IOP Conf. Series: Materials Science and Engineering **420** (2018) 012080 doi:10.1088/1757-899X/420/1/012080

In vitro screening of endophytic fungi associated with mangroveas biofertilizer on the growth of black rice (*Oryza sativa*L. "Cempo Ireng")

Boike Sopiani Tumangger, Fara Nadilla, Nadia Baiduri, Fitriani, Vivi Mardina*

Department of Biology, Faculty of Engineering, Samudra University, Kampus Unsam Meurandeh, Langsa 24415 Indonesia

* vmardina@unsam.ac.id

Abstract. Microorganisms play an important role in maintaining soil fertility and plant health. They can act as biofertilizers and increase the resistance to biotic and abiotic stress. This study focused on the isolation and selection of endophytic fungi associated with mangrove that have ability to promote the growth of *Orizasativa* L. Cempoireng. Assessing the growth of plant was study in vitro test by using PDA as a media. A total of 17 fungi were found in leaf, stem and root of mangrove and classified into 6 morphotypes according to colonies characteristics and form of mycelium. The best isolate (the endophytic fungi with characteristic of white fungus colonies mycelium septa and non-septa revealed the high ability to promote the germination seed and growth of CempoIreng. Overall, the study indicated that the endophytic fungicontributed the positive effect on the growth of *Oryza sativa*L. "Cempo Ireng".

1. Introduction

Mangroves are known as ecological services both in tropical and subtropical regions by providing niches for various flora, fauna as well as microbes [1]. Endophytes fungi are cluster of fungi that colonize the plant interior and contribute to the growth, development, fitness, and adaptation of the host plant. They have been found in almost all plant families including mangrove family. Endophytes fungi often confer considerable benefits to the host plants they inhabit [2]. They can stimulate plant growth by providing nutrients such as phosphate, iron and increase plant resistance to stress such as drought, high salinity, and metallic toxicities. Moreover, endophytic fungus may produce a number of secondary metabolites (auxin, cytokinin, and gibberellin) that contribute to stimulate the growth of plants [3,4].

On the other hand, black rice (*Oryzasativa* L. cultivar "CempoIreng") has been believed as an alternative healthy food for diseases treatmentsbecause of its nutritive and medicinal value. It contains anthocyanin, flavonoids, vitamin B1, Fe, and vitamin E that is higher than brown or white rice [5]. Therefore, the study highlighted the potential endophytic fungus associated with mangrove and investigated their ability to promote the plant growth of *Orizasativa* L. CempoIreng.

2. Materials and Methods

2.1. Materials

The main materials were mangrove that were collected from Kuala Langsa, Langsa, Aceh, Indonesia. All chemicals used for the study were analytical grade and obtained from C.V Multikreasi Medan.

2.2. Methods

2.2.1. Preparation of fungal endophyte isolates

The endophyte isolates were obtained from the roots, stems, and branch of mangrove. The isolation method followed the proposed method of Nawea*et al*. [6] that has been modified on plant surface sterilization. The roots, stems, and leaves of the plant were cut $\pm$ 1 cm and washed with running water. The surface was sterilized gradually through immersion in 70% alcohol for 1 minute, followed by immersion with 5% NaOCl for 2 minutes, and 70% alcohol for 30 minutes. Furthermore, each part of the plant rinsed as much as 3 times with sterile distilled water. Roots, stems, and leaves that have been cut are placed on a 10% PDA medium and incubated in the dark. The isolated plant sterile test was performed by scraping the last rinse of sterile distilled water onto a 10% PDA medium and subsequently incubated for 3-5 days. The mycelium growing on plant tissue pieces was observed daily. The mycelium then was transferred to a 10% PDA medium for purification.

2.2.2. Purification and identification of endophytic fungi

The purification of the spore fungus was carried out by transferring the fungus which has grown by 0.5 cm into the medium of the PDA [7]. An identification book from Nakagiri [8] is used as a reference to identify morphology of endophytic fungi covering macroscopic and microscopic observations. The samecolonies were considered the same isolate, and each representative colony was separated into isolates.

2.2.3. Determination of potential of endophytic fungi as biofertilizers

Sterilization of rice seeds surface wasperformed by soaking the seeds of black rice in sterile water for 15 minutes and 1% NaOCl for 2 minutes. Subsequently, they were rinse with sterile water 2 times then grown on PDA medium. Furthermore, after germination, the seeds were transferred to a pure culture of endophytic fungi aged 4-7 days on PDA medium. Each PDA medium was filled with 15 seeds of rice crops. Observations were carried out by measuring the crown height and root length at 7 days after planting. The observed variables were the height of stemand the length of the root. Determination of the potential fungi as biofertilizer was determined by the response of seeds after endophytic fungi treatments [9].

2.2.4. Data Analysis

The parameters observed were germination, height of stem, root length of black rice plants grown in-vitro. Data were analysed using ANOVA at 5% significant leveland continued with the Smallest Different Test (DMRT). The test criteria were as follows: if the value $F_{count} > F_{table}$, then H_1 was accepted and H_o was rejected, If the value $F_{count} < F_{table}$, then H_o was accepted and H_1 was rejected [10].

3. Results and Discussions

The study obtained a total of 17 isolates of fungi from roots, stems, and leaves of mangrove (table 1) which initially grouped into 6morphotypes according to colonies characteristics and form of mycelium i.e (1) the white fungus colonies with mycelium (PS) and(2) non-septa (PNS),(3) black fungus with mycelium septa (HS) and (4) non-septa (HNS), and (5) yellow fungus with mycelium septa (KS) and (6) non-septa (KNS).

Tabel 1. List of endophytic fungi isolated from mangrove located in Kuala Langsa, Aceh, Indonesia based on morphotypes*

Isolates	Number of isolates			Percentage (%)
	Root	Stem	Leaf	
PS	4	2	1	41,18
PNS	2	1	2	29,4
HS	1	0	0	5,89
HNS	1	1	0	11,76
KS	0	1	0	5,89
KNS	1	0	0	5,89
Total	9	5	3	100

*6 morphotypes of fungi: the white fungus colonies with mycelium (PS) and (2) non-septa (PNS),(3) black fungus with mycelium septa (HS) and (4) non-septa (HNS), and (5) yellow fungus with mycelium septa (KS) and (6) non-septa (KNS).

According to a total of 17 endophytic fungi isolated from mangrove, the endophytic fungi with mycelium septa and non-septa were dominant on frequencies of 41.18% and 29.4% respectively. Furthermore, the observations on three parts of the plant exhibited that endophytic fungi were most widely found in the roots of mangrove. The result was consistent with the research of Paul *et al*. [11] who reported that the root was the most high-frequency isolate compared to the stem and leaf. It can be explained thatroot of plant providessuitable habitat for various micro organism including endophytic fungi.

In order to test the ability of endophyte as biofertilizer, the *in vitro* tests was performed by aseptically germinating and growing Cempoireng in potato dextro agar (PDA). The results revealed that the endophytic fungi had potency to stimulate the germination seed and growth of CempoIreng particularly forthe white fungus colonies with myceliumnon-septa(PNS) (Table 2 and Figure 1).

Tabel 2. *In vitro* test to the ability of endophytic fungi associated with mangrove from Kuala Langsa, Aceh, Indonesia as biofertilizer on the seed germination and growth of Cempoireng.

Treatments		Respons of the germination seed of CempoIreng		Respons of the growth of Cempoireng	
		Normal germination	Non germination	Average of height of stem (cm)	Average length of root (cm)
Positive controls		8 (86,7)	7 (13,3)	1,5	0,2
Endophytic fungi.	PS	14 (93,3)	1 (6,7)	2,4	0,6
	PNS	15 (100)	0	1,9	0,5
	HS	13 (86,7)	2 (13,3)	2,0	0,6
	HNS	12 (80,0)	3 (20)	2,1	0,7
	KS	10 (66,7)	5 (33,3)	1,3	0,2
	KNS	13 (86,7)	2 (13,3)	2,2	0,5

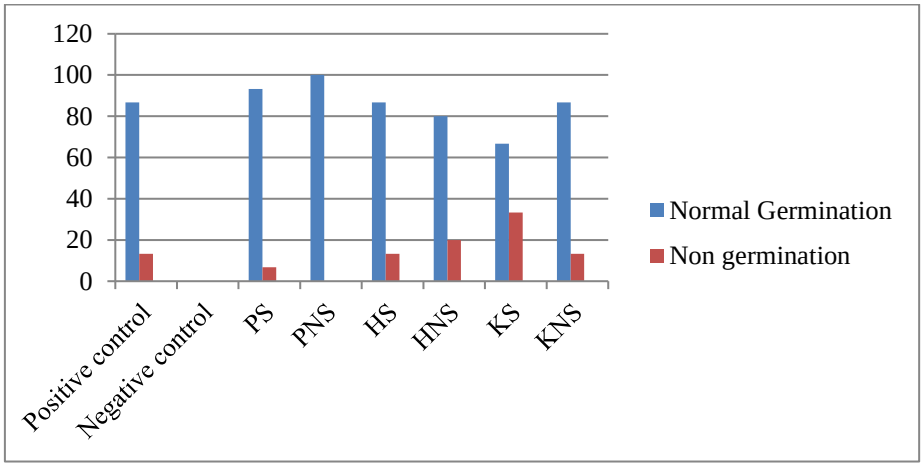

Figure 1. Percentage of in *vitro test* toward the ability of endophytic fungi associated with mangrove from Kuala Langsa, Aceh, Indonesia as biofertilizer on the germination and growth of Cempoireng.

The figure 1 displayed the positive responses of germination of Cempoireng in vitro test approximately 65 % by adding the isolated endophytic fungi associated with mangrove. This finding could be explained byKusumawardani*et al.* [12]who reported that endophytic fungi tested could inhibit the growth of pathogenic fungi through antagonistic mechanisms viz competition mechanisms, parasitism, and antibiosis. Additionally, the effect of endophytic fungi associated with mangrove toward the growth of Cempoireng particularly stem height and root length showed the positive responses as showed in figure 2(a) and 2(b). The isolated fungus was able to increase the stem height of 1.98 cm and the root length of 0.36 cm compared to the control that was1.5 cm and 0.2 cm respectively. These results were supported by Hamayun [13] and Khan [3] who stated endophytic fungi have the ability to produce a number of secondary metabolites viz. auxin, gibberellin and cytokines that promote the growth of Cempoireng. In the same way, Sirrenberg*et al.* [14] reported that the endophytic fungus isolated from *Piriformosporaindica* was able to rise *Arabi-dopsis* growth by increasing the production of auxin hormone which plays a role in increasing the length of the plant stem (apical dominance). As a result it will increases plant growth.

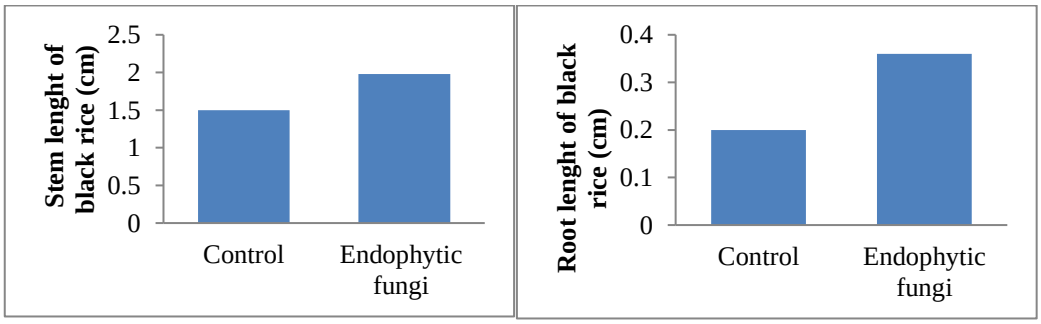

Figure 2. *In vitro* test on the ability of endophytic fungi as biofertilizer toward the growth of stem (a) and root of CempoIreng (b).

4. Conclusions

The present study screened the potential endophytic fungi associated with mangrove plant that was collected from Kuala Langsa, Aceh, Indonesia. The study found a total of 17 endophytic fungi associated with mangrove plant. However, there are 2 colonies which exhibited the best responses on the growth of *Orizasativa* L-CempoIreng particularly toward the stem height and root length.

5. Acknowledgments

The authors are thankful tothe Ministry of Research Technology and Higher Educationfor financial support of Creativity Program of StudentResearch (No. 187/SPK/KM/IV/2018).

6. References

[1] Suciatmih 2015 Diversity of endophytic fungi mangrove plants on Sampiran Beach and Bunaken Island, North sulawesi *Pros Sem Nas Masy Biodiv Indon* **1**(2):177-183.
[2] Kandel S L, Firlincelli A, Joubert pm and Doti S L 2017. An invitro study of biocontrol and plant growth promotion potensial of Salicaceae endophytes. Frontiers in Microbiology **8**(386): 1–16.
[3] Khan SA, Hamayun M, Khan AL, Lee IJ, Shinwari ZK and Kim J 2012 Isolation of plant growth promotic fungi from dicots inhabiting coastal sand dunes of Korea. *JBot***44**(4):1453–1460.
[4] Ilyas M, Kanti A, Jamal Y, Hernita and Agusta A 2009 Biodiversity of endophytic fungi associated with Uncaria gambier Roxb. (Rubiaceae) from West Sumatra *Biodiversitas* 10 (1): 23 – 28.
[5] Kushwaha, U K S 2016. Black rice, research, history and development. Springer International Publishing Switzerland. ISBN 978-3-319-30152-5.
[6] Nawea, Yohanis, dan Robert A. Bara2017Antibacterial Activity Study of Endophytic Fungi Derived from Sonneratia alba Mangrove Growing on Tanawangko Waters*J Pesisir dan Laut Tropis***67**(2): 257 – 268
[7] Nhu, P., Yen. 2017. Isolation and characterization on endophytic bacteria in soybean (Glycin max) cultivatedon aluvial soil of can the city vietnam. International Journal of Inovation in Engenering and Tecnology. Vol 8(1 : 1049-1053
[8] Nakagiri, A. 2005. Preservation of kapang and freezing methods. Workshop on Preservation of Microorganisms. Biotechnology Center-NITE & Research and Development Center for Biotechnology-LIPI, Cibinong: October 17-18, 2005.
[9] Ting ASY, Meon S, Kadir J, Radu S, Singh G. 2005. Endophytic microorganisms as potential growth promoters of banana. *Biocontrol.* Vol 53: 541-555.
[10] Gomez, K and A. Gomez. 2010. *Prosedur Statistik untuk Penelitian Pertanian.*Edisi Kedua. (Diterjemahkan oleh Endang Sjamsuddin dan Yustika S. Baharsjah). Jakarta: Universitas Indonesia. 98-100 p
[11] Paul NC, Deng JX, Sang HK, Choi YP, Yu SH.2012, Distribution and antifungal activity of endophytic fungi in different growth stages of chili pepper (*Capsicum annuum* L.) in Korea. Plant Pathol. J. 28(1):10–19.
[12] Kusumawardani Y, Sulistyiowati LN Cholil A 2015 Potential of endofite fungus antagonists in pepper crops (Piper ningrum L) againts masroom Phytophora capsici leoinian cause of roten disease Journal HPT **3**(1);21-29
[13] Hamayun M, Khan SA, Khan AL, Tang DS,Hussain J, Ahmad B, Anwar Y, Lee IJ.2010. Growth promotion of Cucumber by pure cultures of gibberellins-producting

Phoma sp. GAH7. J Micribiol Biotechnol. 26:889–894.
[14] Sirrenberg A, Goebel C, Grond S, Czempinski N, Ratzinger A, Karlovsky P, Santos P, Feussner I and Pawlowski K 2007 *Piriformospora indica* affects plant growth by auxin production *Physiol Plant.* **131**: 581–589