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Characteristics of rice plant with low methane emission

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ABSTRACT

Globally, methane from rice fields is responsible for about 10% of non-CO2 emissions from agriculture. Rice has an important role in the release of methane into the atmosphere. The release of methane from rice is influenced by genetic and environmental factors. This study is aimed to determine the specific marker characters for the rice with low methane emissions. The research design used randomized block design. Rice was planted by direct seeded system (gogo rancah). The result showed that low CH_4 gas is emitted by Inpari 13 and Mekongga variety. Rice emitted CH_4 gas was influenced by genetic factor with 0.91 heritability value. Rice with lower methane emissions has specific marker characters as follows: little biomass, narrow cavity of root aerenchyma and weak rigidity plant.

Key words : *Rice, Heritability, Characteristics, Methane gas emissions.*

Introduction

Methane (CH_4) is one of the major gases from Greenhouse Gas (GHG) causing global warming. Global warming which result an extreme climate change on earth is engendered by an escalating of the earth's surface temperature. The agriculture sector contributes 67% of total emissions of global GHG emissions. Lowland rice cultivation is one of the agricultural activity which is embedded in the Indonesia agricultural system.

Rice is a staple food for Indonesians. National rice demand is always commensurate with the total of population annually. However, rice cultivation contributes to the release of greenhouse gases into atmosphere, especially methane, which lead to global warming. Rice fields in anaerobic conditions are a source of methane formation. Rice cultivation is one of the sources of CH_4 emissions (21.9%) with an additional rate of 1-2% per year (Khalil and Rasmussen, 1992). Over the previous 300 years, atmospheric methane levels have more than doubled (IPCC, 1996). Among the rice ecosystem, irrigated rice fields, which are expected to expand with population growth, have been contributing to the highest CH_4 emissions compared to that of other land uses (Setyanto, 2004).

Therefore, several attempts are immensely needed to reduce methane emissions while at the same time, increase rice production. One of the mitigation attempts that can be done is to assemble low emission rice. The first step in rice engineering is the selection of varieties to get the specific characteristics of rice plants with low methane emissions. Therefore, this study aimed to determine the specific marker characters for the rice with low methane emissions.

Materials and Methods

The present study was conducted during a rainy season at the experimental field of Agricultural Research Institute (Balingtan) Jakenan, Pati, Central Java. The research instruments used were automatic gas sampling tools i.e. methane gas capture boxes with 100 cm x 100 cm x 120 cm in size, a ruler for measuring plant height, GC-2014 gas chromatography equipped with a FID detector (Flame Ionozation Detector) for CH_4 gas, ruler, camera and scale. The instruments used to observe the anatomical structure of root and stemaerenchyma were microscopes, brushes, razor blades, petri dishes, drip pipettes and glass preparations.

Materials used were rice plants i.e.Ciherang, Mekongga, Inpari 18, IPB3s, Inpari 13, Inpari 31, Inpari 32, and Inpari 33, urea fertilizer, KCl, SP36, manure, pesticides. The materials used to observe the anatomical structure of root and stem aerenchyma were alcohol, nail polish, glycerin, cassava cambium, Safranin solution with 10% concentration, and aquades.

The research design used a randomized block design (RBD) with 3 replications and 8 treatments of varieties planted with a Gogorancah (Gora) system. Observation parameters comprised of plant height (cm), number of tillers, root length (cm), plant biomass (grams) number of root aerenchima, width cavityof root and stem aerenchyma, panicle length, number of panicles per clamp, number of filled grains per clamp, percentage of filled grains per clamp (%), weight of 1000 filled grains (grams), yield of dry milling grains (t/ha), estimation of methane emissions (kg CH_4/ha/season), and estimation of the emission index.

Data were analyzed using analysis of variance (ANOVA). If it shows a significantly different, it will be followed by Honestly Significant Difference (HSD) test. Genetic proportion was analyzed using heritability estimation. Simple regression analysis was carried out on plant parameters with CH_4 flux to determine the agronomic characteristics that influence CH_4 emissions. Correlation analysis between plant parameters and CH_4 flux was conducted to determine the correlation between them.

Results and Discussion

Heritability of Rice Plant with Low Methane Emissions

Heritability of a trait can be defined as a proportion of the genetic variation amount toward the total of genetic variation and integrated with environmental variability (Basuki, 2005). A high heritability value means large genetic variation and small environmental variability (Welsh, 2005). Based on genetic variation and environmental variability, the observed heritability character estimates ranged from 0.04 to 0.91 (Table 1). Characters of plant height, number of tillers, width cavity of stem aerenchyma, panicle length, panicle number, filled grain, 1000 grains weight, yield of dry milling grain, and methane emissions were categorized in a high heritability criteria. Based on those categories, the dominant ability of rice plants to emit CH_4 is presumably influenced by genetic factors. The genetic influences the physiological and morphological processes of rice in emitting CH_4 gas. The variation degrees of CH_4 gas emissions is indicated by the influence of morphological and physiological variations from rice plant varieties (Wihardjaka and Sarwoto, 2015). The high genetic estimate indicates that the character can be inherited. Fehr (1987) elaborated that if the estimated value of heritability is high, the selection is carried out in the initial generations because the character of a genotype is easily inherited to its offspring. Supported also by Lestari *et al*., (2006) that the heritability is useful to know the extent to which the characters are inherited in subsequent offspring. The characters uniformity among genotypes is tested with a large genetic influence of those characters. It shows that the genotype is potentially developed as crossing parents.

According to Islam *et al*., (2012), characters having low genetic variation and high heritability estimates indicate the involvement of non-additive agent on character appearances, and thus, selection based on the phenotype of those characters is not recommended. It was supported by Yamauchi, *et al.* (2013), that the genes play a role in the formation of aerenchyma cavity in rice under flooded conditions which will form Reactive Oxygen Species (ROS) as a response to submerged. The gene encoding metallothionein-2b (MT2b) is expressed to improve dead cell and plant growth. Aerenchyma provides oxygen to the root tips and rhizosphere, and then removes carbon dioxide, ethylene, methane gas from the roots and soil (Colmer, 2003).

Moderate heritability criteria was found in the character of plant biomass, the mean width cavity of root aerenchyma. Based on Martono (2009), the moderate estimate of heritability shows that both

Table 1. Parameters of Rice Plant with Low Methane Emissions

Character	h^2	Criteria
Plant height (cm)	0.89	High
Number of tiller	0.73	High
Root length (cm)	0.09	Low
Biomass (g)	0.27	Moderate
Number ofroot *Ae*	0.04	Low
Root *Ae*cavity (mm^2)	0.37	Moderate
Stem *Ae*cavity (mm^2)	0.68	High
Panicle length (cm)	0.71	High
Number of panicle	0.68	High
Number of filled grains	0.52	High
1000 grains weight (g)	0.87	High
Dry milling grain (ton ha^{-1})	0.75	High
Methane emission (kg CH_4/ha/season)	0.91	High

Note: h^2 = heritability. Ae = aerenchyma. Heritability Criteria: high ($h^2 > 0.5$), moderate ($0.2 \leq h^2 \leq 0.5$), low ($h^2 < 0.2$).

environmental and genetic factors are play a role in determining the characters. The root length character and the number of root aerenchyma cavity had low heritability estimates (0.04 and 0.09) which means that environmental factors play higher role. Root length is strongly influenced by environmental conditions, texture, soil type, water, air, and soil management. The characters of root length and number of root aerenchyma are still hardly inherited to the subsequent generation when they are categorized in low criteria. It is assumedly due to environmental factors. Therefore, the character is not suitable as a marker character.

Based on Yamauchi *et al.,* (2013) study that the carrier gene of flooding-tolerant cause changes in the structure of the cortex into lysigenous aerenchyma cavities. The effect of aerenchyma formation is also greatly influenced by the condition of waterlogged plants. It was similar with Setyanto and Kartikawati study (2008), they founded that the number of aerenchyma formed in rice roots were higher in flooded conditions than those in dried condition.

The results of this study revealed three categories of heritability. Based on Fehr (1987) that the characters having a high heritability estimation are selected in the initial generations because the characters of a genotype are easily inherited to their offspring. On the other hand, if the heritability estimation is low, the selection is carried out on the subsequent generation because it is hardly inherited to the following generation.

Characteristic of Rice Plant with Low Methane Emissions

Morphological Characters

Based on the results of heritability estimation, the character of rice with low methane emission is genetically influenced and expressed on the character of plant height, number of tillers, plant biomass, the mean width cavity of root aerenchyma. The morphological characters are presented in Table 2.

The degrees of CH_4 gas emissions were varied due to the influence of morphological and physiological variations from rice plant varieties (Wihardjaka and Sarwoto, 2015). Methane emissions are a source of greenhouse gases produced

Table 2. Morphological Characters of Rice Plant

Varieties	Plant Height	Number of Tillers	Biomass	Width Cavity of Root Ae	Width Cavity of Stem Aeper clamp	CH_4 Emission
Ciherang	115.5^{bc}	14^{bcd}	210.90^{bc}	4.40 ^{ab}	3035.01^{ab}	221.76^{bc}
Mekongga	112.7^{abc}	14^{cd}	131.94^{a}	3.74 ^{ab}	4299.18^{ab}	193.36^{ab}
Inpari 18	106.6^{a}	12^{abc}	187.91^{b}	4.69 ^{ab}	1616.87^{a}	251.38^{d}
IPB3s	134.7^{d}	10^{a}	184.72^{b}	3.56 ^{ab}	4372.79^{b}	249.94^{cd}
-Inpari 13	115.3^{abc}	13^{abc}	176.03^{ab}	2.92 ^{a}	2075.28^{ab}	169.86^{a}
Inpari 31	119.1^{c}	15^{cd}	188.26^{b}	3.74 ^{ab}	2845.00^{ab}	274.44^{d}
Inpari 32	110.7^{abc}	14^{cd}	259.52^{c}	5.62 ^{b}	2322.17^{ab}	275.24^{d}
Inpari 33	107.9^{ab}	16^{d}	179.22^{b}	3.06 ^{ab}	2740.73^{ab}	223.58^{bc}
HSD 5%	8.70	2.89	9.47	2.69	2690.43	32.86

Note: The numbers followed by the same letters in the same column are not significantly different on HSD test at 5% level. Ae= Aerenchyma.

from the anaerobic process of methanogens, one of which derives from agriculture sector is rice plantation. Characteristics of rice varieties, based on analysis of variance, show different degree of methane emissions. The eight varieties tested, Inpari 18, IPB3s, Inpari 31, and Inpari 32 had high mean of methane emissions. Those four varieties had the following characters: wide cavity of root aerenchyma, high plant, and wide cavity of stem aerenchyma, a lot of number of tillers.

Those morphological characteristics, according to Aulakh *et al.* (2000), confirmed that the more number of tillers, the higher CH_4 transport capacity is because number of tillers can increase the density and number of aerenchyma vessels. However, when the rice plant was at 50 day after plat (DAP) to 98 DAP, emissions of rice varieties tend to wane. It is caused by a decrease in the number of tillers and the rate of photosynthesis which result in reducing the assimilation for methane formation. It was supported by Mulyadi and Wihardjaka (2014) study that the methane flux decreased after the flowering phase (55 DAP) because the rate of photosynthesis fell after the development of grain, and decreased the availability of assimilates for methane formation.

The results showed that Inpari 13 and Mekongga had low emissions compared to other varieties tested. Inpari 13 varietyhad lower value compared to other varieties tested on the following character parameters: plant height, number of tillers, the number of root aerenchyma cavity, it mean width cavity of root aerenchyma and width cavity of stem aerenchyma. Similarly, Mekongga had lower value on the following character parameters: plant height, plant biomass, mean width cavity of root aerenchyma and width cavity of stem aerenchyma. Based on those characters, the ability of rice to release methaneis low because of less biomass and narrow cavity of root aerenchyma. This specific marker character is supported by Susilawati *et al*., (2009) study that confirmed that the more biomass, the higher the CH_4 emissions were released, and Aulakh *et al.* (2000) study that found the more number of aerenchyma cavity, the higher CH_4 emissions were released.

Appearance of rice plants with high biomass can be presumed by knowing the rigidity level of the rice plant. The morphological plants with low biomass can be presumed by the rigidity level of stems and weak leaves. It is due to rice biomass is determined from the content of lignin, cellulose and hemicellulose (Yulianto *et al*., 2009). Lignin compound is a phenylpropanoid polymer which can harden cell walls. High lignin content will determine plant rigidity.

The results of this present study indicated that the yield component greatly influences the production result based on the character of each rice plant. Rice yield determines the emission index. Emission index is a comparison between the amount of grain produced and GHG released through rice plants. The higher the emission index, in turn,the lower emission that the variety has long with high grain yield (Mulyadi and Wihardjaka, 2014). Rice varieties affect the result of the emission index. Based on the estimation of CH_4 emission index, it showed that Inpari 13 had the lowest value (29,296 kg CH_4/ton grains) compared to the other varieties tested. It is not significantly different with Mekongga's emission index (29,854 kg CH_4 / ton grain), whereas, Ciherang, Inpari 32, and Inpari 33 had high CH_4 emission index (Table 3).

Anatomical Characters

Tissue of rice aerenchyma is a modified tissue of parenchyma that forms air cavities functioned as gas exchange in waterlogged conditions. The results of this study indicated that the number and the width of aerenchyma were varied (Figure 1).

Based on observation of tissue anatomy, the tissue anatomy of rice root aerenchyma affects the level of methane emissions. The tissue of rice aerenchyma is a modified tissue of parenchyma that forms air cavities functioned as gas exchange in waterlogged conditions. Inpari 32 variety had the highest width of aerenchyma, while Inpari 13 had the lowest width of aerenchyma. The higher number of aerenchyma cavities are, the more CH_4 emissions are released. It was supported by Purnobasuki and Suzuki (2001) that aerenchyma gas spaces are important for plants that grow in flooded and anaerobic sites or habitats, because these gas spaces provide an internal pathway for oxygen transport.

In the observation of aerenchyma cavity, Figure 2 shows the correlation between the width cavity of root aerenchyma and CH_4 flux with the variable y = 49.038x + 70.453. It means that the increase cavity of each 1 mm^2 contributes 119,941 mg CH_4 m^{-2} $days^{-1}$.

Inpari 13 and Mekongga had narrow cavity of root aerenchyma. The main influence of the aerenchyma cavity in emitting CH_4 gas is on the width

Table 3. The Mean of Rice Methane Emission and emission index

Varieties	Flux CH_4(kg CH_4/ ha/season)	Dry milling grain (t/ha)KA:14%	Emission index (kg CH_4/ton grain)
Ciherang	221.76^{bc}	5.98^{ab}	37.23^{abc}
Mekongga	193.36^{ab}	6.51^{ab}	29.85^{a}
Inpari 18	251.38^{d}	6.09^{ab}	41.26^{bc}
IPB3s	249.94^{cd}	5.69^{a}	44.07^{c}
Inpari 13	169.86^{a}	5.82^{a}	29.30^{a}
Inpari 31	274.44^{d}	7.12^{bc}	38.54^{bc}
Inpari 32	275.24^{d}	7.78^{c}	35.41^{ab}
Inpari 33	223.58^{bc}	5.86^{a}	38.43^{bc}
HSD 5%	32.86	1.18	8.51

Note: The numbers followed by the same letters in the same column arenot significantly different on HSD test at 5% level; tn: not significant different

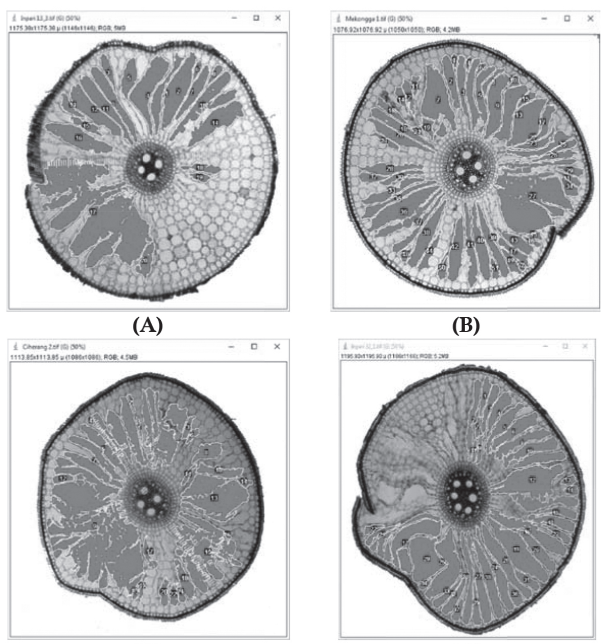

Fig. 1. Tissue Anatomy of Rice Root: (A) Inpari 13, (B) Mekongga, (C) Ciherang, (D). Inpari33 Ae: aerenchyma cavity, scale : 10 µm

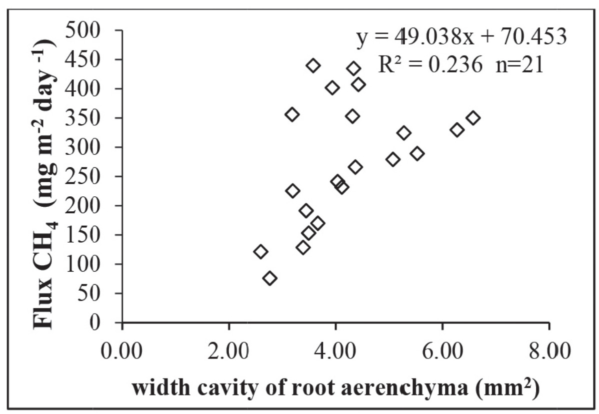

Fig. 2. Correlation Graph between Methane Emission and The width cavity of root aerenchyma.

cavity of the aerenchima. Therefore, the width of root aerenchyma cavity is directly proportional to the ability of rice to produce a very high CH_4 flux (Setyanto, 2004)

Conclusion

Rice plants emitting CH_4 gas are assumedly influenced by genetic factors with a heritability of 0.91. Rice with low methane emissions has the following marker characters: little biomass, narrow cavity of root aerenchyma, and weak morphological rigidity of stems and leaves. Inpari 13 and Mekongga varieties emit low CH_4 gas.

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