure 4. Species composition in the Mycorrhizal community “A”, Bacterial community “B”, and Fungi community “C”.

The relative abundance of bacterial was found to be 10 species, both in dry habitat-healthy plants (KS+) and diseased plants (KS-) and in wet habitat-healthy plants (BS+) and wet-diseased plants (BS-) "Fig. 5B". In the KS+ habitat, the smallest bacterial abundance was Erwinia 4.17%, Bacillus 2 4.17%, and Rhizobium 4.17%. In the KS- habitat, the smallest abundance was found in Agrobacterium species 7.69%, Bacillus 2 7.69%, and Rhizobium 7.69%. In the BS+ habitat, the smallest was Achromobacter 5.56%, Bacillus 2 5.56%, Rhizobium 5.56%, and Azotobacter 5.56%. In the BS- habitat, the highest relative abundance (Achromobacter 13.33%, Pseudomonas 11.53%, Bacillus 1 11.53%, and Streptomyces 11.53%).

The relative abundance of fungal species was quite variable “Fig. 5C”. Rhizopus sp was the largest. In the dry habitat-healthy plants (KS+), 4 species were found, namely: Rhizopus sp 40%, Gloeosporium sp 20%, Gliocladium sp 20%, and
Trichoderma sp 20%. Meanwhile, the abundance of fungal species in the dry habitat-diseased plants (KS-)was Rhizopus 20%, Gliocladium 20%, Gloisporium 20%, Aspergillus niger 20%, and Paecilomyces sp. 20%. In contrast to the relative abundance of bacterial groups in the healthy plant-wet habitat (BS+) and diseased plant-wet habitat (BS-), the abundance of fungi was found to be less. Five species in BS+ habitat viz: Fusarium (16.67%), Aspergillus Niger (16.67%), Gloisporium sp (16.67%), Gliocladium sp (16.67%), Rhizopus sp (33.33%). While in the BS- habitat, 4 species of fungi were found, namely: Aspergillus Niger 25%, Gloisporium sp 25%, Gliocladium sp 25%, and Rhizopus 33.33%.

We have investigated the links between soil chemistry and microbe diversity. It was found that VAM diversity was not significantly correlated with the content of N and P elements in the soil and even tended to be negatively correlated with N. The correlation of Glomus-N was \( r = -0.636 \), Gigaspora-N \( r = -0.218 \), and Scleroytis-N \( r = -0.504 \). As with the K element in the soil, there was a positive and significant correlation with VAM but only in the Glomus species or genus \( (r = 0.756) \) “Table 1”.

### Table 1. Recapitulation of correlation analysis between nutrients and mycorrhizae in the rhizosphere of duku main growing area along the Batanghari riverbank.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nutrient in soil (Rhizosphere)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>C</td>
<td>-0.262&lt;sup&gt;in&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>0.500&lt;sup&gt;in&lt;/sup&gt;</td>
</tr>
<tr>
<td>K</td>
<td>-0.667&lt;sup&gt;in&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca</td>
<td>-0.310&lt;sup&gt;in&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glomus</td>
<td>-0.636&lt;sup&gt;in&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gigaspora</td>
<td>-0.218&lt;sup&gt;in&lt;/sup&gt;</td>
</tr>
<tr>
<td>Scleroytis</td>
<td>-0.504&lt;sup&gt;in&lt;/sup&gt;</td>
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Note: * = significant at a 5% level

### Discussions

The ecosystem of lowland duku plantations along the banks of the Batanghari River in dry and wet conditions affects the composition and richness of microbes in the root rhizosphere of duku plants. This offers more possibilities for biodiversity research, to observe the richness in species diversity, especially bacteria and fungi. In the highlands, Peters et al. 7 reported that the species richness of almost half of the plant and microbial taxa showed a decreasing trend with increasing altitude. In the lowland duku plantations along the banks of the Batanghari River in Jambi, however, the fluctuation in species diversity was influenced by wet or dry soil conditions. Our results found 3 main microbial community groups in the lowland ecosystem along the Jambi duku plantation under dry and wet soil conditions/habitats.

The diversity and distribution of the main microbial communities in dry and wet habitats were mycorrhizae, bacteria, and fungi communities. Mycorrhiza was found as the smallest community in both habitats of Jambi duku plantations, but there was no relative difference between the two, a slight decrease occurred in the wet habitat (4.01%). The largest community was the bacteria group, which in contrast increased in the wet habitat (1.64%). The fungal group tended to increase in wet habitats (2.31%) “data processed from Fig. 3.

There was no difference in the abundance of microorganism species in dry and wet habitats, except for species of the fungus group. Vieira et al. 8 showed that Vesicular Arbuscular Mycorrhizae (VAM) diversity at lower altitudes is generally small. This statement was in line with the low altitude VAM diversity findings in Jambi duku
plantsations. The reason for this phenomenon may be that lowland Jambi duku plantations experience more human disturbance and a less extreme climatic environment. Hence, mid-elevation areas are favorable for VAM sporulation and growth. Zhang et al., 9 and Shi et al. 10, however, had different opinions that altitude has no significant effect on VAM diversity. Other previous studies have shown that environmental factors, especially geographical and soil factors, have an important impact on VAM diversity. Different ecological factors will affect the growth, development, colonization, and reproduction of AMF, which will lead to differences in VAM diversity in Indonesia 11-12. Meanwhile, the ecology and soil of lowland Jambi duku plantations are in the same geographical environment, so these circumstances do not contribute to the diversity of mycorrhiza and bacteria. The strength of this statement can be seen from the explanation of Montiel-Rozas et al. 13 that VAM diversity and richness are only influenced by soil factors and properties which are the main driving force of VAM fungal communities.

Previously, most VAMs were thought to be aerobic organisms whose survival is disturbed when exposed to anoxic or hypoxic environmental conditions. Mycorrhizae may play a central facilitating factor in the evolutionary adaptation of plants to critical changing water availability throughout the global climate 14. Most VAMs were originally regarded as terrestrial organisms. Their presence and function in aquatic, wet, or waterlogged habitats were not of concern. However, several recent studies have explored the potential of these mycorrhizae in stressful environments. Recent studies have shown that VAM is common in waterlogged land, and suited for poor-quality and low-fertility soils 15-18. Anoxic or hypoxic conditions caused by waterlogging do not prevent VAM colonization 16,19. In addition, previous researcher 19 reported that in all wetland types, Glomeraceae was found to be the family with the most species. There were also reports that the rhizosphere of wetland plants inhabits various VAM species 20. VAM can enhance the flooding tolerance of Quercus robur 21, and trifoliate orange 22, and on the other hand, VAM symbiosis helps in the physiological regulating of plants thereby increasing their tolerance to drought. The mechanism is by colonizing plant roots and developing an extensive network of extraradical hyphae in the soil rhizosphere that help absorb water and increase water uptake during drought conditions. 23, and accumulates proline to enhance wet tolerance 24. Interestingly no Gigaspora was found in the wet habitat.

Soil and plant nutrients were found to significantly impact the soil microbial diversity index, especially AMF. Whether it was wet or dry, P concentration did not significantly affect VAM diversity, nor did bacterial diversity. This result was inconsistent with the findings of Maitra et al. 25 who confirmed that the VAM diversity index showed a positive response to P. Previous studies also showed that N addition increased VAM diversity in N-deficient soil 26,27, which was inconsistent with previous studies that plant root N content had a significant effect on VAM diversity, yet soil N content did not affect it. These results suggested that plant roots had a greater impact on VAM diversity than the soil impact. It also showed that VAM tends to be symbiotic with plants to absorb nutrients, thus increasing mycorrhizal diversity 26. In contrast, the results of our study showed that VAM diversity was not significantly correlated with the content of N and P elements in the soil and even tended to be negatively correlated with N elements in the soil. The correlation of Glomus-N (r = -0.630), Gigaspora-N (r = -0.218), and Sclerotyis-N (r = -0.504) “Table 1”. This can be interpreted that the lower the N content in the soil, the smaller the chance of finding VAM in the environment or rhizosphere along Jambi duku plantations. Thus, the sudden death of duku trees in dry and wet ecosystems/habitats was due to the limited number and diversity of VAM due to very low N availability in the soil. This statement is based on the management of duku cultivation carried out by the community so far is still very traditional without the application of fertilizers.

As with the K element in the soil, there was a positive and significant correlation with VAM but only in the Glomus species or genus (r = 0.756). This indicated that the higher the K content in the soil, the greater the chance of finding AMF-Glomus. Likewise with Gigaspora although not as significant (r = 0.655).

Kalium is one of the most abundant elements in soil composition, yet its very low availability limits plant growth and ecosystem productivity 25. Potassium also plays an important role in several plant functions, including membrane polarization, plant growth, and stomatal aperture function 26, which promotes high rates of
photosynthesis resulting in large amounts of carbohydrates, such as glucose and sucrose. These carbohydrates are transferred from their source (from the leaves) to the root tissue of the host plant which encourages FMA to approach the roots and form mycorrhiza-root associations of host plants which then colonize the roots which is one of the FMA protection mechanisms against pathogen interference. In addition, plants benefit from higher mycelial absorption of water and mineral nutrients. Potassium accumulation in AM fungi is seen in mycelia, vesicles, and spores. Plant K+ nutrition via the arbuscular mycorrhiza pathway is still rarely studied. However, assessment of potassium distribution in AM fungi revealed strong K+ accumulation in spores, hyphae, and vesicles.

Further investigation related to the comparison of microbial diversity in the rhizosphere of duku plants that experienced sudden death on one side and healthy duku plants on the other side, it was found that in the diseased duku plants, the percentage of mycorrhiza was lower (10.13%) while the percentage of bacteria was higher (65.82%). Meanwhile, in healthy plants, the percentage of mycorrhiza was found in a relatively higher percentage (14.08%) and fewer bacteria (59.15%). But if observed based on habitat, our results showed that in healthy plants in a dry habitat, the percentage of mycorrhiza species/genus Gigaspora was higher (28.27%) while in diseased plants 25%. In the wet habitat of healthy plants, the percentage of Gigaspora was higher (33.33%), in diseased plants no Gigaspora was found even though Sclerocistys species was the highest (66.67%) (Fig. 3A).

The sudden death outbreak in Jambi duku plants caused by Phytophthora seems to be closely related to the parent tree, as well as the microbial diversity in the soil in the rhizosphere. The study conducted by Hayati reported that duku mother trees in the field along the Batanghari River in Batanghari and Muaro Jambi districts consisted of 2 groups with 40% similarity and were not related to the location where they grew. The finding of diseased and healthy duku plants in dry and wet ecosystems indicated that the group is susceptible to the disease. An interesting finding in a recent study was that in the wet ecosystem/habitat, no Gigaspora genus/species was found in the rhizosphere of diseased trees, on the other hand, all three genera of VAM were found in healthy mother trees in the same ecosystem. This suggested that in wet ecosystems/habitats, Gigaspora plays an important role as one of the plant microbes in mutualistic symbiosis with duku trees that can protect against the pathogen that causes the sudden death of duku. However, in dry ecosystems/habitats, all three VAM genera/species (Gigaspora, Sclerocistys, and Glomus) play an important role in protecting duku trees against pathogen interference. The mechanism analysis may involve increasing plant nutrition, changing the morphological structure of plant roots, regulating the synthesis of secondary metabolites, improving the plant rhizosphere microenvironment, directly competing with pathogenic microorganisms for invasion sites and nutrients, and promoting plant disease resistance and defense system formation.

Without major differences in root mycorrhizal communities, N uptake patterns in lower plants varied under different upper parts of plants. The VAM-duku tree symbiosis can cause the plant root system to grow and thicken, increase branching, accelerate cell wall lignification, thicken the root tip epidermis, and increase the number of cell layers. This symbiosis also alters the morphological structure of the roots, thus slowing down the process of root infection by pathogens.

Boutaj et al. also showed that Gossypium hirsutum symbiotic with G. mosseae and G. etunicatum caused palisade tissue to increase, vessels to deform, gelatinous substance to be produced in vessels, cells to deform and shrink, cell walls to thicken significantly. Lignification, a significant reduction in the number of vacuoles, loss of folds in mitochondria, and a series of structural changes in the root system are all beneficial to enhance the resistance of host plants to Verticillium dahliae. VAM can form mycelial and callus networks stacked by non-esterified pectin in the root epidermis and endodermis of the host plant, which inhibits the penetration of the pathogen into the root cell tissue and furthers infection. These changes in root anatomy in tomato plants alter the kinetics of Phytophthora infections. This mechanism may also occur in healthy duku trees in both dry and wet ecosystems/habitats.

Healthy duku trees in both dry and wet ecosystems/habitats along the Batanghari riverbanks showed a high level of VAM colonization in the rhizosphere. This may occur because mycorrhizal colonization serves as a key, widely accepted index for assessing symbiotic relationships. High VAM
colonization on the roots can thus provide good protection to the host plant (duku tree) against pathogen interference. Furthermore, according to Tahat et al. 40, there is a positive relationship between the number of spores and root colonization in plants. Referring to this opinion, it can be argued that healthy duku mother trees in both dry and wet ecosystems/habitats produce higher VAM spores compared to the production of VAM spores on diseased mother trees. Our research data showed that the number of VAM spores is less (about 250/10 g sample) in dry habitat-diseased plants, while in dry habitat-healthy plants, the number of spores was relatively higher (about 200-500/10 g sample) (data not shown). Differences in spore production may be caused by host plant characteristics that vary in adaptability to growing conditions such as soil temperature, pH, and moisture.

The role of Gigaspora and the positive correlation of Glomus with soil K, and the negative correlation of VAM with N and P, needs to be further examined. Plants that grow faster, have more stable growth, and larger root sizes, often have more extensive root systems that allow for higher spore formation which in turn increases the percentage of colonization on roots compared to smaller hosts. Host type and its growth period are the most critical factors for spore production, multiplication, and colonization37,38. It has been shown that extensive colonization of host plant roots results in healthy plant growth and better root system development40. Furthermore, Jaiti et al. 33 emphasized that the regulation of jasmonic acid (JA) biosynthesis plays a main role in symbiosis. In line with that statement, Song et al. 34 also showed that the lack of JA synthesis significantly decreased mycorrhizal colonization rate and arbuscular abundance. Studies have shown that JA and ethylene (ET) are generally defense/resistance compounds against saprophytic pathogens, while SA (salicylic acid) has inhibitory effects on biotrophic pathogens. JA, ET, and SA are associated with systemic induced resistance in plants (ISR) when pathogens infect plants41.

While soil water content and plant biodiversity influence bacterial diversity along natural gradients 42 the existence of different variations in plant colonization may be related to the specific nature of protection in each parent tree. Some of the bacterium isolated in this study were also found by Jaffar 43 to play a significant role as an indicator of metal pollution in the soil, in addition to the use of mycorrhiza for biological remediation 44.

Conclusion

The biodiversity of microbes in dry and wet habitats in healthy and diseased plants was a variation in species. In the wet habitat-healthy plants, the relative abundance of Sclerocytis, and Glomus were lower than that of the wet habitat-diseased plants. Gigaspora was only found in the rhizosphere of diseased plants. The bacteria group was found to be more abundant in dry habitats than in wet habitats. The fungal group was present in less quantity in dry than in wet habitats. Mycorrhizae exhibited the lowest presence rate at the duku plant rhizosphere. The presence of mycorrhizae was correlated negatively with N, and P in the rhizosphere, whilst the presence of Glomus sp. was positively correlated with soil K.

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Authors’ Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Jambi.

Authors’ Contribution Statement

The Authors IH, HM, and WW supported the study, interpreted the data, proofread the manuscript, and approved the final version of the manuscript written by IH.

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