Akta Agrosia

Diversity of Endophytic Fungal Community Associated with Local Rice Varieties Commonly Grown in Kuantan Singingi, Riau Province, Indonesia

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Doi: 10.31186/aa.26.1.1-6

ARTICLE INFO

Keywords:
endophyte,
fungal community,
local rice variety,
beneficial microorganism

Article history:
Received: April 18, 2023
Accepted: June 25, 2023
Published: June 30, 2023

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ABSTRACT

In a natural ecosystem, the plant establishes a mutual relationship with beneficial endophytes contributing huge impact on its host plant. Therefore, exploring beneficial endophytic fungi identified in a wide range of host plant species and their interaction is an interesting area of study. The present work aimed to characterize the fungal endophytic communities associated with local rice varieties. Healthy local rice variety commonly grown in Kuantan Singingi (Padi Kuning, Padi Gondok, Padi Sironda Putih, and Padi Sironda Merah) were selected and sampled. The samples were taken from different plant parts (root, stem and leaf). Descriptive statistics were used to present the results of this study. Results obtained from the present study showed that a total of 8 isolates were found in Padi Kuning, 9 isolates in Padi Rondok, 11 isolates in Padi Sironda Putih, and 11 isolates in Padi Sironda Merah. From different tissue parts of the plant, a total of 14, 12, and 13 isolates was found in stem, leaf, and root, respectively. Comparisons of the stem, leaf and root samples demonstrate a similarity in the endophyte assemblages among the local rice varieties and plant parts.

INDRODUCTION

Rice (*Oryza sativa* L.) is the world most important cereal crops. According to Food and Agricultural Organization of the United Nations (FAO), It is the primary dietary energy source for 17 countries in Asia and the Pacific, eight in Africa, and nine in North and South America. In Indonesia, rice is one of the most consumed staple foods belonging to the Poaceae family in Indonesia due to its high nutrition value.

The different varieties of rice have been cultivated in Kuantan Singingi Indonesia such as white, red, yellow, and black-seeded rice and each of them harbor different nutritional values, as white rice provides a good source of carbohydrates, along with calcium, iron, thiamine, pantothenic acid, folate, and vitamin E. Colored rice varieties, such as red, are known to have high iron and zinc content whereas black rice is known to have high protein content. The grain also helps reduce cardiovascular diseases, some cancer and improves gut health. Besides the nutritional

ISSN: 1410-3354 / e-ISSN:2615-7136

Cited this as: Andriani, D., Seprido, C. Ezward. 2023. Diversity of Endophytic Fungal Community Associated with Local Rice Varieties Commonly Grown in Kuantan Singingi, Riau Province, Indonesia. Akta Agrosia 26(1):1–. doi: 10.31186/aa.26.1.1-6

attributes, rice is also a versatile crop cultivated in diverse climatic conditions, including dry and wetland environments at high and low altitudes. Ezward *et.al*. (2020) reported that there were as many as 24 types of local rice genotypes which were widely cultivated by the Kuantan Singingi community.

The biotic stresses changes have seriously affected rice cultivation, making future production in jeopardy. Over the years, many efforts have been tried to make rice plants tolerant to the aforementioned abiotic stresses. Genetic cross-breeding has been used conventionally to produce superior rice varieties with improved tolerance and increased yield. The hybrid vigors are reported to produce a 30% higher grain yield than the traditional varieties. However, the drawbacks of using these interventions is that the farmers must buy new seeds each season, as planting the seeds from the previous hybrid plant can display varying traits and inconsistent yield. In such atypical circumstances, the utilization of a unique symbiotic association of endophytic fungi and plants represents a sustainable alternative.

Endophyte is defined as an important group of widespread and diverse plant symbionts that live asymptomatically and sometimes systematically within plant tissues without causing symptoms of disease. Endophytic fungi represent an important and quantifiable component of fungal biodiversity in plants that impinge on plant community diversity and structure. Ezward *et.al*. (2021) studying 24 local rice varieties in Kuantan Singingi reported that 3 varieties were susceptible to planthopper attack and the remaining were resistant to moderately resistant to planthopper attack.

Endophytic fungi is usually found in plant tissues without causing symptoms to the host plant (Faeth, 2010). Several endophytic fungi have been identified and have the ability as biocontrol, Aspergillus niger can inhibit the growth of pathogenic fungi because it produces hydrolytic enzymes such as lipase, protease, cellulase, and pectinase (Schuster et al 2002). *Nigrospora* was able to increase the resistance of rice plants to the brown rice planthopper *Nilaparvata lugens* Stahl (Budiprakoso, 2010). Sucipto *et al* (2015) reported that 4 isolates of 14 endophytic fungi inhibited *P. oryzae* activity *in vitro*. Durham (2004) studying blast disease in local rice variety of Kencana Bali in the greenhouse reported that the 4 isolates of its endophytic fungi were able to suppress blast disease development with suppression levels between 30–70%.

It is widely reported that endophytic fungi of rice vary among varieties, location and plant parts. However, study on endophytic fungi of local rice varieties in Kuantan Singing is limited. Therefore, the objectives of the research were to study the diversity of endophytic fungi of different plant parts of local rice varieties commonly grown in Kuantan Sengingi, Indonesia.

MATERIALS AND METHODS

Sampling

Endophytic fungi samples were taken from rice plant parts (stem, root, and leaf) of local variety from Inuman and Pangean districts. The local rice varieties used were padi kuning, padi gondok, padi sironda putih, dan padi sironda merah. The samples were taken from healthy plants at early vegetative stage with no little pest or pathogen infection, cut in 5 cm length and placed in a sample bag. The endophytic fungi were then isolated in the Basic Laboratory of the Faculty of Agriculture.

Isolation and Characterization of Endophytic Fungi

Isolation of endophytic fungi from the roots, stems and leaves of rice plants was carried out using a modified method of Rodriques (Wilia *et al,* 2010). The collected plant parts were washed in running water, cut into 5 mm x 5 mm size, disinfected 2 times with 70 % ethanol for 30 seconds and 3% sodium hypo chloride for 60 seconds, then rinsed by sterilized water four times. The excessive water was dried by sterile paper towel and plated on medium potato dextrose agar (PDA) pH 5.5. Isolated fungi passed the sterility test were then purified by re-culturing on PDA media for a culture collection preparation.

Colonies of endophytic fungi that grew were observed for the character of colony growth, namely: the color and surface of the colony, radial lines from the center of the colony to the edge of the colony, and concentric circles.

Recorded Variables

Recorded variables were number of isolates obtained from observing the macroscopic characters of the fungi from samples grown on PDA media. Colony growth character was microscopically determined at the colony color, the surface shape, and the colony growth direction on the 3rd, 7th, and 10th days.

All the recorded variables were performed in triplicates. Descriptive statistics were used to characterize the fungal endophytic communities associated with local rice varieties.

RESULTS AND DISCUSSION

Isolation and Diversity Analysis

A total of 49 different fungal isolates were identified from three tissue sections of four indigenous rice plants collected from various locations of Kuantan Singingi. Endophytic fungal occurrence was highest in Padi Sironda Putih and Padi Sironda Merah (11 isolates) followed by Padi Gondok with 9 isolates, and the lowest in Padi Kuning with 8 isolates (Table 1).

Irrespective of rice varieties, the diversity analysis reveals that stem tissues harbored the highest number of isolates (14 isolates) followed by root tissue (13 isolates) and leaf tissue (12 isolates) for the lowest. The diversity analysis also showed that the occurrence of endophytes were almost equally distributed in stem, leaf, and root section of all rice varieties except for Padi Sironda Merah. In the case of Padi Sironda Merah, the highest diversity of endophytes (5 isolates) occurred in stem section, while the endophytes occurrence in leaf and root parts (3 isolates) were equally distributed (Table 1). These result suggest that the recovered fungal endophytes are not tissue and even host specific. This assures wide applicable nature of the isolates on multiple host plant.

Table 1. Occurrence of endophytic fungi isolates in different parts of local rice varieties.

Rice Variety	Plant Parts			Total
	Stem	Leaf	Root	
Padi kuning	3	2	3	**8**
Padi gondok	3	3	3	**9**
Padi sironda putih	3	4	4	**11**
Padi sirondah merah	5	3	3	**11**
Total	14	12	13	

When these experimental data were connected with the exploratory results, it reveals that there was a close association between the dominance of endophytic fungi, planted rice varieties, and location where the local rice was cultivated. These factors seem to have strong effects on the dominance, diversity of endophytic fungi, and the potential yield of the local rice variety. In the case of Padi Sironda Putih and Sironda Merah, for instance, these two varieties are widely cultivated in Pangean sub-district community. With 11 isolates of endophytic fungi being harbored, these two rice varieties had agronomic characteristics as follows: plant height of 126 cm, harvesting age of 121 days, an average number of productive tillers of 7.06, and an average number of grains per panicle of 267.10 (Ezward , et al. 2020). Pangean sub-district is one of the sub-districts in Kuantan Singingi district with flat and partly hilly land conditions at 65 meters above sea level. Rice cultivation in this sub-district is concentrated in Tonga Island Village, Ingu Island, Kumpai Island, with rainfed rice fields. Most of these rice fields are located along the banks of the Kuantan river. The average temperature in Inuman district ranges from 20 to 35^0C.

There were differences in the number of isolates in each plant part. For instance, 14 isolates were found in the stems, 12 isolates were found in the leaves, and 13 isolates were found in the roots. These results suggest that there was diversity in the presence of endophytic fungi in plant tissue. Higher plants are equipped with various layers that make up the structure of the plant body so that they have a diversity of microbes that exist in each of these arrangements. Fungi are the dominant microbes that often colonize every

arrangement of the layers of the plant structure so the fungi are divided into several types, namely surface colonizers of leaves and twigs (epiphytes), internal tissues of leaves (leaf endophytes), bark (bark endophytes), and wood (xylem endophytes and wood decomposing) (Stone *et al*. 2004).

Each isolated plant part, i.e., the stem, leaves, and roots, was occupied by the endophytic microbes. According to Rodriguez *et al*. (2009), all plants in natural ecosystems have a symbiosis with endophytic fungi. Based on transmission and ecological interactions, endophytic microbes are divided into four classes, class 1 endophytes infect narrow-leaved hosts, generally have vertical transmission, and produce mycotoxins, class 2 (two) have broad-leaved host ranges and have vertical and horizontal transmission. Class 3 (three) and class 4 (four) endophytes infect broadleaf hosts, have horizontal transmission, and infect shoots and roots. The transmission system of endophytic fungi is vertically through the outer layer of seeds, seeds, or seedlings, while the transmission system of endophytic fungi is horizontally through airborne spores (Hodgson *et al*. 2014). Rice plant belongs to the Poaceae family and was classified as the narrow-leaved plant where the transmission of endophytic fungi occurs horizontally. The horizontal spread of endophytic fungi allows endophytic fungi to move more freely, so they are often found on stems and leaves.

The diversity of fungi that colonize plant tissues is also influenced by the nutritional factors needed by the fungus for its growth and development. In plant tissue, it is rich in carbon elements and other elements which are important nutrients for the survival of microorganisms, one of which is fungi.

Colony Characteristics

The results of the study reveal that several similarities were found in the characteristics of the endophytic fungi isolated from Padi Kuning, Padi Gondok, Padi Sironda Putih, and Padi Sironda Merah. White colony color with a velvety colony surface and symmetrical growth direction were not found in Padi Gondok, but present in the other three varieties. Cream colony color with a velvety surface and symmetrical growth direction was not found in Padi Gondok, but founded in the other three varieties (Table 2). Due to these similar characteristics of the colony, they were hypothetically the same type of endophyte. According to Anggraeni and Usman (2015), there are morphological similarities in several colonies of endophytic fungi isolated from suspected to have come from the same isolate.

The results of the study also demonstrtae several differences in the characteristics of endophytic fungi isolated from various local rice genotypes. Greenish colony color with a velvety colony surface shape and symmetrical growth direction was only found in Padi Kuning, endophytic fungi with white colony color with a colony surface shape Coarse and symmetrical growth directions were only found in Padi Gondok. Endophytic fungi with a cream colony color with a smooth colony surface shape and symmetrical growth direction were only found in Padi Gondok Endophytic fungi with a white colony color a

Table 2. Characteristics of endophytic fungi on different plant parts of local rice varieties

Rice variety	Colony color	Colony surface	Growth direction
Padi	grey	velvet	cymetrical
kuning	white	velvet	ctmetrical
	creamy	velvet	cymetrical
	Greenish	Velvet	cymetrical
Padi	grey	velvet	cymetrical
gondok	White	smooth like	cymetrical
		cotton	cymetrical
	creamy	smooth like cotton	
Padi	grey	velvet	cymetrical
sironda	white	velvet	cymetrical
putih	white	rough	cymetrical
	creamy	velvet	cymetrical
	greenish	rough	cymetrical
Padi	greenish	rough	cymetrical
sironda	grey	velvet	cymetrical
merah	Grey	smooth like	cymetrical
		cotton	cymetrical
	Pink	smooth like	cymetrical
		cotton	cymetrical
	black	rough	cymetrical
	white	velvet	cymetrical
	creamy	velvet	
	greenish	sooth like	

rough colony surface shape and a symmetrical growth direction are only found in Padi Sironda Putih. In Padi Sironda Merah, the most common characteristics observed were gray color with smooth colony surface shape and symmetrical growth direction, gray color with smooth colony surface shape and symmetrical growth direction, brown color black with a rough colony surface shape and symmetrical growth direction and greenish colony color with smooth colony surface shape and symmetrical growth direction.

Characteristics of Endophytic Fungi in Different Plant Parts

The results of the study show that there were several similarities in the characteristics of endophytic fungi isolated from stems, leaf, and root. Characteristics of endophytic fungi with gray colony color, velvety surface of the colony, and symmetrical growth direction were found in all plant parts. Endophytic fungi with white colony color with a velvety colony surface and symmetrical growth direction were found in all plant parts. Characteristics of endophytic fungi with cream colony color, with a velvety colony surface and symmetrical growth direction were found in all isolated plant parts (Table 3).

Table 3. Characteristics of endophytic fungi in different plant parts.

Plant part	Colony color	Colony surface	Growth direction
Stem	grey	velvet	cymetrical
	white	velvet	ctmetrical
	white	smooth like cotton	cymetrical
	creamy	smooth like cotton	cymetrical
	creamy	velvet	cymetrical
	greenish	rough	cymetrical
	pink	smooth like cotton	cymetrical
	black	rough	cymetrical
Leaf	grey	velvet	cymetrical
	white	velvet	ctmetrical
	creamy	velvet	cymetrical
	white	rough	cymetrical
	grey	smooth like cotton	cymetrical
	creamy	velvet	cymetrical
Root	grey	velvet	cymetrical
	white	velvet	ctmetrical
	greenish	velvet	cymetrical
	creamy	velvet	cymetrical
	greenish	rough	cymetrical
	greenish	smooth like cotton	cymetrical

The results of the study also found several differences in the characteristics of endophytic fungi isolated from different parts of the plant. White colony color with a smooth colony surface shape and symmetrical growth direction as well as endophytic fungi with colony color characters of cream, pink, and black. with the surface of each colony being velvety, smooth, and rough, and the direction of growth of each being symmetrical, was only observed in stem. Meanwhile, endophytic fungi with gray colony color, a smooth colony surface, and symmetrical growth was observed in leaf section. In root section, different endophytic fungi with greenish color of the colonies with a smooth surface shape and symmetrical growth directions were observed.

CONCLUSION

From four field-grown indigenous rice samples in several districts of Kuantan Singingi, Indonesia, eight isolates were found in Padi Kuning, nine isolates in Padi Gondok, 11 isolates in Padi Sironda Putih, and 11 isolates in Padi Sironda Merah. There were some similarities in the characteristics of edophytic fungi isolated from four field-grown indigenous rice. From different tissue parts of the plant samples, a total of 14, 12, and 13 isolates was found in stem, leaf, and root, respectively. Comparisons of the stem, leaf and root samples demonstrate a similarity in the endophyte assemblages among the local rice varieties and plant parts. Several similarities were observed in the characteristics of the endophytic fungi isolated from four field-grown indigenous rice samples

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