directly by plant roots can dissolve in groundwater, causing pollution, thus posing a threat to human health [1] and also making plants more susceptible to pests and pathogens [6]. To reduce the negative effects of the excessive use of nitrogen in rice cultivations, its application needs to be limited, which would also allow obtaining nitrogen-efficient rice varieties. Herniwati and Nappu [6] stated that nitrogen optimization based on plant needs can increase rice productivity in sustainable farming systems. Nitrogen reduction to 282-286 kg urea ha^{-1} in Inpari 4 resulted in high productivity (6.97 ton ha^{-1}) if compared to nitrogen doses commonly used by farmers (> 300 kg urea ha^{-1}) to 6.41 ton ha^{-1}. Toiman et al. [24] reported that NPK 15-15-15 300 kg ha^{-1} application in Inpari 33 produced 1000 grain weight which was not significantly different from 200 kg ha^{-1}, 26.97 g and 26.45 g respectively. This result indicated that NPK 200 kg ha^{-1} is already enough in improving production parameters as well as prevent environmental pollution due to residual nitrite in soil.

To reduce the negative effects of the excessive use of nitrogen in rice cultivations, its application needs to be limited, which would also allow obtaining nitrogen-efficient rice varieties. The use of chemical fertilizers in the form of urea, SP-36, ZA, and NPK in farmers, is generally related to subsidized fertilizers. Anas et al. [27] reported that the use of subsidized fertilizers in Melati II Village, Perbaungan District, Serdang Bedagai Regency, was already inefficient from a technical point of view. The use of subsidized fertilizers, especially urea fertilizers, has exceeded the usage limit in accordance with the recommendations (250 kg ha^{-1}), which is an average of 369.86 kg ha^{-1}. According to Patti et al. [28], excessive use of urea in rice has the potential to cause plants to fall easily because rice stems become softer. Plants that fall down eventually reduce the yield potential and quality of rice plants, Siallagan et al. [29] added that excessive and inefficient use of N fertilizer leads to increased production costs and environmental pollution. The efficiency of nitrogen use (EPN) in the mid-season rice growing season in China is < 30%, indicating that 70% of nitrogen is lost to environmental ecosystems. The efficiency of nitrogen use is the result of nitrogen absorption efficiency and nitrogen utilization efficiency, which varies from 30.2 to 53.2%. The causes of nitrogen loss from the environment are excessive application of N fertilizers, low plant population, as well as improper application of fertilizing.

In higher plants, low-N stress results in physiological, morphological, and molecular changes. Secondary metabolites produced by rice plants are used as defense agents and for a variety of biological activities, and they mainly include phenolic acids, flavonoids, terpenoids, steroids, and alkaloids [7]. The findings of numerous studies indicated that nitrogen fertilizer had an impact on the amount of phenolic compounds in plants. Research on the effect of nitrogen on the phenolic content of artichoke plants [8] shows that compared to the application of 0 kg/ha and 120 kg/ha fertilizer, the application of 80 kg/ha fertilizer gave the highest yield of DPPH content in artichoke var. Albic and Rubik. Increasing the dose of nitrogen is not always followed by an increase in phenolic content.

Further research shows that Rice flavonoids are divided into flavones, flavanols, flavanonols, isoflavones, and flavanones. Flavones are the most common flavonoids found in non-pigmented rice varieties [9].

The metabolites produced in rice plants can be profiled using gas chromatography mass spectrometry (GCMS). When exposed to stress, the leaves and roots of the 'FL478' salt-tolerant rice variety presented higher concentrations of amino acids, sugars as well as higher relative water content [10]. There were 142 metabolites in the leaves of the 'SH527' and 'G9' rice varieties cultivated under control (307 kg urea ha^{-1}) and low nitrogen (102 kg urea ha^{-1}) conditions. In variety 'G9' under low nitrogen conditions, an increase was observed in nine metabolites, i.e., PEP, pyroglutamate, malate, 2-oxoglutarate, 3-sulfinoalanine, glutamate 5-methylester, glycerate-2-phosphate, histidinol phosphate, and sorbose, indicating their important role in low nitrogen tolerance [11]. The purpose of this study was to determine how the reduction of nitrogen doses in the 'Way Apo Buru' and 'Inpari 33' varieties affects growth parameters, yield and the quality of rice seeds, as well as ascorbic acid content, dissolved protein, flavonoids, DPPH, and secondary metabolites.

2. Materials and Methods

Experimental plots were set up in the greenhouse of the Experimental Gardens, at the Faculty of Agriculture of University of Jember using polybags. Three treatments of nitrogen including 0%, 40%, and 100% of 250 kg Nitrogen, were applied for two rice varieties i.e. 'Way Apo Buru' and 'Inpari 33'. The basic doses of P, K, and ZA (Zwavelzure Amonium) fertilizers were adjusted to the dose of the standard fertilizer. Nitrogen fertilizer was applied three times after planting, i.e., 7, 21, and 50 days after planting, which corresponded to 10%, 40%, and 50% of the treatment. The standard doses used for rice plants per hectare were 250 kg of urea, 100 kg of ZA, 75 kg of SP-36 (36% P_2O_5), and 100 kg of KCl. A factorial randomized block design with two treatment factors, namely, the dose of N fertilizer (urea) and irrigated rice varieties, was used in this experiment; measurements were conducted in triplicate.

Growth parameters, i.e., plant height (cm) were measured, and the yield of rice seeds were assessed based on the number of filled grains, total of panicle length (cm), and weight of 1000-grains (g). The quality of rice was determined based on flavonoid compounds, the 1.1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay, and secondary metabolites content; the analyses were conducted at the Plant Analysis laboratory of Jember University.

In addition, profiling of physiochemical compounds of metabolites in rice plants was carried out using GCMS (Agilent Technologies 7890). The rice samples were dried in an oven for approximately 3 days or until they were completely dry. Subsequently, they were blended until smooth and were macerated in methanol pa for approximately 5 days. Then, 10 ml of extract was transferred into a tube and was left to dry at 60 ℃ for 1 hour. After drying, it was dissolved again in the remaining extract of as much as 200 μL and was then injected into GCMSD. The rice extract samples were analyzed using HP Ultra 2 capillary columns. Capillary column length (m) 30 X 0.20 (mm) ID X 0.11 (um) film thickness. The initial temperature was 80 ℃ and was held for 0 minutes; it was then increased at the rate of 3 ℃/min to 15 ℃ and held for 1 minute. Finally, it was raised from 20 ℃/min to 28 ℃ and held for 26 minutes. The injection port, ion source, interface, and quadrupole temperatures were 25 ℃, 23 ℃, 28 ℃, and 14 ℃, respectively. The carrier gas was helium, and the column mode was constant flow at 1.2 mL/min; the injection volume was 5 L with a split ratio of 8:1.

3. Results

The differences in nitrogen application in rice were not significantly affected in the weight of 1000-grains both on Way Apo Buru and Inpari 33. Nitrogen application resulted in higher 1000-grains weight if compared to plant without nitrogen application (Table 1).

Table 1. Weight of 1000-grains (g) in different nitrogen percentage treatments and rice varieties (Note: ns = not significant)

Nitrogen (%)		
0	**40**	**100**
26.11 ns	26.97 ns	26.52 ns
Rice Varieties		
Way Apo Buru	**Inpari 33**	
26.54 ns	26.53 ns	

The tallest plants and highest percentage of filled grains were obtained from variety 'Inpari 33' (Fig. 1).

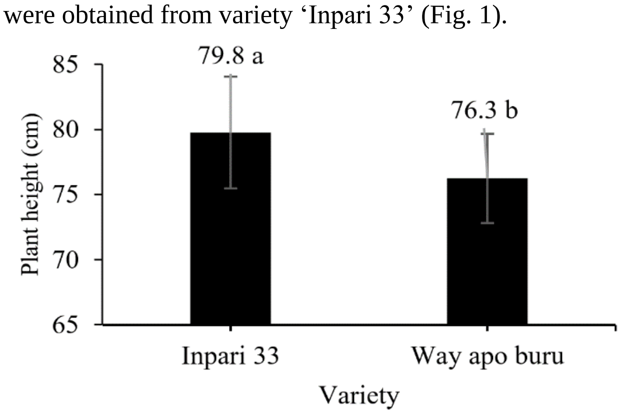

Figure 1. Differences between the two rice varieties in plant height

This is in line with the results of research using the same rice varieties, that Inpari 33 is a superior variety which genetically has productivity 30-50% higher than Way Apo Buru which is local superior rice [25]. Application of 100% nitrogen resulted in the highest number of filled grains to 2424 grains. Plants without nitrogen application and 40% nitrogen resulted in number of filled grains which is not significantly different (Fig. 1). Moreover, application of 250 kg urea ha^{-1} resulted in the highest number of filled grain in rice to 2424 grains. Meanwhile the rice plant without urea application and 100 kg urea ha^{-1} were not significantly different in filled grain (1677–1632 grains). In other research, Okegbade et al. [22] state nitrogen application to 30 kg ha^{-1} resulted in the optimum yield in all rice varieties (NERICA L-19, L-20, L-41, L-42, L-60), while nitrogen levels to 60 kg ha^{-1} resulted in the lowest mean of yield. An increase in nitrogen dose is not always accompanied by an increase in yield, [23] reported that nitrogen application to 119 kg ha^{-1} in okra resulted the highest pod dry weight to 0.48 g but not different from nitrogen application to 357 kg ha^{-1} (0.46 g). The highest percentage of pod dry matter has resulted from nitrogen application to 119 kg ha^{-1} (1.27%). Meanwhile, [5] stated that nitrogen in fertilizer functions as a substitute for nucleotides, proteins, enzymes, and chlorophyll which is involved in vegetative and generative phases of plants.

The total panicle length is higher in the application of 100% N, meanwhile, rice plants without nitrogen application resulted in the shortest panicle. The increasing nitrogen level also increased the panicle length (Fig. 2).

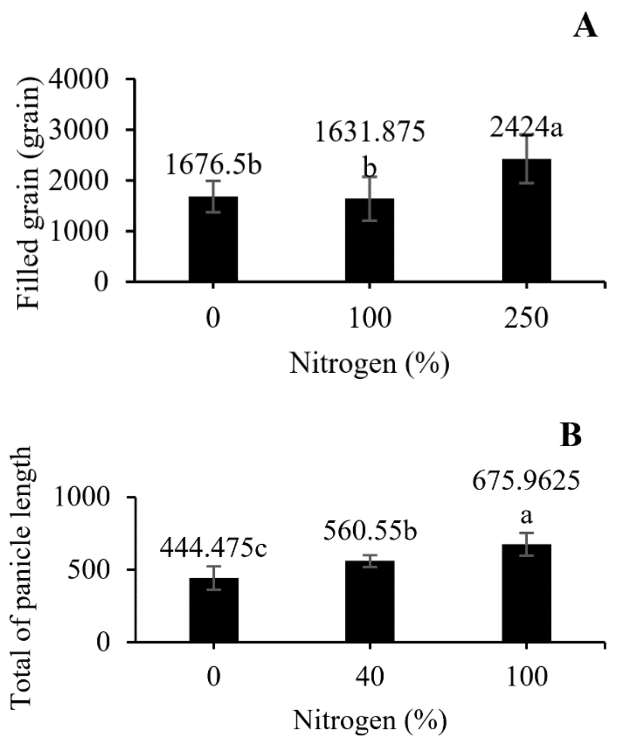

Figure 2. Differences in filled grain (**A**) and total panicle length (**B**) depending on nitrogen dosage

Growth media with low N produced rice plants that are responsive to nitrogen application, thereby increasing panicle length. The same result was reported by [5], where 40 kg/ha N MWUR 1 resulted in higher panicle length than MWUR 2, 3, 4, and the NERICAs. The increase of nitrogen levels to 120 kg/ha also increased the panicle length in all rice varieties in Western Kenya. Awan et al. [12] stated that nitrogen plays an important role in panicle formation and elongation.

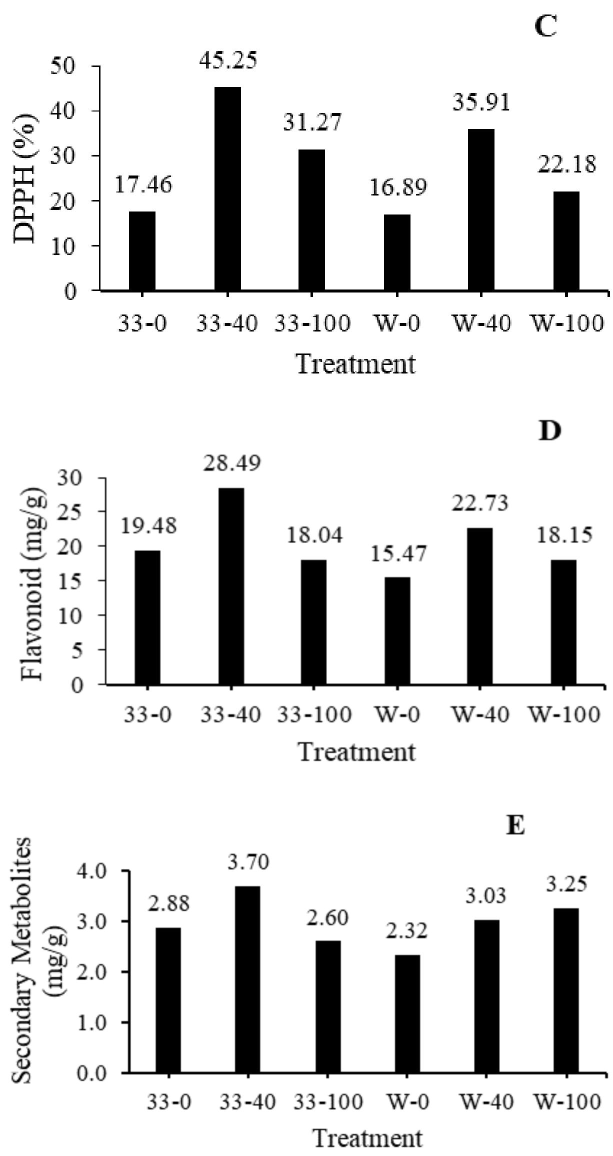

Figure 3. Effects of nitrogen dosage on (**C**) DPPH, (**D**) flavonoids, and (**E**) secondary metabolites content exclusively in 'Way Apo' (W) and 'Inpari 33' (33) rice grains

The percentages of DPPH, flavonoids, and secondary metabolites content were higher in 'Inpari 33' treated with 40% 250 kg N. 'Inpari' 33 rice variety which was applied by 40% nitrogen, resulted in the highest secondary metabolites content (3.70 mg/g). Secondary metabolites in Way Apo Buru increased along with the increase of nitrogen levels. Application of 100% N in Way Apo Buru resulted in the highest secondary metabolites 3.25 mg/g (Fig. 3). secondary metabolites compound in rice variety is an indicator of plant resistance. Inpari 33 rice variety has better resistance to pests and diseases than Way Apo Buru varieties.

The heatmap cluster comprises a dendrogram and color gradient. In the dendrogram located along the sides, samples (columns) and metabolite compounds (rows) are grouped independently. The concept of similarity in cluster analysis is measured using the Euclidean distance, and the ward linkage is applied as an unsupervised clustering method. Through a color gradient, the heatmap shows the value of the data (which have been standardized) for each row and column, and it is similar to contour plots. A two-way view of the data matrix wherein individual cells are displayed is shown using colored rectangles. The color of each cell is proportional to its position along the color gradient. The combination of varieties and nitrogen fertilizer concentrations on the column matrix are displayed as heatmap columns, and metabolite compounds on the row matrix are displayed as heatmap rows. Patterns in the heatmap indicate the relationship between samples and metabolite compounds.

The percentage of compounds detected in the 'Way Apo Buru' and 'Inpari 33' samples treated with nitrogen fertilizer was calculated, and the results were expressed via cluster heatmap analysis. Samples of the combination of 'Way Apo Buru' and 'Inpari 33' treated with 100% 250 kg N showed the presence of the highest number of compounds compared with the other five treatment combinations; in particular, 9,12-octadecadienoic acid was the most abundant compound detected in the rice seeds used in the study (Fig. 4). The peak areas in the heatmap were converted to z-scores, which were calculated by subtracting the mean abundance subtracted from the abundant, and dividing it by the standard deviation across all samples [14]. The z-score of each characteristic was plotted on a blue-red color scale, where blue and red indicate low and high abundances, respectively.

Through the dendrogram, metabolite compounds were divided into three groups. The first included gamma-sitosterol; tetramethylcyclam; campestrol; cyclopropane carboxamide, 2-cyclopropyl-2-methyl-n-(1-cyclopenthyl); stigmasterol; and vitamin E. The second included 2-monoolein; hexadecanoic acid, methyl ester; (9e,12e)-9-12-octadecadienoic acid; cis-13-octadecadienoic acid; 11- octadecenoic acid, methyl ester; 9,12-octadecadienoic acid; anoheal; 9,17-octadecadienal; and hexadecenoic acid. The third included glycerol 2-monooleate; carbonic acid, 2-dimethylaminoethyl isobutyl ester; trans-13-octadecenoic acid, methyl ester; n-hexadecanoic acid; pyridine-3-carboxamide, oxime, n-(2-trifluomethylphenyl); fumaric acid, 2-dimethyylaminoethylhexyl ester, 9,12-octadecadien-1-ol; 2-furancarboxaldehde 5-(hydroxymethyl), and hexadecanoic acid, methyl acid. These metabolite compounds were clustered based on similar patterns of mutual associations between their content and the combination of varieties and fertilizer doses (samples). The pattern of mutual relations was used as a reference to determine the combination of rice varieties and doses of nitrogen fertilizers that result in the highest number of metabolite compounds. The dendrogram illustrating the treatment of samples (combination of varieties and nitrogen fertilizers) shows each sample in a different cluster.

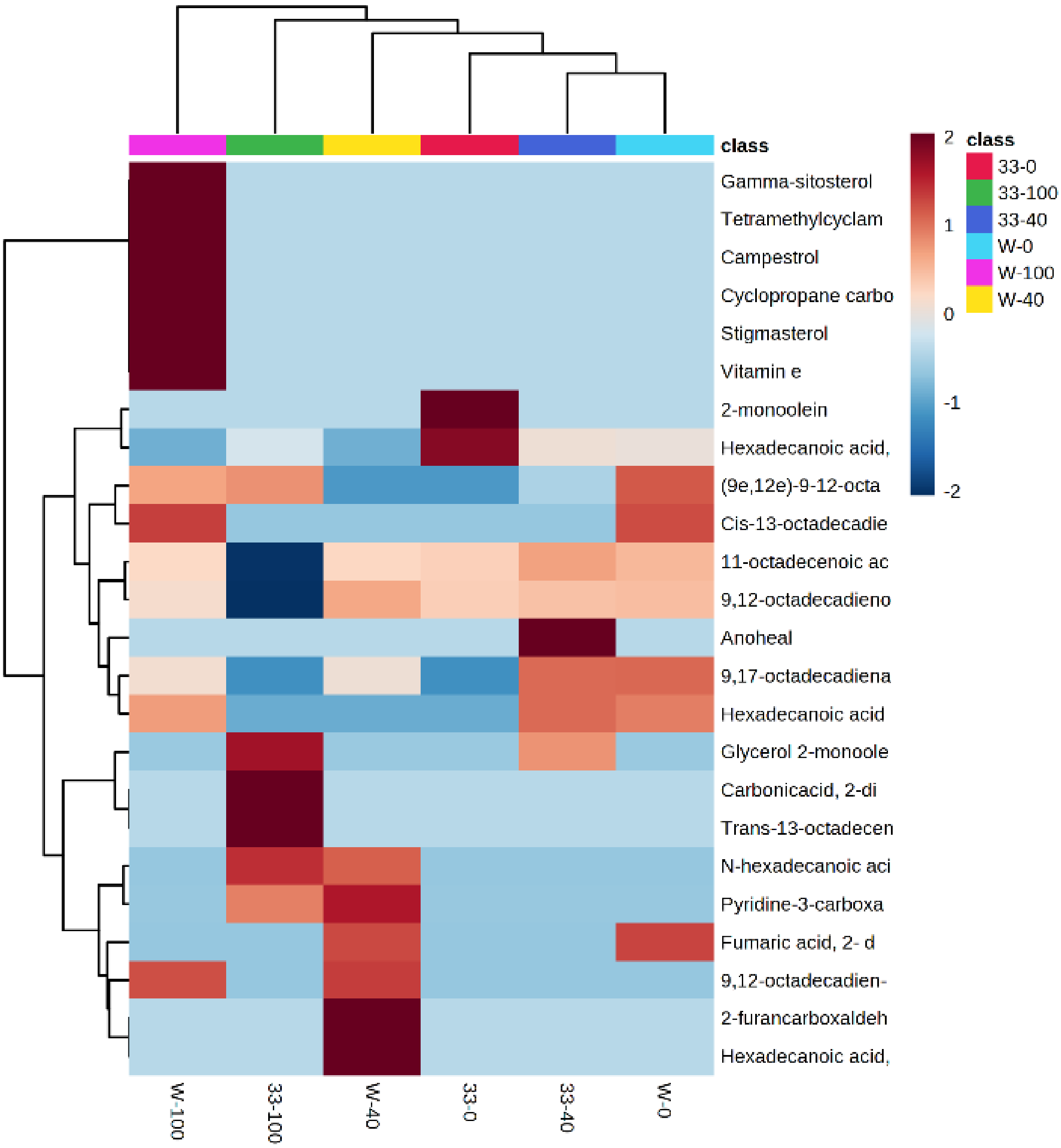

Figure 4. Cluster heatmap of rice metabolites obtained via GCMS analysis. W-0 to W-100 and 33-0 to 33-100 represent the nitrogen fertilizer concentrations of 0% ZA, 40% ZA and 100% ZA applied to the 'Way apo' (W) and 'Inpari 33' (33) varieties, respectively. The color gradient shows the normalized values (the color scale is included above the figure)

In Fig. 5, the analysis of the six treatments shows that the roportion of cumulative variability (principal component analysis, PCA) explained by the two main components of the total original sample was 78% of them were successful in explaining about 50% variance. The rice seed characteristics of 'Way Apo Buru' and 'Inpari 33' were similar when these varieties were treated with a nitrogen fertilizer at 40% 250 kg N.

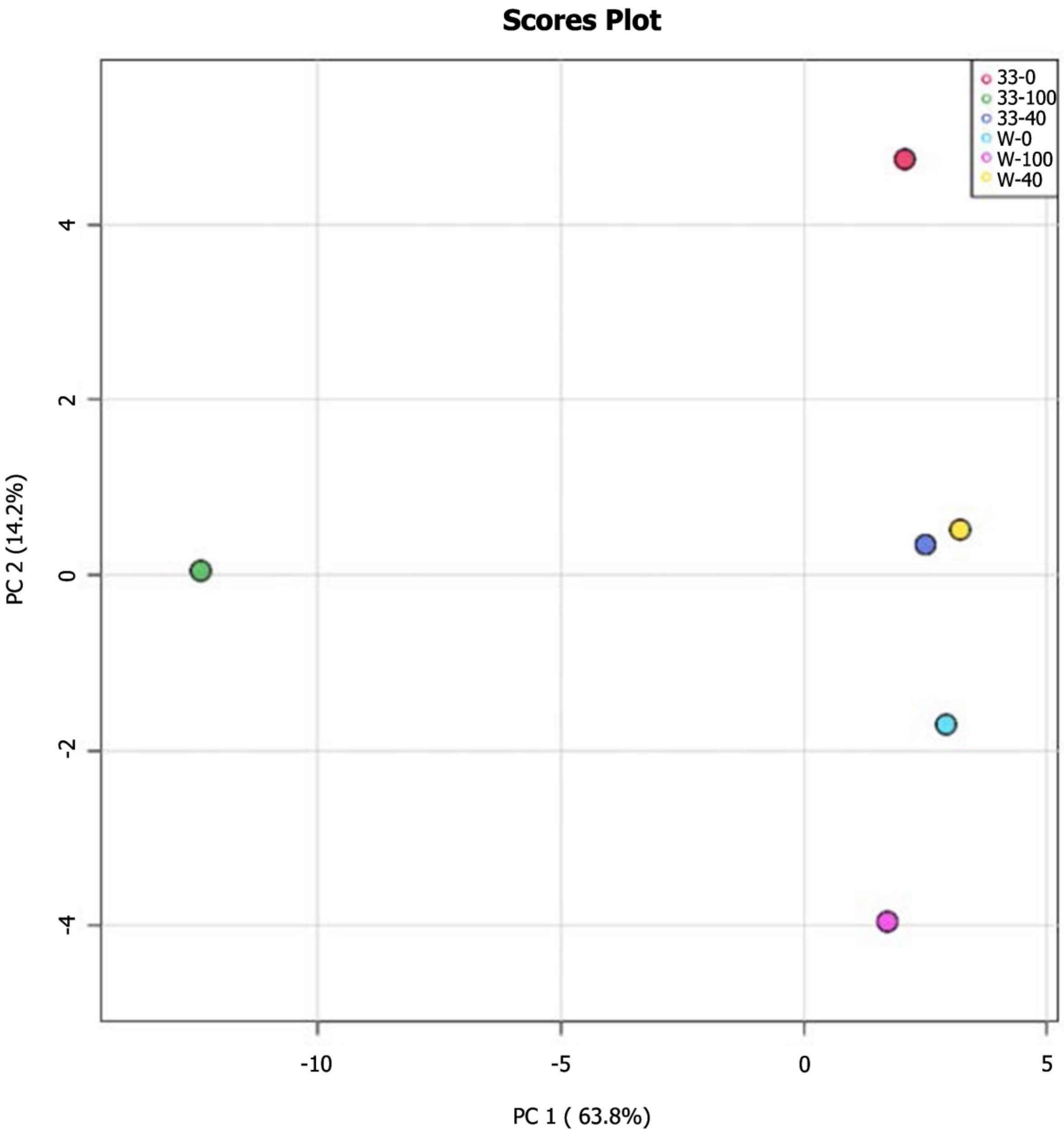

Figure 5. Principal component analysis of the treatment samples (combination of rice varieties and dosage of nitrogen fertilizer) showing the two main axes, i.e., PC1 and PC2. red: 33-0, green: 33-100, blue: 33-40, turquoise: W-0, yellow: W-40, purple: W-100, each no repetition

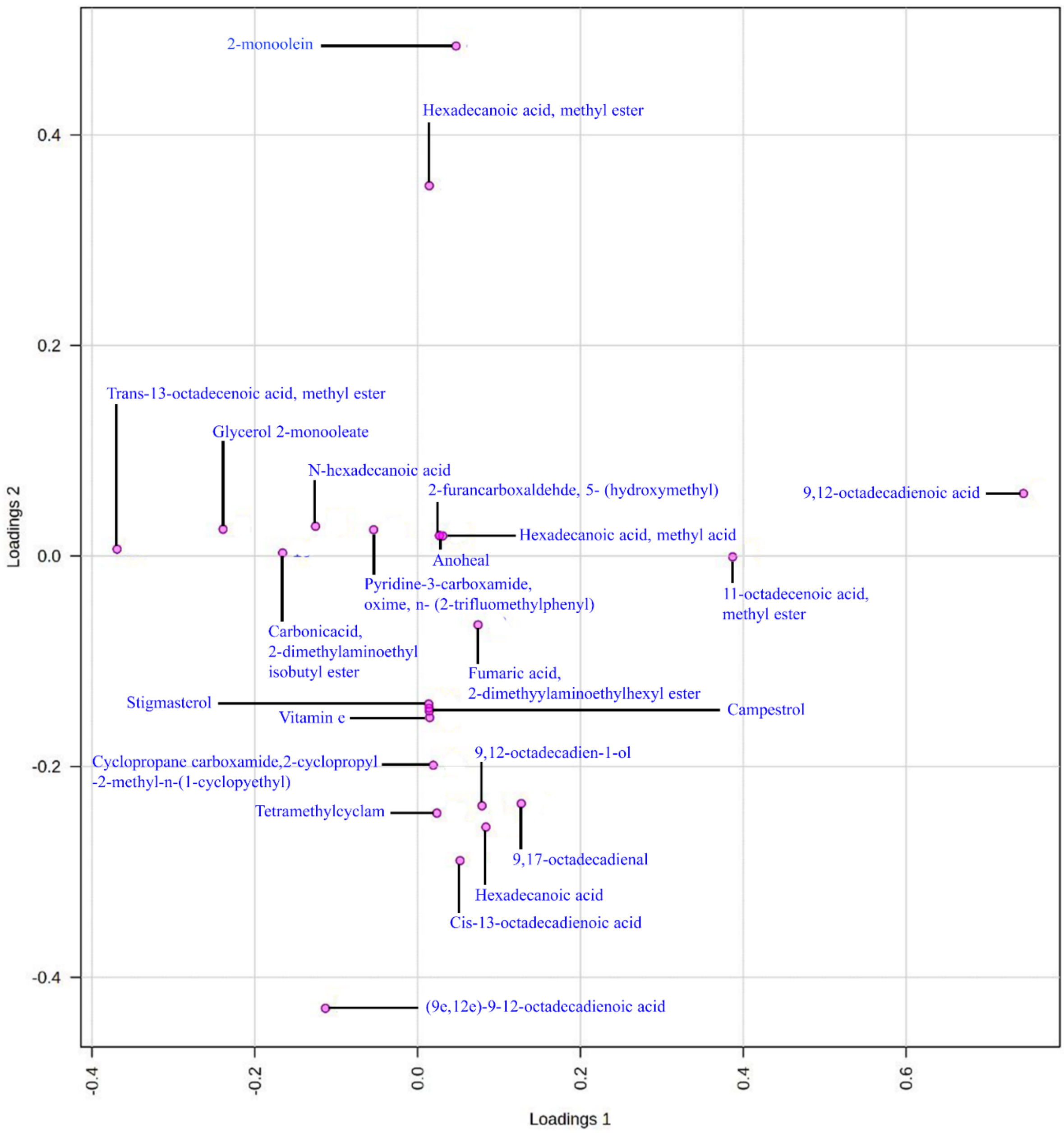

Figure 6. Loading plot of metabolite compounds of a combination of rice varieties and dosage of nitrogen fertilizer.

The loading plot in Fig. 6 shows the similarity among the characteristics of compound metabolites in each variety. In the plot, certain metabolite compounds are described as group characteristics. Four metabolites could be categorized as unique compounds in this study: 2-monoolein; hexadecanoic acid, methyl acid; 9, 12 octadecadienoic acid; and 11-octadecenoic acid, methyl ester.

4. Discussion

Nitrogen is a major factor limiting rice yield under field conditions. Inpari 33 is taller than Way Apo Buru rice varieties (Table 1). These differences were due to the varieties' different genotypes. 'Inpari 33' is genetically superior in terms of grain production compared with 'Way Apo Buru'; it is also a new high-yielding variety, while 'Way Apo Buru' is a type of local superior rice in terms of shorter harvest time, pest, and disease resistance, and water requirement. 'FL78', which is a salt-tolerant recombinant inbred line, produced taller plants (42 cm) compared to 'IR64' under control conditions (32 cm) [10]. Nitrogen application in rice plants promoted plant growth and yield, and improved rice qualities by increasing the number of tillers, leaf area index, seed formation, seed filling, and protein synthesis [4].

'Inpari 33', which is an inbred variety, resulted in a higher percentage of filled grains (73.2%) compared with 'Way Apo Buru' (63.5%). Two inbred rice varieties ('Huanghuazhan' and 'Yuenongsimiao') produced the highest percentage of filled grains between 2017 and 2018 without any nitrogen treatment, but this percentage decreased as nitrogen was applied at concentrations of up to 180 kg ha^{-1} [15]. In the present study, the local 'Way Apo Buru' variety resulted in the lowest number of empty grains when no urea was applied. The application of nitrogen at 100% 250 kg N resulted in a higher number of empty grains in both 'Way Apo Buru' and 'Inpari 33'. The same result was reported in [16], where 'Sigupai', a local rice variety from Aceh, produced a lower percentage of empty grains (24.54%) than 'Inpari 23 Bantul' (40.15%) in the control treatment (without nitrogen application), while the higher dosage of 180 kg of N ha^{-1} resulted in a higher percentage of empty grains in both these varieties. Based on the results of the study on rice, increasing the dose of N fertilizer in both varieties increased the chlorophyll content. the existence of a relationship between nitrogen concentration and chlorophyll content by identifying a strong correlation between the concentration of this nutrient in the leaves and their green color, which is a measure of chlorophyll content [17].

Chlorophyll content, photosynthetic rate, panicle length, and yield components of upland rice varieties were influenced by nitrogen content [5]. Likewise, this element plays a vital role in plant life and contributes to increasing the grain yield, in addition to being essential for cell division, elongation, and chlorophyll formation [18]. The height of rice plants also depends on differences in varieties, as observed, for example, for two rice variety groups, i.e., 'Ciherang', 'Mekongga', 'Inpari 4' [19] and 'Inpari 30', 'Inpari 3', 'Inpari 33', 'Inpari 43', and 'Hipa 5 Cepa' [20]. The application of different nitrogen doses had a significant effect on chlorophyll fluorescence; specifically, a decrease in the fertilizer dose reduced the value of this parameter in the leaves. The application of the standard nitrogen dosage—namely, 250 kg of urea ha^{-1} (100% 250 kg N)—resulted in the highest chlorophyll fluorescence value (36.68) compared to the application of 40% and 0% 250 kg N (i.e., 33.48 and 30.25, respectively) (Fig. 1). The growing medium used in this study had low nitrogen content (0.17%), so that the application of the standard dose was able to increase the amount of chlorophyll in the leaves.

The application of 100% 250 kg N in 'Way Apo Buru' resulted in the greatest panicle length increasing the urea dosage to 120 kg ha^{-1} in local varieties such as 'Nerica' and 'Mwur' favored plants during their reproductive stage, resulting in longer panicles [5]. In contrast, insufficient nitrogen supply produced smaller leaves, lower chlorophyll content, and lower biomass, and consequently, decreased grain yield and quality. Conversely, excessive nitrogen application exhibited negative effects on the environment in the form of water and atmospheric pollution. Scavenging activities for DPPH and ascorbic acid, and high content of ascorbic acid indicated a low antiradical capacity. Increasing the dose of nitrogen fertilizer led to an increase in antioxidant activity [21].

In the present study, 2-monoolein was found only in 'Inpari 33' not subjected to treatment. In both 'Way Apo Buru' and 'Inpari 33', the concentrations of hexadecanoic acid, methyl ester compounds decreased as fertilizer concentration increased. The heatmap colors of the eight classes in Fig. 5 show that the red area corresponds to 'Inpari 33' and 'Way Apo Buru' without treatment, while the blue area corresponds to the two varieties treated with 100% 250 kg N.

In both 'Way Apo Buru' and 'Inpari 33', the 9,12-octadecadienoic acid content slightly increased under the 40% 250 kg N treatment, but decreased under 100% 250 kg N. In particular, a drastic decrease under the latter treatment was observed in 'Inpari 33'. In contrast, 11-octadecenoic acid, methyl ester showed different patterns between the two varieties. In 'Way Apo Buru', it slightly increased up to 40% 250 kg N and drastically decreased under 100% 250 kg N. On the other hand, in 'Inpari 33', the concentration of 11-octadecenoic acid, and methyl ester remained unaltered under both fertilizer dosages.

The high content of ascorbic acid indicates a low antiradical capacity [21]. Increasing the dose of nitrogen fertilizer led to increasing antioxidant activity. The results identified different metabolite groups, each with different characteristics, proportions, and amounts. Lipids were almost the main group in rice, showing the highest proportion compared to other metabolites.

Nitrogen is a very important nutrient in plant metabolism, as it is an essential component of numerous macromolecular compounds, including nucleic acids, lipids, and proteins. Nitrogen compounds in plants also play a role in the ornithine cycle and the shikimate pathway. Previous studies conducted on cyanobacteria of the genus Pseudanabaena showed that the decrease in nitrogen influenced the composition and content of lipids. In line with the results other researchers Zhaoa et al. [26] stated that some metabolites of pyroglutamate, glutamate, 2-oxoglutarate, sorbose, glycerate-2-P, and phosphoenolpyruvic acid are involved in low nitrogen fertilization tolerance.

5. Conclusions

This study showed that high doses of nitrogen fertilizer affected panicle length and the number of filled grains in the 'Way Apo Buru' and 'Inpari 33' rice varieties. The difference in nitrogen application in rice has not significantly affected the weight of 1000-grains on Way Apo Buru and Inpari 33. It was indicated that the land still had sufficient nitrogen content for the growth of this variety. Moreover, DPPH, the flavonoid, and secondary metabolites contents detected the highest amount in

'Inpari33' variety under the 40% 250 kg N. This study used GCMS analysis to reveal that four specific metabolites characterize the two varieties examined at all nitrogen doses applied.

Acknowledgements

The authors thank the Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) Bogor, Indonesia for technical assistance with GCMS analysis.

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