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Studies on Morpho-physiological Fingerprints of Rice Cultivars in Rice Crop in Rice-Rice-Rice, Maize-Maize-Rice and Vegetable-Vegetable-Rice Cropping Systems

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ABSTRACT

Background: Continuous rice cultivation has a negative effect on sustainable rice production. Crop rotation can increase the yield and soil quality. This study aimed to provide information on the morpho-physiological fingerprints of rice cultivars in different crop rotation systems.

Methods: Twelve rice cultivars were evaluated in terms of their morpho-physiological in three crop rotation systems, *i.e.*, rice-rice-rice, maize-maize-rice (M-M-R) and vegetable-vegetable-rice, at the Agrotechnology Innovation Center, Universitas Gadjah Mada at Berbah District, Sleman Regency, Special Province of Yogyakarta, Indonesia, from December 2020 to June 2021.

Result: The results revealed an interaction between rice cultivars and the crop rotation system, with the effects observed on the nitrogen, phosphorus and potassium contents in the leaf tissue (NC, PC and KC, respectively), crop growth rate, total dry weight per clump, empty grain per clump (EG) and grain weight per clump (GWC). The increase in NC, PC and KC positively affected the increase in GWC. The GM 8 cultivar in the M-M-R crop rotation system showed lowest EG of 3% and highest GWC of 133.90 g clump^{-1}.

Key words: Crop rotation, Fingerprints, Morpo-physiological, Rice cultivar.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. Rice is a staple food and source of income for farmers in Indonesia (FAO, 2019). The volume of rice imports increases significantly every year. In 2021, rice production in Indonesia decreased by 233.91 thousand tons or (0.43%) compared with 2020 (Statistics Indonesia, 2022). This finding was caused by many factors, such as the paddy field conversion of 245.47 thousand hectares in 2021, global climate change, increased pest and disease attack and decreased soil fertility (Mulyani *et al.,* 2011; Statistics Indonesia, 2022; Suryanto *et al.,* 2020). These problems can be overcome using superior rice cultivars and crop rotation arrangements (Alam *et al.,* 2021).

Rice cultivars are one of the essential factors in increasing rice production. Rice cultivars are effective, cheap and quickly adopted by farmers. The role of plant breeders has become vital in creating cultivars that can attain high and stable productivity in all environmental conditions (Alam *et al.,* 2019; Piepho *et al.,* 2016). Universitas Gadjah Mada is currently conducting a multi-location test on ten promising rice lines in 15 locations throughout Indonesia. This test is one of the stages in the release of varieties (Agrotechnology Innovation Center, 2022).

The preliminary study in Sleman, Klaten and Banyumas Regencies, Indonesia showed that GM 2, GM 8 and GM 28 cultivars had high productivities by 6.17, 6.98 and 7.18 tons ha^{-1}, respectively (Aristya *et al.,* 2021). Crop rotation arrangement is crucial for sustainable agriculture. Rotation of different crops can be used as a strategy to overcome the negative impacts of intensive monoculture farming (*Grzebisz et al.,* 2018). Continuous rice planting can cause a decrease in rice yield and soil quality (Meena *et al.,* 2019). Deep *et al.* (2018) informed that rice-maize crop rotation produced highest rice yield of 10.20 tons ha^{-1} compared with rice-rice, rice-wheat, rice-pulse and rice-oilseed crop rotation with yields of 7.80, 8.40, 8.50 and 7.40 tons ha^{-1}, respectively.

This study aimed to provide information on the morpho-physiological fingerprints of rice cultivars in different crop rotation systems. This study provides new information related to the effect of the crop rotation system on new rice cultivars.

MATERIALS AND METHODS

The study was conducted at the Agrotechnology Innovation Center, Universitas Gadjah Mada, Kalitirto Sub-District, Berbah District, Sleman Regency, Special Province of

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Yogyakarta, Indonesia, from December 2020 to June 2021. The total rainfall observed during the experiment was ±727 mm. The mean air temperature and relative humidity were 26.28°C and 66.26%, respectively.

The three crop rotation systems were prepared using a randomized complete block design with three blocks as replications. Twelve rice cultivars consisted of ten promising rice lines sourced from Universitas Gadjah Mada, Indonesia and two rice varieties sourced from the Indonesian Center for Rice Research (ICRR), West Java, Indonesia. The ten promising rice lines consisted of V11, GM 28, GM 2, GM 8, Mutant Lampung Kuning, Mutant Rojolele 30 Pendek, Mutant Rojolele 30 Tinggi, Mutant V12T, Mutant Mayangsari and Mutant Lakatesan and the two rice varieties for the control consisted of Inpari 33 and Inpari 30 Ciherang Sub 1. The three crop rotation systems consisted of rice-rice-rice (R-R-R), maize-maize-rice (M-M-R) and vegetable-vegetable-rice (V-V-R) crop rotation.

Rice seeds were sown in a screen house and then transplanted to a paddy field after 21 days. Soil tillage was carried out using a hand tractor before the rice was transplanted. Rice planting distance was 25 cm × 25 cm and one seed was planted per planting hole. Fertilization and other cultivation techniques were applied as recommended by the ICRR (Alam *et al.,* 2021; Faridah *et al.,* 2021).

The observations consisted of morphological and physiological characteristics. The physiological characteristics consisted of chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), nitrate reductase activity (NRA), nitrogen content in the leaf tissue (NC), phosphorus content in the leaf tissue (PC) and potassium content in the leaf tissue (KC). The growth analysis consisted of leaf area index (LAI), net assimilation rate (NAR), crop growth rate (CGR). The physiological characteristics and growth analysis were observed in maximum vegetative phase. The morphological characteristics consisted of total dry weight per clump (TDW), empty grain per clump (EG) and grain weight per clump (GWC) (Gross, 1991; Horwitz and Latimer Jr, 2006; Hunt, 1990; Jones, 1984; Krywult and Bielec, 2013). The morphological characteristics were analysed during harvesting. The observations were made at the General Soil Laboratory and the Crop Production and Management Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia.

The data must have normal distribution and homogeneous variance prior to analysis of variance (ANOVA). The data were analyzed by ANOVA ($p<0.05$), followed by HSD-Tukey test ($p<0.05$) as a post hoc test (Welham *et al.,* 2015). The relationship between parameters and the similarity of characteristics between rice cultivars were analyzed by correlation and cluster analyses, respectively (Widyawan *et al.,* 2020). The ANOVA was performed using SAS software version 9.4 for Windows with PROC MIXED (SAS Institute Inc, 2013). Correlation and cluster analyses were performed using Rstudio software with the crorrplot, GGally and Pheatmap packages (Raivo, 2019; R Core Team, 2017).

RESULTS AND DISCUSSION

Physiological fingerprints

The results of ANOVA for the NC, PC and KC showed an interaction between rice cultivars and the crop rotation systems. The GM 8 cultivar in the M-M-R crop rotation system manifested the highest NC value of 1.93%, but this result was not significantly different from that of the GM 8 cultivar in the V-V-R crop rotation system (1.75%). Inpari 33 cultivar in the R-R-R crop rotation system revealed the lowest NC of 1.16% (Fig 1a).

The GM 8 cultivar in the M-M-R crop rotation system exhibited the highest PC value of 0.26%, but it was not significantly different from those of the Mutant Lampung Kuning and Mutant Rojolele 30 Tinggi cultivars in the M-M-R crop rotation system and GM 8 in the V-V-R crop rotation system, with values of 0.22%, 0.22% and 0.23%, respectively. The lowest PC (0.09%) was observed in Inpari 30 Ciherang Sub 1 in the V-V-R crop rotation system (Fig 1b). The GM 8 cultivar in the M-M-R crop rotation system showed the highest KC at 0.72%. By comparison, the lowest KC was 0.19%, which was observed in Inpari 30 Ciherang Sub 1 in the V-V-R crop rotation system (Fig 1c).

The difference in NC, PC and KC values is due to variations in genetic factors for each rice cultivar and crop rotation system. The continuous R-R-R had low NC, PC and KC values. Thus, the GM 8 cultivar can potentially become a cultivar with more optimal nitrogen/phosphorus/potassium absorption capability than other cultivars. Lu *et al.* (2018) informed that the short-term (4 years) and long-term (30 years) nitrogen content of the tissue in the rice-rice-fallow crop rotation system is lower than that in the rice-rice-rapeseed crop rotation system.

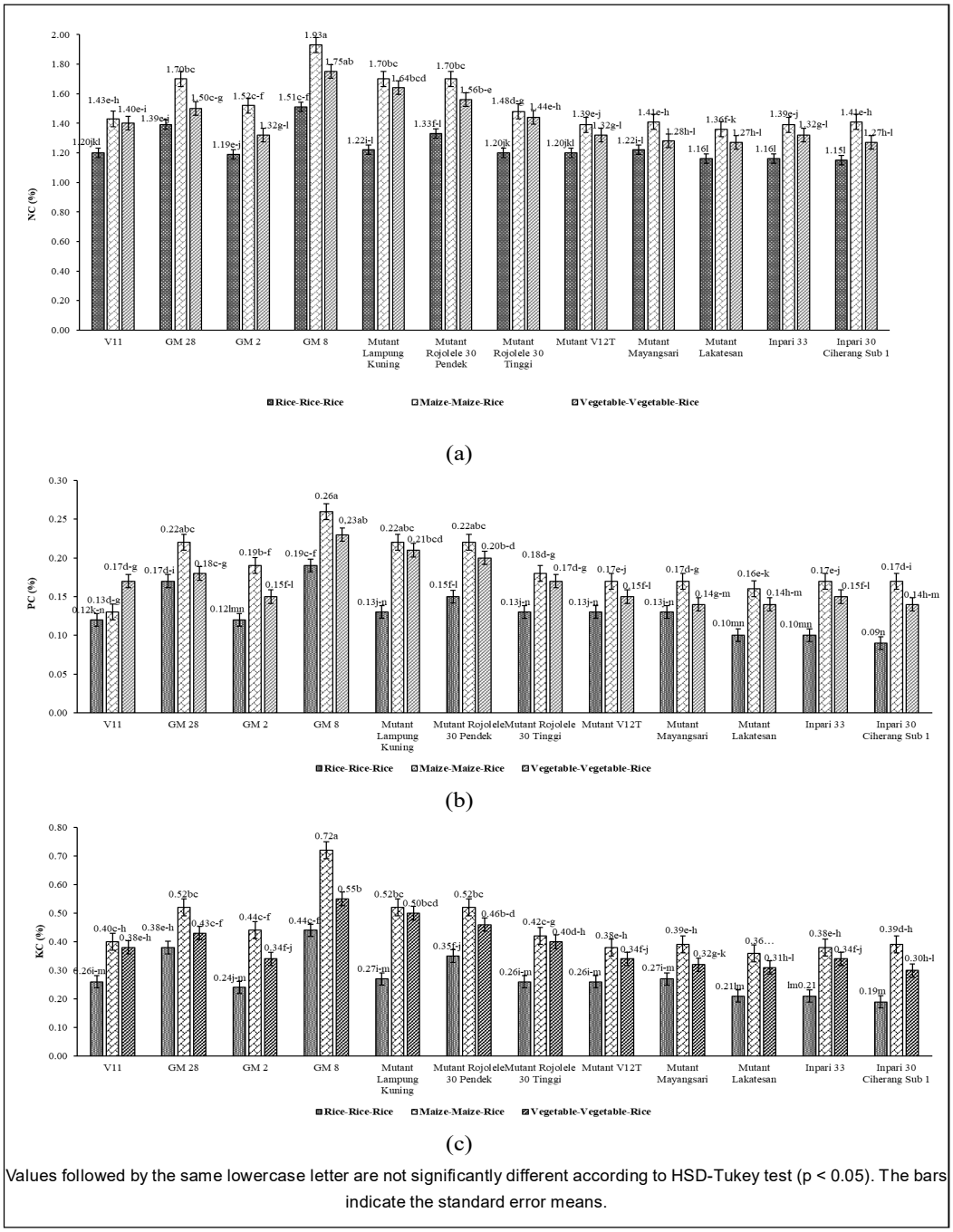
The ANOVA results showed no interaction between rice cultivars and the crop rotation system in terms of CA, CB, TC and NRA (Table 1). The rice cultivars and crop rotation systems did not show significant differences in terms of CA, CB, TC and NRA. The CA values in rice cultivars ranged from 0.37 g g leaf^{-1} to 0.42 g g leaf^{-1} and those of CB ranged from 0.33 g g leaf^{-1} to 0.46 g g leaf^{-1}. The TC and NRA values in rice cultivars were between 0.62-0.88 g g leaf^{-1} and 2.23-3.39 µmol NO_2^- g^{-1} h^{-1}, respectively. The CA and CB values in the crop rotation system were in the range of 0.38-0.43 and 0.39-0.40 g g leaf^{-1}, respectively. The TC and NRA values were in the range of 0.74-0.79 g g leaf^{-1} and 2.61-3.25 NO_2^- g^{-1} h^{-1}, respectively.

Morphological fingerprint

The ANOVA result on LAI and NAR showed no interaction between rice cultivars and crop rotation systems (Table 2). The Mutant Lakatesan cultivar had the highest LAI value of 0.89, which was significantly different from those of cultivars V11, GM 28, GM 2 and GM 8 (0.49, 0.49, 0.50 and 0.53, respectively). The M-M-R crop rotation system was not significantly different from that of V-V-R. However, both were significantly different from the R-R-R crop rotation system. The LAI values of M-M-R and V-V-R were 0.64 and 0.75,

respectively. The NAR values for rice cultivars and crop rotation systems did not differ significantly. The NAR values for rice cultivar ranged from 0.37 g cm^{-2} $week^{-1}$ to 0.56 g cm^{-2} $week^{-1}$ and it was between 0.38-0.42 g cm^{-2} $week^{-1}$ in the crop rotation systems.

The ANOVA result on the CGR, TDW, EG and GWC showed an interaction between the rice cultivars and crop rotation systems (Fig 2). The GM 8 cultivar in the M-M-R crop rotation system showed the highest value of 61.73 g g^{-1} $week^{-1}$ and the lowest was that of the Inpari 30 Ciherang

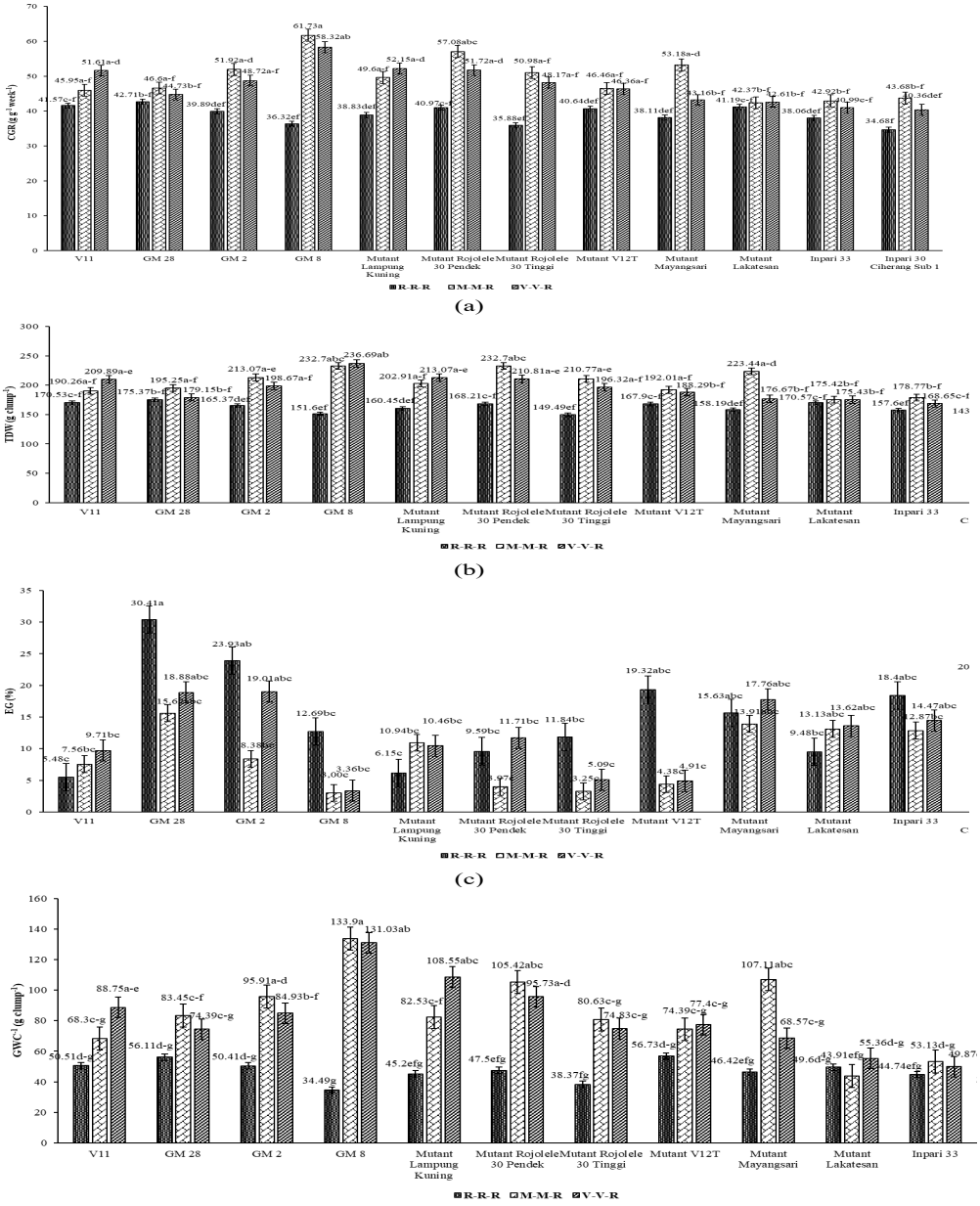


Values followed by the same lowercase letter are not significantly different according to HSD-Tukey test ($p < 0.05$). The bars indicate the standard error means.

Fig 1: a). NC- Nitrogen content in the leaf tissue; b). PC- Phosphorus content in the leaf tissue; c). KC- Potassium content in the leaf tissue.

Sub 1 cultivar in the R-R-R crop rotation system (34.68 g g^{-1} $week^{-1}$) (Fig 2a). V11, GM 28, GM 2, GM 8, Mutant Lampung Kuning, Mutant Rojolele 30 Pendek and Mutant Rojolele 30 Tinggi cultivars in the M-M-R and V-V-R crop rotation systems generally showed the highest TDW (Fig 2b).

The GM 28 cultivar in R-R-R crop rotation system showed the highest EG of 30.41% and the lowest was observed on the GM 8 cultivar in the M-M-R crop rotation system (3%) (Fig 2c). The GM 8 cultivar in the M-M-R crop rotation system had the highest GWC of 133.90 g $clump^{-1}$



Values followed by the same lowercase letter are not significantly different according to HSD-Tukey test ($p<0.05$). The bars indicate the standard error means.

Fig 2: a). CGR- Crop growth rate; b). TDW- Total dry weight per clump; c). EG- Empty grain per clump; d). GWC- Grain weight per clump.

Table 1: Chlorophyll content and nitrate reductase activity in different rice cultivars and crop rotation systems.

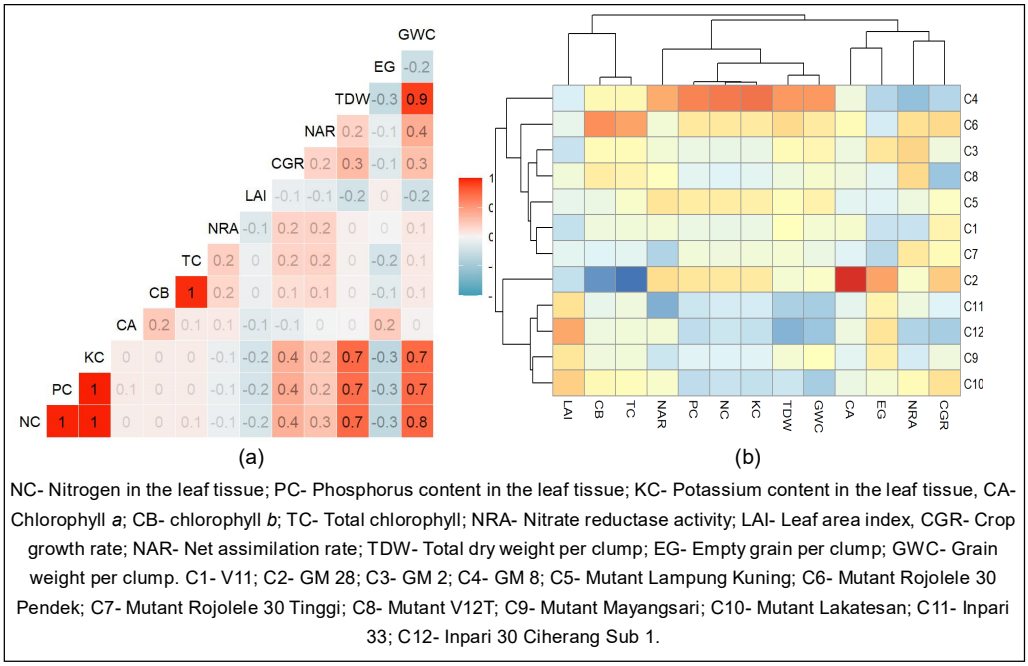
Treatments	Chlorophyll *a* (g g leaf^{-1})	Chlorophyll *b* (g g leaf^{-1})	Total chlorophyll (g g leaf^{-1})	Nitrate reductase activity (NO_2^- g^{-1} h^{-1})
Rice cultivars				
- V11	0.40a	0.39a	0.76a	2.49a
- GM 28	0.56a	0.33a	0.62a	2.89a
- GM 2	0.39a	0.41a	0.79a	3.39a
- GM 8	0.39a	0.41a	0.80a	2.23a
- Mutant lampung kuning	0.37a	0.38a	0.77a	2.75a
- Mutant rojolele 30 pendek	0.42a	0.46a	0.88a	3.33a
- Mutant rojolele 30 tinggi	0.37a	0.37a	0.74a	3.24a
- Mutant V12T	0.39a	0.42a	0.81a	3.38a
- Mutant mayangsari	0.37a	0.39a	0.76a	2.54a
- Mutant lakatesan	0.38a	0.41a	0.79a	3.04a
- Inpari 33	0.37a	0.38a	0.76a	2.75a
- Inpari 30 ciherang sub 1	0.39a	0.39a	0.76a	2.36a
Crop rotation systems				
- Rice-rice-rice	0.43a	0.39a	0.74a	2.73a
- Maize-maize-rice	0.38a	0.39a	0.77a	2.61a
- Vegetable-vegetable-rice	0.39a	0.40a	0.79a	3.25a
Means	0.40	0.39	0.77	2.87
Interactions	(-)	(-)	(-)	(-)

Notes-Values followed by the same rows are not significantly different according to the HSD-Tukey test ($p<0.05$). (-) showed no interaction was observed between the rice cultivars and crop rotation systems.

Table 2: Leaf area index and net assimilation rate in different rice cultivars and crop rotation systems.

Treatments	Leaf area index	Net assimilation rate (g cm^{-2} week^{-1})
Rice cultivars:		
- V11	0.49c	0.40a
- GM 28	0.49c	0.56a
- GM 2	0.50bc	0.39a
- GM 8	0.53bc	0.39a
- Mutant lampung kuning	0.57abc	0.37a
- Mutant rojolele 30 pendek	0.56abc	0.42a
- Mutant rojolele 30 tinggi	0.55abc	0.37a
- Mutant V12T	0.61abc	0.39a
- Mutant mayangsari	0.75abc	0.37a
- Mutant lakatesan	0.89a	0.38a
- Inpari 33	0.76abc	0.37a
- Inpari 30 Ciherang Sub 1	0.86ab	0.39a
Crop rotation systems		
- Rice-Rice-Rice	0.48b	0.43a
- Maize-Maize-Rice	0.64a	0.38a
- Vegetable-Vegetable-Rice	0.75a	0.39a
Means	0.62	0.40
Interactions	(-)	(-)

Notes- Values followed by the same rows are not significantly different according to the HSD-Tukey test ($p<0.05$). (-) showed no interaction was observed between the rice cultivars and crop rotation systems.



NC- Nitrogen in the leaf tissue; PC- Phosphorus content in the leaf tissue; KC- Potassium content in the leaf tissue, CA- Chlorophyll *a*; CB- chlorophyll *b*; TC- Total chlorophyll; NRA- Nitrate reductase activity; LAI- Leaf area index, CGR- Crop growth rate; NAR- Net assimilation rate; TDW- Total dry weight per clump; EG- Empty grain per clump; GWC- Grain weight per clump. C1- V11; C2- GM 28; C3- GM 2; C4- GM 8; C5- Mutant Lampung Kuning; C6- Mutant Rojolele 30 Pendek; C7- Mutant Rojolele 30 Tinggi; C8- Mutant V12T; C9- Mutant Mayangsari; C10- Mutant Lakatesan; C11- Inpari 33; C12- Inpari 30 Ciherang Sub 1.

Fig 3: a). Correlogram between rice variables; b). Heatmap of clusters between rice cultivars in different crop rotation systems.

and the lowest values were observed in GM 8 and Inpari 30 Ciherang Sub 1 cultivars in the R-R-R crop rotation system (34.49 and 35.40 g clump^{-1}, respectively) (Fig 2d). Cropping system berbasis jagung.

In the study rice-rice rotation showed low mean values for all parameters, resulting in decreased soil fertility and an uninterrupted cycle of pests and plant diseases (Ashworth *et al.,* 2017). Suprihatin *et al*. (2020) stated that R-R-R crop rotation showed the lowest rice productivity compared with rice-rice-maize and rice-rice-soybean crop rotations. This is due to multi-nutrient deficiencies (Baishya *et al.,* 2017).

Fig 3a shows the results of the correlation analysis. The closer to the red color, higher is the positive correlation. GWC was significantly positively correlated with TDW, NC, PC and KC, with correlation values of 0.9**, 0.8**, 0.7** and 0.7**, respectively. The cluster analysis provided information on the proximity of rice cultivar characteristics in the various crop rotation systems (Fig 3b). Three clusters formed and each group had similar characteristics. The first cluster consisted of Mutant Lakatesan, Inpari 33 and Inpari 30 Ciherang Sub 1 cultivars. The second cluster included GM 2, Mutant Lampung Kuning, Mutant Rojolele 30 Pendek and GM 8 cultivars and the third cluster comprised V12T, Mutant Rojolele 30 Tinggi, V11, GM 28 and Mutant Mayangsari cultivars.

The relatively long harvesting age (127 days after planting) resulted in a higher EG in the GM 28 cultivar compared with other cultivars. This finding can lead to high yield losses due to global climate changes, which cause El-Nino and La-Nina (FAO, 2015). Aristya *et al.* (2021b) provided information that GM 2, GM 8 and GM 28 cultivars are relatively resistant to brown planthopper (*Nilaparvata lugens*), leaf blast (*Pyricularia oryzae*) and bacterial leaf blight (*Xanthomonas oryzae* PV. oryzae). Neupan *et al.* (2021) stated that continuous maize cultivation resulted in higher bacterial and fungal contents in the soil than continuously planted rice and beans.

Conclusion

The increase in NC, PC and KC positively affected the increase in GWC. The GM 8 cultivar in the M-M-R crop rotation system showed the lowest EG of 3% and the highest GWC of 133.90 g clump^{-1}. The first cluster consisted of Mutant Lakatesan, Inpari 33 and Inpari 30 Ciherang Sub 1 cultivars. The second cluster included GM 2, Mutant Lampung Kuning, Mutant Rojolele 30 Pendek and GM 8 cultivars and the third cluster comprised V12T, Mutant Rojolele 30 Tinggi, V11, GM 28 and Mutant Mayangsari cultivars.

Conflict of interest: None.

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