Rhizosphere Fungi Abundance on Acid Dry and Tidal Soils in Borneo Prima Citrus Fields, East Kalimantan

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Abstract. Acid dry and Tidal land in East Kalimantan is one of the sub-optimal land ecosystems, that needs to increase the quality of its fertility to support the production of citrus of the Borneo Prima variety. One of them with rhizosphere microbes can increase fertility, decomposition process and as biological control agents to plant diseases. The aim of the study was to determine the abundance of rhizosphere fungi on citrus trees on dry acid and tidal soils and their relationship with soil physico-chemical factors. Rhizosphere fungi was isolated from the soil around citrus plant roots, East Kalimantan were analysed for their abundance and their correlation with soil physio-chemical factors. The results showed that found 29 rhizosphere fungi. The highest importance value index was found TR25 rhizosphere fungi isolates in the acid dry land block A and TR 17 in block B. The highest importance value index is at TR1 in block A tidal land, and TR23 in block B. Principal Component Analysis results showed that there is a strong relationship between the abundance of rhizosphere fungi and soil physicochemical. The highest number of rhizosphere fungal colonies was on tidal land B. The number of colonies was directly proportional to humidity.

1 Introduction

The Borneo Prima tangerine variety (Citrus reticulata) is a local superior variety native to East Kalimantan which has been released by the Minister of Agriculture as East Kalimantan germplasm to be developed nationally (East Kalimantan Provincial Agriculture Office 2012). This variety is known to be able to adapt in a wide lowland area in Indonesia. It is known that the Province of East Kalimantan has an area of agricultural land of 46,265.79 ha [1] some of which are tidal land of around 10,716 ha while the rest is acid dry land. Tidal land in this area has been used for the development of food crops, especially rice around 46.3% with an average productivity level of 2 t/ha and others for plantation and horticultural crops [2]. The development of citrus in East Kalimantan is in Rantau Pulung,
East Kutai Regency and Tanah Grogot, Paser Regency. This potential variety has not been optimally produced, due to several factors, including biotic and abiotic pressures. The most disturbing biotic pressure on Borneo Prima citrus plantations is the attack of the pathogen *Lasiodiplodia theobromae* which causes diplodia stem rot (DSR). It is feared that the threat of this disease will cause a decrease in production and plant death. Visually, symptoms of disease attack can be found in citrus plantations on dry land and Tidal land in East Kalimantan. Until now, citrus cultivation in East Kalimantan is still relatively environmentally friendly. Therefore, DSR control is also expected to be able to utilize potential biological microbial resources to control BBD disease.

Some antagonistic microbes have been proven to be able to control this disease. *Trichoderma harzianum*, *T. Asperellum* and *T. viride* [3][4]. *Bacillus subtilis*, *Gliocladium* sp. [4][5], *T. virens*, and *T. Pinophilus* [3]. These microbes are mostly present in the rhizosphere. Soil biological quality will increase with the presence of soil microorganisms, especially in the rhizosphere. The rhizosphere is the zone around roots and is interconnected with root exudates (proteins and sugars), respiration and biogeochemical reactions [6]. The population of microorganisms in the rhizosphere is generally higher more numerous and varied compared to non-rhizosphere soils. The activity of rhizosphere microorganisms is influenced by the exudate produced by plant roots [7]. Some rhizosphere microorganisms play a role in nutrient cycling and soil formation processes, plant growth, influencing the activity of microorganisms, as well as biological control of root pathogens. Rhizosphere fungi are more has the potential to spread in the soil compared to rhizosphere bacteria [8]. Some rhizosphere microorganisms play a role in nutrient cycling and soil formation processes, plant growth, influencing the activity of microorganisms, as well as biological control of root pathogens. Rhizosphere fungi are more has the potential to spread in the soil compared to rhizosphere bacteria [9]. Rhizosphere fungi are known to play an important role in nutrient cycles and soil formation processes, plant growth (Plant Growth Promoting Fungi) [10].

The abundance of rhizosphere fungus is influenced by several factors, among others, substrate, humidity, temperature, and organic matter. Substrate is the main source of nutrients for fungi. New nutrients can be utilized after the mold has excreted extracellular enzymes that can break down complex compounds from the substrate into simpler compounds and are able to excrete α-amylase enzymes to convert starch into glucose. The glucose compound is then absorbed by the fungus [11]. Moisture is very important for mold growth. Common low-grade fungus such as Rhizopus or Mucor require an environment of 90% relative humidity, whereas Aspergillus, Penicillium, Fusarium and many other hyphomycetes can thrive at lower relative humidity of 80%. Molds are classified as xerophilic and can survive at 70% relative humidity, for example *Wallemia sebi*, *Aspergillus glaucus*, many strains of *Aspergillus tamarii* and *Aspergillus flavus* [12]. Rhizospheric fungus, such as *Aspergillus*, *Fusarium*, *Humicola*, and *Penicillium* are common and have been reported to be found in many vegetable and fruit agricultural fields [13]. Temperature can affect the diameter of colonies. The optimum temperature for colony growth is at 28°C and the smallest colony growth occurs at 39°C, generally yeast prefers a pH below 7.0 [14]. Certain fungus grows at a fairly low pH, ie pH 4.5-5.5. Observations in the of Rhizopus, Penicillium, Alternaria, Fusarium, Mucor could grow at 35°C and all fungal isolates grew at temperatures of 20, 25 and 30°C but no fungal isolates grew at 40°C. At pH 5 and 7 all fungal isolates grew, but at pH 3 only Mucor spp. which can grow slowly. At pH 9-11 no fungal isolates grew [15]. The aim of the study was to determine the abundance of rhizosphere mold on Borneo Prima citrus plants in 2 types of land, to analyse its correlation with physicochemical characters.
2 Materials and methods

2.1 Sampling

The research was carried out in early 2023 on dry land and tidal land. Samples were obtained using a purposive sampling survey method on Borneo Prima citrus varieties infected with *L. theobromae* in Padang Prapat village, Tanah Grogot subdistrict, Paser regency (figure 1).

The land used for sampling is in Padang Prapat Village, one of the villages in Tanah Grogot District, Paser Regency, East Kalimantan Province, Indonesia, with a geographic location at coordinates 116.214407 BT/ -1.842763 LS. Sampling on acidic dry land at the coordinates -1°50'27", 116°14'54", 61,2m.173° and -1°50'35", 116°14'28", 62,9m. 272° dan lahan pasang surut pada -1°50'37", 116°15'20", 66,4m, 101° dan -1°50'29", 116°15'29", 62,1m.358°. For each type of land, samples were taken from 2 different gardens, with the criteria of containing a population of ± 100 plants. Samples were taken diagonally at several points (5%). Soil was taken from healthy plants around the canopy to a depth of ± 10 cm [16], each amount was ± 10 g, then combined with samples from other points.

![Fig. 1. Map of Borneo Prima citrus soil sampling locations](image)

2.2 Isolation and purification of rhizosphere microbial isolates

Isolation using the 10^-1-10^-5 serial dilution technique using Potato Dextrose Agar (PDA) media with the pour plate method [17] using 0.85% NaCl solution which is isotonic with cytoplasm cell. 28 g of PDA dissolved in 1000 ml and distilled water. Stir vigorously and dissolve with a hot plate then sterilize in an autoclave for 15 minutes at 121°C. Then it is allowed to cool after that it is poured into a Petri dish and allowed to solidify. The isolation process was carried out by pipetting 0.1 ml of the soil sample solution in each dilution series into a petri dish containing PDA media, then incubating at 25°C and observing the growth of the fungus every day. Observations were made for 2-7 days. The selection of purified microbial colonies was based on differences in colony morphological characters, both in terms of colour, elevation, surface texture, radial lines, concentric circles, and...
exudate drops in order to obtain pure isolates. Purification of fungal isolates using the point method in the transfer process into PDA media.

2.3 Soil physicochemical characterization

Environmental parameters measured included soil and air temperature, soil and air humidity, soil acidity (pH), light intensity, altitude, and organic matter content. Soil temperature, air temperature, air humidity, light intensity, altitude was measured directly at the time of sampling, while pH, moisture and soil organic matter were measured in the laboratory. Air temperature is measured using a thermometer, light intensity is measured using a luximeter, and altitude is measured using Garmin's Global Positioning System (GPS). Soil pH parameters were measured by means of 5 grams of soil sample mixed with 25 mL of distilled water, homogenized, and precipitated. Soil sample suspensions were measured using a calibrated pH meter using buffer solutions of pH 10, 7, and 4.

Soil and moisture content were measured by the gravimetric method. The 100 g soil sample was put into a porcelain cup. The soil is then dried in an oven at 105°C for 24 hours (a). Soil moisture is calculated using the formula, namely soil moisture = (100-a) 100-1 x 100% [18]. After 24 hours of baking at 105°C (a), 2 grams of soil samples were taken and then put into a porcelain cup. The soil sample is burned in a furnace at 500°C until the soil turns gray-red or the ash weight becomes constant (b). Soil organic matter is calculated using the formula, namely soil organic matter = ½ (2-b) x 100% [19].

2.4 Abundance and domination of Borneo Prima citrus rhizosphere fungi in 2 types of land

Types of fungal isolates from purification were selected macroscopically. Then the Importance Value Index (IVI) of an isolate was determined based on the average values of relative abundance, relative dominance, and relative frequency. The calculation of significant values follows the formula from [20].

2.5 The relationship between the physicochemical characteristics of the soil and the rhizosphere fungus colonies

Correlation between physical and chemical characters of soil and fungus colonies was analysed using Principal Component Analysis (PCA).

2.6 Data Analysis

The IVI of the species was estimated by the use of the following formula as described by [20].

\[
IVI = \frac{(RF + RD + RD_0)}{3}
\]

\( IVI \) = Important Value Index
\( RF \) = Relative frequency (RF)
\( RD \) = isolates relative density (RD)
\( RD_0 \) = isolates relative dominant (RDo)

Soil physicochemical variables including temperature, humidity, pH, and soil organic matter were analysed by one-way ANOVA and if there was a difference, it was continued with the BNJ test p value <0.05 using SPSS software version 24.
Important soil physicochemical variables in a field were analyzed using Principal Component Analysis (PCA), to determine the correlation value and the form of the relationship between soil physicochemical and the number of fungus colonies determined using a biplot with PAST4.12b software.

3 Results and discussion

3.1 Actual Land Conditions

The citrus fields for the Borneo Prima variety has an area of 10 hectares throughout the village of Padang Prapat, spread over dry acid and tidal land (Figure 2). The average production of Borneo Prima is 40 kg/tree, less than the Siam variety which can harvest 50-60 kg/tree. However, the price of Borneo Prima can be 2.5 times that of the Siam variety, whose market price is IDR 10,000 per kg. From field observations it is known that the production of prime Borneo oranges tends to be higher in tidal areas (table 2). Because irrigation is more guaranteed for plant needs. Meanwhile, on dry land, acidic water conditions are more limited.

Fig. 2. The actual condition of Borneo Prima citrus land on land types A) acid dry, B) tidal

<table>
<thead>
<tr>
<th>Land types</th>
<th>Block</th>
<th>Light intensity (Lux)</th>
<th>Temperature (°C)</th>
<th>Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inner canopy</td>
<td>Outer canopy</td>
<td></td>
</tr>
<tr>
<td>Acid dry</td>
<td>A</td>
<td>5.20 ± 0.15</td>
<td>6.70</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.11 ± 0.06</td>
<td>4.50</td>
<td>33.8</td>
</tr>
<tr>
<td>Tidal</td>
<td>A</td>
<td>1.73 ± 0.01</td>
<td>2.54</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.40 ± 0.22</td>
<td>4.25</td>
<td>45</td>
</tr>
</tbody>
</table>

The average plant height is over 2 meters, and the crown is not well developed (Fig.1). The stems of the plants are generally not clean, mostly overgrown with moss and pathogenic fungus. The environmental conditions were recorded to be quite hot with temperatures between 28-45°C, and low humidity between 40-74%. Even though the temperature was quite high, the sunlight recorded in the afternoon was low, both in the shade and outside the canopy (Table 1).

Table 2. Plant conditions, cultivation and citrus production of Borneo Prima in 2 land types
<table>
<thead>
<tr>
<th>No</th>
<th>Cultivation and production</th>
<th>Acid dry land</th>
<th>Tidal land</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Planting system</td>
<td>Monoculture in the yard, around residential areas</td>
<td>Surjan Intercropping with rice</td>
</tr>
<tr>
<td>2</td>
<td>Number of plants</td>
<td>200 plants</td>
<td>400 plants</td>
</tr>
<tr>
<td>3</td>
<td>Plant age</td>
<td>8 – 12 years</td>
<td>12 years</td>
</tr>
<tr>
<td>4</td>
<td>Plant spacing</td>
<td>4x 4 - 5 x5</td>
<td>4 x 8</td>
</tr>
<tr>
<td>5</td>
<td>Plant condition</td>
<td>2 meters and the crown is not well developed. The stems of the plants are generally not clean, mostly overgrown with moss and pathogenic fungus.</td>
<td>Plant conditions generally lack plant management, including branch pruning, production pruning and sanitary pruning are not carried out optimally</td>
</tr>
<tr>
<td>6</td>
<td>Fertilization</td>
<td>Manure, Ponska, and TSP</td>
<td>Manure, Ponska, and TSP</td>
</tr>
<tr>
<td>7</td>
<td>Irrigation</td>
<td>Irrigation with rainwater or watering using a water pump from the river</td>
<td>Flooding of water around the orange plantations</td>
</tr>
<tr>
<td>8</td>
<td>Pruning</td>
<td>Almost Not done</td>
<td>Trimming shape</td>
</tr>
<tr>
<td>9</td>
<td>Pests and Diseases</td>
<td>mildew, antracnose</td>
<td>mildew, anthracnose, diplodia, phytophthora.</td>
</tr>
<tr>
<td>10</td>
<td>Pest and disease control</td>
<td>Using pesticides, including Marshall, Mite, Antracol, Dithane</td>
<td>Using pesticides, including Marshall, Mite, Antracol, Dithane. (not yet familiar with vegetable pesticides/biological agents).</td>
</tr>
<tr>
<td>11</td>
<td>Production</td>
<td>30 - 75 kg / tree</td>
<td>50 - 100 kg / tree</td>
</tr>
<tr>
<td>12</td>
<td>Price</td>
<td>Rp. 15,000–Rp.25,000/ kg</td>
<td>Rp. 10,000-Rp. 30,000/kg</td>
</tr>
</tbody>
</table>

### 3.2 Soil physicochemical character

From the results of the analysis of the samples taken on the 4 indicators of the physicochemical condition of the soil, it was found that the pH of the soil in the acid dry land of block A, tidal blocks A and B was not significantly different with a pH 4.08-4.34, but lower and significantly different from the soil pH in acid dry land block B. Moisture in dry land (blocks A and B) is lower (63.25-66.19%) and significantly different from the soil in tidal land (blocks A and B). with humidity above 72.14%-75.00%). Meanwhile, the soil temperature in tidal land is lower (25.67- 26.50 °C) than the soil temperature in acid dry land (27.33-28.17°C). The results of the organic matter content test conducted in the MIPA UB laboratory showed that the organic matter in the tidal land was much higher than in the low tide (29.75-3.67%) (Fig.3).
3.3 Abundance of Borneo Prima citrus rhizosphere Fungus in 2 types of land

The Importance Value Index (IVI) reflects the role (dominance) of isolate types in a particular community. This important value provides an overview of the role of a type of rhizosphere fungus in the soil ecosystem and can also be used to determine the dominance of an isolate in the community. The results of the isolation of rhizosphere fungus in Borneo Prima orange plantations found 29 rhizosphere fungus. Each type of land has different rhizosphere fungus with the highest importance in Borneo Prima. The highest number of isolates was in acidic dry land A (11 isolates) followed by tidal land B (10 isolates) and tidal B (8 isolates) and the least in dry land B (7 isolates). There are 5 highest INPs in acid dry land A, namely TR25, TR23, TR37, TR2 and TR 18. In dry land B, namely TR 17, TR 36, TR 37, TR22, TR 23 and TR 15. In Tidal A, isolates with INP The highest were TR 1, TR 23, TR 20, TR 3. At tidal B, the dominant isolates were TR 23, TR 16, TR 34, TR 10 and TR 3. TR3 rhizosphere fungus isolates were found in 3 types of land, namely acid dry land block A, tidal land blocks A and B. The highest important value index in acid dry land block A was isolate TR25, while in block B it was isolate TR17. The highest value index for rhizosphere fungus is in tidal fields in block A, namely TR1, while in block B, it is TR23 (Figure 4).

In this study, the abundance of rhizosphere fungus on Borneo Prima citrus plants on acidic dry land in different tidal areas was not too great. This could possibly happen because these 2 types of land receive enough fertilizer (see table 1). Manure, Ponska and
TSP are given to these two types of land. Microbial diversity is greatly influenced by the availability of organic material in the roots in the soil because organic material is used as a building block and energy source for soil microbes [21]. The availability of nutrients in the form of exudate secreted by plants helps the germination of fungal spores.

According to [22] the quality and quantity of organic matter in the soil has a direct influence on the abundance/number of fungi in the soil because most fungi are heterotrophic. The results of the identification of fungal isolates have not been finalized. However, some of them were identified morphologically as *Trichoderma* sp1, *Aspergillus* sp1, *Aureobasidium* sp1, *Penicillium* sp1, *Paecylomycetes* sp1, *Mucor* sp1, *Rhizopus* sp1, *Aspergillus* sp2, *Trichoderma* sp2, *Cunninghamella* sp1. The rhizosphere fungi exist everywhere. This is caused by various vegetation factors and adaptability to the environment is quite high. *Trichoderma*, *Aspergillus*, *Penicillium*, and *Rhizopus* are found in the soil around the clove plant rhizosphere [23].

*Fusarium* sp., *Fusidium* sp., *Penicillium* sp., and *Trichoderma* sp. Found from the rhizosphere of citrus Siam Pontianak organic farming [24]. *Aspergillus* and *mucor* are known to easily adapt to environmental conditions so they are easily found in various treatments [25]. The Aspergillus genus is known to be able to increase plant growth by secreting phytohormones in cucumber plants (*Cucumis sativus*) [26] and has been reported to produce many metabolite compounds that are important in the medical field such as antimicrobial and anticancer substances [27]. The Mucor genus can act as a decomposer that helps fertilize the soil and is able to produce proteases, namely enzymes that play a role in the nitrogen cycle in the soil and produce metabolite compounds that are important in the medical field such as antitumor substances [28].

![Fig. 4. Rhizospheric fungal community structure based on the Important Value Index on Borneo Prima plants (KA = Dry Acid Block A; KB = Dry Acid Block B; PA = Tidal Block A; PB = Tidal Block B. TR1-TR44 = Code of rhizosphere fungi isolates)](image-url)
3.4 Relationship between rhizosphere mold abundance and soil physicochemical characteristics

Based on data on the physicochemical conditions of the soil (pH, soil moisture, soil temperature, organic matter) and the number of colonies (table 3) found in different land types, the eigenvalues and variance percentages (table 4) can be determined which are used to analyse PCA.

<table>
<thead>
<tr>
<th>Land types</th>
<th>pH</th>
<th>Soil moisture (%)</th>
<th>Soil temperature (0C)</th>
<th>Organic matter</th>
<th>Number of colonies (103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Acid Block A</td>
<td>4.08 ± 0.15</td>
<td>27.33 ± 0.29</td>
<td>66.19 ± 1.04</td>
<td>4.42 ± 0.03</td>
<td>19.67 ± 8.39</td>
</tr>
<tr>
<td>Dry Acid Block B</td>
<td>5.01 ± 0.06</td>
<td>28.17 ± 0.29</td>
<td>63.25 ± 1.44</td>
<td>1.72 ± 0.47</td>
<td>16.33 ± 1.53</td>
</tr>
<tr>
<td>Tidal Block A</td>
<td>4.34 ± 0.01</td>
<td>26.50 ± 0.29</td>
<td>72.14 ± 0.80</td>
<td>29.75 ± 0.65</td>
<td>103.33 ± 5.77</td>
</tr>
<tr>
<td>Tidal Block B</td>
<td>4.08 ± 0.22</td>
<td>25.67 ± 0.29</td>
<td>75.00 ± 1.76</td>
<td>32.85 ± 1.60</td>
<td>126.67 ± 5.77</td>
</tr>
</tbody>
</table>

Table 4. PC. Eigen value and % variance 5 factor

<table>
<thead>
<tr>
<th>PC</th>
<th>Factor</th>
<th>Eigen value</th>
<th>% variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>4.23</td>
<td>84.57</td>
</tr>
<tr>
<td>2</td>
<td>Soil moisture</td>
<td>0.65</td>
<td>12.90</td>
</tr>
<tr>
<td>3</td>
<td>Soil temperature</td>
<td>0.10</td>
<td>1.94</td>
</tr>
<tr>
<td>4</td>
<td>Organic matter</td>
<td>0.02</td>
<td>0.47</td>
</tr>
<tr>
<td>5</td>
<td>Number of colonies</td>
<td>0.01</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Based on the PCA summary results on the 5 factors whose relationship you want to know, it shows that only 1 factor that has been formed has an eigenvalue > 1, while the others are < 1 (table 3). The results of principal component analysis (PCA) of the main physico-chemical components of soil show a PC1 value of 84.57% and PC2 of 12.90%. The physico-chemical values of block A's tidal land are similar to block B's tidal land. Meanwhile, the physicochemical values of acid dry land A are similar to block B's acid dry land. The number of rhizosphere mold colonies is direct proportional to soil moisture and soil organic matter, which meaning that the higher the organic material and humidity, the higher the number of rhizosphere mold colonies. However, the number of rhizosphere mold colonies was inversely proportional to soil temperature and pH (Figure 5).

Sufficient soil organic matter can stimulate metabolic activity in the roots, causing more intensive root and shoot growth. Optimal root growth is characterized by the production of
root exudates which are increasingly released into the environment/soil. This root exudate will stimulate rhizosphere mold to grow and develop in plant roots, especially in the rhizosphere zone and enter into symbiosis with the plant [29][30]. The composition of this root exudate is also will determine the structure and community of fungi in the rhizosphere [31]. This is because root exudates are chemoattractant compounds which will colonize with molds to live in the rhizosphere zone because this exudate is a source of nutrition for molds so that the rhizosphere mold community will actively affect the composition of the rhizosphere. In return, this rhizosphere mold population will degrade organic and inorganic compounds present in the soil into simpler compounds and bring them to the rhizosphere zone so that they are more easily absorbed by plants [32]. The response of fungi to soil physical and chemical properties and types of vegetation varies, and soil environmental factors are the main factors influencing the diversity of soil fungi [33]. By analyzing the relationship between fungal diversity and soil environmental factors, it can indirectly reflect whether the soil is healthy, and has significance as a guide in recording the dynamic changes of soil fungal communities and restoration of the ecological environment [34].

![Principal component analysis](image)

**Fig. 5.** Principal component analysis

### 4 Conclusions

Twenty nine rhizosphere fungus isolates were found in the Borneo Prima citrus plantation endemic of *L.theobromae* with values. The abundance of rhizosphere fungi varies between acid and tidal dry land types. The highest abundance of isolates was found in acidic dry land A (11 isolates). The highest importance value index was found TR25 rhizosphere fungi isolates in the acid dry land block A and TR 17 in block B. The highest importance value index is at TR1 in block A tidal land, and TR23 in block B. TR3 rhizosphere fungus isolates were found in 3 types of land, namely acid dry land block A, tidal land blocks A and B.

Based on PCA analysis, a relationship was found between the physical and chemical properties of soil and rhizosphere fungal colonies. The number of rhizosphere fungal
colonies is directly proportional to soil moisture and soil organic matter. However, it is inversely proportional to temperature and soil pH. These findings will be followed by molecular identification of the isolate and further testing to determine the potential of the microbe in controlling *L. theobromae*, the cause of DSR disease which is endemic to citrus plants.

References


