

Journal homepage: http://jurnal.uns.ac.id/tanah



Methane flux from high-yielding Inpari rice varieties in Central Java, Indonesia

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ABSTRACT
Rice cultivation is a source of greenhouse gas emissions, particularly methane (CH ₄). One
of the factors that affect CH ₄ emissions from rice fields is rice cultivar. In this study, a field
experiment was conducted to determine CH4 emissions from various high-yielding inbred
varieties and the relationship between CH4 emissions and rice growth parameters. The
field experiment was conducted in Jaken, Pati Regency, Central Java province, during the wet season of 2014/2015. The experiment was arranged using a randomized block design
with three replications and several cultivar treatments (Inpari 13, 18, 19, 20, 23, 24, 29, 30,
31, 32, and 33, with Ciherang as the comparison cultivar). The data collected includes CH ₄
flux, plant height, tiller number, biomass, grain yield, and root aerenchyma area. The CH ₄ flux was measured at several critical growth stages. The Inpari 24, Inpari 13, and Inpari 19
demonstrated CH ₄ emissions reduced by as much as 36.1%, 32.8%, and 21.3%, respectively, compared to Ciherang. The Inpari 13 and Inpari 24 varieties had significantly lower emission indices than Ciherang and the other Inpari varieties, with 17 and 20 g CH ₄ per 1 kg grain yield, respectively. CH ₄ flux was found to correlate significantly with tiller number per hill, total biomass, and root aerenchyma area at the panicle initiation growth stage.

How to Cite: Wihardjaka, A., Yulianingsih, N., and Yulianingrum, H. (2020). Methane flux from high-yielding Inpari rice varieties in Central Java, Indonesia. Sains Tanah Journal of Soil Science and Agroclimatology, 17(2): 128-134 (doi: 10.20961/stjssa.v17i2.42729)

1. Introduction

High-yielding rice varieties are among the innovations to increase food crop productivity in order to satisfy food demands that increase along with increases in the human population. The superior rice varieties have been intensively introduced since the green revolution, and the number of high-yielding varieties has experienced a significant increase recently. Between 2010 and 2016, 67 new rice varieties were released in Indonesia, consisting of 37 irrigated inbred varieties (known as Inpari), 12 hybrid varieties, 10 upland inbred varieties (known as Inpago), and 7 swampy inbred rice varieties (Inpara) (Jamil et al., 2015).

Agriculture contributes 52% of global anthropogenic methane (CH₄) and 84% of nitrous oxide (N₂O) emissions (Zhang et al., 2019). Rice, an anthropogenic CH₄ emission source, contributes 12% of total anthropogenic methane (Brye et al., 2017; Qin et al., 2015; Zheng et al., 2014). Estimates of CH₄ emissions from rice fields range from 39 to 112 Tg CH₄ per year, or 5%–19% of global CH₄ emissions (Ma, Wang, Zhou, Yan, & Xiong, 2012), along with the increase in rice requirements and the rate of population growth. As different rice varieties emit different levels of greenhouse

gases, the management of variety selection can regulate the release of these gases, especially CH_4 , from rice fields (Qin et al., 2015). CH_4 flux is affected by interactions of genotype, microorganisms, water, and soil conditions (Balakrishnan, Kulkarni, Latha, & Subrahmanyam, 2018). The reduction of CH_4 emission from rice fields can be achieved through improved cultivation practices, adoption of precision input management, and selection of high-yield, low-emission rice cultivars (Sapkota et al., 2018; Zhang et al., 2019).

The rates of production and release of CH₄ by rice varieties vary depending on physiological characteristics, plant morphology, availability of organic matter in the soil, physicochemical properties of the soil, and microbial activity of methanogens and methanotrophs in the rice rhizosphere (Smartt et al., 2016; Yun, Choi, Choi, & Kim, 2013). Unlike inbred strains and conventional varieties, types of rice varieties such as hybrid rice influence the diversity of CH₄ production and emissions from the rhizosphere of rice (Balakrishnan et al., 2018). Rice varieties release CH₄ into the atmosphere mostly through their plants (namely, aerenchyma tissue), with a small portion released through diffusion and ebullition (Wang et al., 2013; Zheng et al., 2014).

According to Balakrishnan et al. (2018), as much as 60%–90% of CH₄ emissions occur through transport in plant tissues. During the active tillering stage of rice, methane transport occurs via a diffusion mechanism (Xu et al., 2015).

Most farmers still use old superior varieties even though these are sensitive to pests and disease. In the past decade, breeders have produced new superior varieties of inbred rice with high yield potential that are relatively more tolerant to pests: Inpari. Information on greenhouse gas emission capacity is not yet available for most types of Inpari. Some rice varieties cultivated in rainfed rice fields release lower levels of CH₄ than the rice variety IR64. These varieties are Way Apoburu, Dodokan, Silugonggo, Cisantana, Sintanur aromatic, and a local variety of Mentik (Wihardjaka, 2015), with the lowest CH₄ flux produced by the Way Apoburu variety.

The adaptation of Inpari varieties by farming communities is low even though the Inpari variety is relatively more tolerant of environmental stress and plant pests. In addition, information about the ability of the Inpari superior varieties to emit greenhouse gases, especially CH₄, remains relatively limited. Currently, farmers still cultivate rice varieties released before 2010, with 30.31% using Ciherang (released in 2000), 12% using IR64 (1989), 11% using Mekongga (2004), 6.5% using Situ Bagendit (2004), and 4.4% using Cigeulis (2003) (Jamil et al., 2015). This study aims to determine CH₄ flux from several new types of Inpari superior varieties and the relationships between CH₄ emissions and plant growth parameters in rice fields.

2. Materials and Method

The field experiment was conducted in Jaken, Pati Regency, Central Java province, during the wet season of 2014/2015. The experimental site was located on 111°11′76.7″ E and 06°46″45.9′ S at an altitude of 12 m above sea level. The soil type in the experiment site was Inceptisols, which has loamy textural class at 0–20 cm depth (49% sand, 46% silt, 5% clay), with acid reaction (pH H₂O 5.0, pH KCl 3.9), low C content (0.43%), low N-total content (0.05%), available P of 50.7 ppm, and low cation exchangeable capacity (5.74 cmol kg⁻¹), with exchangeable cations of Ca, Mg, K, and Na as high as 3.03, 0.45, 0.04, and 0.06 cmol kg⁻¹, respectively.

Several new rice varieties of Inpari released from 2010 to 2013 were arranged using randomized block designs with three replications. The varieties reported in this study are Inpari 13, Inpari 18, Inpari 19, Inpari 20, Inpari 23, Inpari 24,

Inpari 29, Inpari 30, Inpari 31, Inpari 32, Inpari 33, and Inpari 33, with Ciherang as the control. The selection origin and year released for each variety are shown in Table 1. The 15 daysold rice seedlings per hole were transplanted in each plot of 2 m X 3 m with spacing of 20 cm X 20 cm. Before transplanting, the paddy soil was tilled perfectly. In each plot, fertilizers were applied: 120 kg N, 18 kg P, 50 kg K, and 2000 kg compost per hectare. Compost was applied together with soil tillage. The N fertilizer was applied in three stages: 1/4 before transplanting, 1/2 at the active tillering phase, and 1/4 at the panicle initiation phase. The P fertilizer was applied once before transplanting, while the K fertilizer was applied twice ($\frac{1}{2}$ before transplanting and $\frac{1}{2}$ at the panicle initiation phase). Intensive plant maintenance was carried out, including control of plant pest organisms based on conditions in the field. During plant growth, water was maintained so that the plants remained submerged. Data collected included plant growth (i.e., plant height, tiller number); grain yield in the harvest area; aerenchyma diameter of the root; CH₄ flux; and emission index. Plant height and tiller number were measured from 12 hills per plot. The aerenchyma area of the root was observed at the panicle initiation phase using root paraffin slices prepared from each variety, with safranin and fastgreen staining performed in the Science Laboratory of Sebelas Maret University at Surakarta, Central Java.

A gas sample was taken using the closed chamber method at the growth phase of active tillering (35 days after transplanting [DAT]), maximum tillering (50 DAT), panicle initiation (60 DAT), flowering (75 DAT), maturity (90 DAT), and before harvest. The plexiglass chamber (40 cm width x 40 cm length x 100 cm height) was laid above the cross-section during the collection of gas samples. The gas sample was taken using a 10 mL syringe at the time points of 0, 5, 10, 15, 20, and 25 minutes for each plot. The gas samples were analyzed using a gas chromatography-equipped flame ionization detector (FID) to determine CH₄ concentration. The CH₄ flux was computed using the following formula [1] from Qin et al. (2015).

Remarks: $F = CH_4$ flux (mg CH₄m⁻² day⁻¹), V = chamber volume (m³), A = surface area of chamber (m²), dC/dt = change of CH₄ concentration per time unit (ppm minute⁻¹), T = average temperature in chamber (°C).

Variety	Selection origin	Plant age (days)	Released year
Ciherang	IR18349-53-1-3-1-3/IR19661-131-3-1-3//IR64	116-125	2000
Inpari 13	OM606/IR18348-36-3-3	99	2010
Inpari 18	BP364B-33-3-PN-5-1/Bio530B-45-9-3-1	102	2011
Inpari 19	BP342B-MR-1-3/BP226E-MR-76	104	2011
Inpari 20	S2823E-KN-33/IR64//S2823E/KN/33	102	2011
Inpari 23	Bii738 ^{RS} (Gilirang/BP342F-MR-1-3//Gilirang)	113	2012
Inpari 24	Bio 12-MR-1-4-PN-6/Beras Merah	111	2012
Inpari 29	IR69502-6-SKN-UBN-1-B-1-3/KAL-9418F//Pokhali/Angke	110	2012
Inpari 30	Ciherang/IR64sub1/Ciherang	111	2012
Inpari 31	Pepe/BP342B-MR-1-3-KN-1-2-3-6-MR-3-BT-1	112	2013
Inpari 32	Ciherang/IRBB64	120	2013
Inpari 33	BP360E-MR-79-PN-2/IR71218-38-4-3//BP360E-MR-79-PN-2	107	2013

Table 1. Information on the varieties of Inpari used in this study

Source: Jamil et al. (2016)

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Data were statistically analyzed using analysis of variance followed by the Duncan multiple range test with the significance level set at 5% to identify statistically significant differences among rice varieties. The relationships between CH₄ flux and agronomic parameters of the rice crop were tested with simple regression and correlation.

3. Results

3.1 Methane Flux from Inpari Varieties

The different characteristics of various rice varieties play an important role in regulating CH₄ emissions in rice fields. Figure 1 shows the fluctuations of CH₄ flux from several Inpari varieties compared to the Ciherang variety. The CH₄ flux at vegetative growth is higher than at the reproductive phase. The CH_4 flux at 35, 50, 60, 75, and 90 DAT ranged from 101 to 188, 105 to 275, 77 to 190, 76 to 277, and 23 to 119 mg CH₄ $m^{\text{-}2}\,day^{\text{-}1}$ for the vegetative growth and reproductive phases, respectively. The average CH₄ flux from Ciherang and Inpari was 143.6 and 154.1 (35 DAT), 165.3 and 191.4 (50 DAT), 156.0 and 131.0 (60 DAT), 153.7 and 182.0 (75 DAT), and 54.9 and 67.6 mg m⁻² day⁻¹ (90 DAT), respectively. Thus, the lowest CH₄ flux occurs at 60 and 90 DAT.

3.2. Grain Yield of Inpari Varieties

Table 2 shows that the only Inpari variety that yielded significantly more grain than Ciherang was Inpari 32 (p = 0.0005). The Inpari 19, Inpari 24, Inpari 29, and Inpari 30 varieties yielded significantly less grain than Ciherang. The Ciherang, Inpari 18, Inpari 31, and Inpari 32 varieties have the highest tiller numbers, with more than 20 tillers per hill (Table 3). Those with low grain yields have low numbers of effective tillers; for example, Inpari 24 and Inpari 29 had lower maximum and productive tiller numbers than Ciherang (Table 3). Plant growth between varieties is significantly different despite the varieties being planted with the same transplanting system; p-values for differences in plant height, maximum tillers, and productive tillers were below 0.0001 (Table 3). The number of productive tillers, which produce better grains, were increased in the Inpari 13 and Inpari 24 varieties by 91% and 96%, respectively. The percentage of effective tillers from other Inpari varieties averaged between 50% and 86.5%.



Figure. 1. CH₄ flux from high-yielding rice varieties of Inpari in different growth phases (35, 50, 60, 75, and 90 days after transplanting)

Variaty	Grain yield in 14% moisture	CH₄ flux	Emission index
variety	content (kg ha ⁻¹)	(kg CH₄ ha⁻¹ season⁻¹)	(kg CH₄ ha⁻¹ grains)
Ciherang	4908 bc	122 d	0.025 cdef
Inpari 13	4745 bcd	82 f	0.017 f
Inpari 18	4965 ab	164 ab	0.033 abc
Inpari 19	3991 de	96 ef	0.024 def
Inpari 20	4199 bcde	114 de	0.027 bcde
Inpari 23	4188 bcde	155 ab	0.037 a
Inpari 24	4003 de	78 f	0.020 ef
Inpari 29	4076 cde	122 d	0.030 abcd
Inpari 30	3602 e	130 cd	0.038 a
Inpari 31	4990 ab	178 a	0.036 ab
Inpari 32	5745 a	171 ab	0.030 abcd
Inpari 33	4670 bcd	150 bc	0.032 abcd
CV (%)	10.06	10.13	15.32

Table 2. Grain yield,	CH ₄ flux, and emission index fro	m high-yielding varieties fror	n Inpari, Central Java, Indonesia

mean in the same column followed by the same letter were not significantly different at 0.05 according to Duncan Remark: multiple range test at 0.05

Variety	Tiller number per hill		
	Plant height (cm)	Maximum	Productive
Ciherang	106.3 bc	20.1 ab	10.6 de
Inpari 13	112.8 ab	7.6 f	6.9 f
Inpari 18	105.8 bc	20.9 a	14.0 abc
Inpari 19	104.6 c	14.9 d	9.2 e
Inpari 20	82.2 e	17.9 bc	12.9 bc
Inpari 23	106.1 bc	15.3 cd	9.3 e
Inpari 24	94.1 d	11.1 e	10.6 de
Inpari 29	111.1 abc	15.9 cd	9.9 e
Inpari 30	97.1 d	14.1 d	12.2 cd
Inpari 31	115.6 a	21.1 a	15.8 a
Inpari 32	107.9 bc	21.0 a	14.9 ab
Inpari 33	107.3 bc	16.9 cd	9.3 de
CV (%)	3.97	9.91	10.01

Remark: means in the same column followed by the same letter were not significantly different at 0.05 according to Duncan multiple range test



Figure 2. Relationship between tiller number and CH₄ flux from Inpari rice varieties (** significant at 0.01 level)



Figure 3. Relationship between either plant biomass or root aerenchyma and CH₄ flux from Inpari rice varieties (** significant at 0.01 level).

4. Discussion

According to Kartikawati et al. (2017), Ciherang, the comparison cultivar used in this study, is a variety that emits relatively low levels of CH₄. We found that the Inpari 13 and Inpari 24 varieties released less CH₄ than the other varieties studied in all critical growth phases, including Ciherang. Moreover, the highest CH₄ flux from the Inpari varieties occurs at 50 and 75 DAT. Tang et al. (2015) reported that the peak of CH₄ flux occurred at the booting stage, followed by the tillering stage. According to Qin et al. (2015), the CH₄ flux rate was lower at the beginning of the vegetative phase and

increased significantly at maximum tillering until the panicle initiation growth phase that was mainly supported by anaerobic soil conditions.

The ability of rice varieties to release CH₄ in certain growth phases is influenced by the diversity of available organic substrates in the rhizosphere of rice crops. The root exudates are a source of available organic substrates for methanogenic bacteria to produce CH₄. According to Naher et al. (2009), the components of root exudates vary among the genotypes of rice plants. Rice crops produce root exudates from the maximum tillering stage to panicle initiation (Zhang et al., 2019). The main

components of root exudate are sugar, organic acids, and amino acids that are used as sources of substrates and carbon for microbial activity in the rhizosphere, including CH_{4} -producing bacteria (Naher et al., 2009; Zhang et al., 2019). The decrease in CH_4 flux at the maturity phase of rice crops tends to be caused by blocking the root aerenchyma from CH_4 diffusion (Wang et al., 2017).

In varieties with high numbers of effective tillers, the number of tillers that did not produce panicles and instead decomposed into the soil was decreased. The dead tillers that have decomposed into the soil are a source of substrate for methanogenic bacteria that produce CH₄. In addition, the rice plants are important conduits of CH₄ from soil to the atmosphere, sometimes accounting for more than 90% of the total CH₄ emissions (Sapkota et al., 2018). According to Yu et al. (2013), the organic matter used as a substrate source for methanogenic activity comes from the soil organic matter, organic carbon released by roots (including root exudates), dead roots, and fresh organic supply such as straw and dead tillers. The various inbred varieties of Inpari were significantly different in their levels of CH₄ released into the atmosphere (p < 0.0001) and in their emission indices (p < 0.0001). The Inpari varieties that had the lowest CH₄ emissions compared to Ciherang were Inpari 24, Inpari 13, and Inpari 19 (Table 2). The CH₄ emissions from Inpari 24, Inpari 13, Inpari 19, and Ciherang were 78, 82, 96, and 122 kg CH₄ ha⁻¹ season⁻¹, respectively. This means that the Inpari 24, Inpari 13, and Inpari 19 varieties have the ability to reduce CH₄ emissions by as much as 36.1%, 32.8%, and 21.3%, respectively, compared to Ciherang. The varieties of Inpari with significantly higher CH₄ emissions than Ciherang were Inpari 33, Inpari 23, Inpari 18, Inpari 32, and Inpari 31, which had emission values of 150, 155, 164, 171, and 178 kg CH_4 ha⁻¹ season⁻¹, respectively. The CH₄ emissions from Inpari 20, Inpari 29, and Inpari 30 did not differ significantly from those of Ciherang. The emission index can be used to illustrate the magnitude of greenhouse gas released from rice crops while producing grains. The index of emission efficiency for Ciherang was 25 g CH₄ per 1 kg of grains produced. As seen in Table 2, some varieties of Inpari were significantly more efficient than Ciherang, namely Inpari 13, Inpari 24, and Inpari 19, which had emission indices of 17, 20, and 24 g CH₄ per 1 kg grains produced, respectively. The Inpari varieties that were less efficient than Ciherang were Inpari 30, Inpari 23, and Inpari 31, with emission indices of 38, 37, and 36 g CH₄ per 1 kg grains produced, respectively.

Some rice varieties produce high grain yield without emitting high levels of CH_4 . The emission index is considered when developing high-yielding varieties with low emission to support food security efforts; thus, these varieties are candidates for further development through improving genetic characteristics.

The amount of CH₄ different rice varieties release into the atmosphere depends on their plants' morphological properties, as well as other factors such as water regimes, organic matter availability, physicochemical properties of the soil, and rice cultivation practices. Plant growth parameters such as the number of tillers, plant biomass, and area of root aerenchyma are used to determine the relationship between plant morphology and methane flux. Figure 2 shows that tiller

number per hill of rice crop correlated positively and significantly with CH_4 flux in both the maximum tillering stage and the panicle initiation growth stage, which are described by the curves Y = -0.83X2 + 33.39X - 118.5 (R² = 0.4065; n = 36) and Y = 0.10X2 + 4.20X + 35.33 (R² = 0.6757; n = 36), respectively (X = tiller number per hill, Y = CH_4 flux). At the maximum tillering growth stage, the highest CH_4 flux occurs on the average tiller number of 19–21 tillers.

The CH₄ flux from rice varieties is also determined by the total biomass and area of root aerenchyma. The transport of CH4 and oxygen through the aerenchyma plays a role in supplying substrate to methanogenic bacteria and methanotrophs through root exudates (Kim, Bui, Chun, McClung, & Barnaby, 2018). Figure 3 shows that CH₄ flux correlated significantly with the plant biomass at the panicle initiation growth stage, which is described by the equation Y = 0.015X2 - 0.126X + 74.209 (R² = 0.5667; n = 36; Y = CH₄ flux and X = plant biomass). Root biomass plays a role in regulating CH₄ flux, especially the root's ability to oxidate CH₄, the abundance of CH₄-producing methanogens, and the availability of root exudates. Rice varieties have the ability to produce exudate components (Naher et al., 2009), and the relationship between the CH₄ flux and biomass varies among varieties, as they differentially allocate photosynthetic products to root exudation rather than to other parts (Qin et al., 2015). Up to 40% of net carbon retained during the photosynthetic process is released into the rhizosphere of the rice plant via, for example, root exudation (Dundek et al., 2014).

Figure 3 also shows that the area of root aerenchyma was correlated with CH₄ flux at the panicle initiation growth stage, which is showed described by the curve Y = -5.49X2 + 91.36X – 165.63 (R² = 0.7023; n = 12), where Y = CH₄ flux and X = area of root aerenchyma. According to Figure 3, the highest CH₄ flux was observed in the varieties with root aerenchyma are in between 8 and 9 mm², with root aerenchyma areas of <8 mm² and >9 mm² tending to decrease CH₄ flux. The research findings by Kartikawati et al. (2017) and Wihardjaka (2015) also showed that CH₄ flux correlated significantly with characteristics of rice growth, such as tiller number and plant biomass. Furthermore, characteristics of plant physiology such as the production of organic substrates and the potency of oxygen translocation are additional factors that accelerate the activity of methanogenic bacteria (Qin et al., 2015)

5. Conclusion

Among the rice varieties studied, the CH₄ flux correlates significantly with tiller number per hill, the weight of plant biomass, and the area of root aerenchyma. The Inpari 13 and Inpari 24 varieties release less CH₄ than Ciherang and the other Inpari varieties. Both of these varieties are more efficient in releasing CH₄ per 1 kg grain produced, with their efficiency indices of 17 and 20 g CH₄ kg⁻¹ grain yield translating to efficiency improvements of 32% and 20%, respectively. The lower CH₄ emissions from Inpari 13 and Inpari 24 are determined by their tillers, which were highly effective in yielding grains. The Inpari 13 and Inpari 24 varieties may thus replace the Ciherang variety, which is still used by most farmers in rice fields.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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