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Short communication: Pattern of chlorophylls content declined during partial submergence for rice varieties cultivated in North Sulawesi at the vegetative stage

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Abstract. *Nio SA, Ludong DPM, Siahaan R. 2022. Short communication: Pattern of chlorophylls content declined during partial submergence for rice varieties cultivated in North Sulawesi at the vegetative stage. Biodiversitas 23: 2451-2456.* Submergence or flooding stress limits plant growth in various physiological, anatomical, and morphological aspects. This study examined leaf chlorophyll content as a physiological response in some rice varieties cultivated in North Sulawesi during partial submergence imposed at the vegetative stage. Ten rice cultivars, i.e., Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31, were grown in plastic trays on planting media in the greenhouse, then watered to field capacity using a mixture of water and fertilizer every second day for two weeks. The plants were submerged 18 cm from the media's surface, and the content of leaf chlorophylls (total chlorophyll, chlorophyll a, and chlorophyll b) were measured using a spectrophotometer (λ 649 and 665 nm) at 0, 3, 6 and 9 days of partial submergence. The chlorophylls (total, a, and b) content decreased during the 9 days of treatment. The highest chlorophylls contents were observed in rice cv. Superwin and the chlorophylls content at day 0 (before partial submergence commenced) were higher than at 3, 6, and 9 days after treatment ($p<0.05$). The data presented here supported that leaf chlorophylls content is a potential physiological indicator of partial submergence stress in rice, including the North Sulawesi local rice, at the vegetative phase.

Keywords: Chlorophyll, partial submergence, rice, vegetative

INTRODUCTION

Flooding is a severe problem impacting rice production worldwide in rainfed lowlands and flood-prone places. Climate change exacerbates the problem by increasing flood risks, particularly in areas prone to monsoon rains in Asia (Bui et al. 2017). Flooding disasters have significantly impacted agricultural production in Asia, particularly in several Southeast Asian countries such as the Philippines, Myanmar, and Indonesia, resulting in a significant decrease in crop yield (Li et al. 2020). Sasidharan et al. (2017) divided the effects of excess water on crops into four categories, i.e., waterlogging or soil flooding (only the root system is flooded), partial waterlogging or soil flooding (only the root system is flooded), submergence (both the root system and the above-ground shoot are submerged), and partial submergence (part of the root system and above-ground shoot are under the water).

The metabolism activity of most cultivated plants is disrupted in flooded land. A temporary or long period of flooding often existed in the tropical lowland area with excessive rainfall, which inhibited plant metabolism, including photosynthesis. This condition is caused by a lack of oxygen rather than an excess of water (Gribaldi et al. 2014). Floods submerged the leaves, causing them to turn yellow (senescence) and inhibiting carbon fixation in the photosynthesis process during and after flooding. When carbon fixation was inhibited, photosynthesis was reduced, and the leaves were damaged (Bui et al. 2017). The duration and depth of flooding significantly impacted the content of chlorophyll a, chlorophyll b, the ratio of chlorophyll a / b, chlorophyll a + b, net photosynthesis in Dystilium chinese seedlings (Xiaoling et al. 2011). Some other studies also evaluated the chlorophyll content as a physiological response to flooding exposure, such as in wheat seedlings (Koshravi et al. 2018), soybean (Dhungana et al. 2019), maize (Lama et al. 2020; Qi et al. 2021) and young Chinese cabbage (Šola et al. 2021).

Food crops, such as rice (*Oryza sativa* L.), can adapt to wet soil conditions, but rice plants will generally die if plant parts are submerged for at least a week due to a lack of oxygen (Bui et al. 2019). The most sensitive rice plants responded to flooding during the vegetative phase. Floods during the vegetative phase resulted in non-optimal growth and inhibition of tiller growth, resulting in a decrease in plant production (Yulianida et al. 2014). This current study utilized the biodiversity of cultivated rice varieties in North Sulawesi, including local rice, which has not been maximized as a potential source of germplasm to meet national food requirements. Flood-resistant properties of cultivated rice in North Sulawesi were limited to be studied. The morphological response to partial submergence in rice grown in North Sulawesi was investigated based on a percentage of live plants, plant height, shoot dry weight, shoot length, root dry weight, root length, root volume, shoot to root ratio, stem elongation rate, number of leaves, the rate of decrease in the number of leaves, the rate of increase in plant height and the rate of stem elongation. The longer the partial-submergence treatment lasted, the lower the leaf number, the higher the plant height, and the longer the shoot elongation (Nio et al. 2018). However, the leaf chlorophyll content under partial submergence has not been examined in rice cultivated in North Sulawesi. Thus, the experiment evaluated leaf chlorophyll content as a physiological response during partial submergence at the vegetative stage in some rice varieties cultivated in North Sulawesi. This study would help elucidate the physiological response underlying chlorophyll content under partial submergence by measuring leaf chlorophyll content in ten rice varieties cultivated in North Sulawesi during 9 days of partial submergence.

MATERIALS AND METHODS

Procedures

This study was carried out from May until October 2019 in the greenhouse in the Tingkulu District, Manado – North Sulawesi, Indonesia (T 23-44ºC, Rh 34-83%) and in the Laboratory Ecology, Biology Department, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University. There were 10 rice varieties cultivated in North Sulawesi used in this study, namely Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 in a completely randomized design with 10 replications. Superwin, Pulo Manado, TB, Ombong, and Burungan are North Sulawesi local rice cultivars. This research included seed selection, planting, plant maintenance, partial submergence treatment, data collection, and data analysis.

Seed selection was conducted by soaking the seeds in saltwater for 2 hours. The seeds were sterilized three times using 0.1% NaOCl solution for two minutes each, then rinsed with water (Nio et al. 2021). Before planting, each plastic tray containing soil was watered until field capacity (Nio and Ludong 2014).

Seeds were planted in plastic trays on planting media (50 seeds per tray for each cultivar), then watered to field capacity every two days using a mixture of water and fertilizer (10 g of Gandasil D fertilizer in 10 L of water). The Gandasil D® solution comprised 20% total nitrogen, 15% P_2O_5, 15% K_2O, 1% $MgSO_4$, Mn, B, Cu, Co, Zn, as well as aneurin, lactoflavin, and nicotinic acid amide. Watering plants to field capacity continued until the plants were 2-weeks-old (Nio and Ludong 2014). For 9 days, the trays and plants were placed in a bucket filled with water, with the plants submerged 18 cm from the media's surface. Leaf chlorophyll was extracted with 95 percent ethanol, and total chlorophyll, chlorophyll a, and chlorophyll b contents were measured at wavelengths 649 and 665 nm using a spectrophotometer SP–3000 nano Optima® (Nio et al. 2019). The content of chlorophylls (total, a, and b) in rice leaves was measured on day 0 (before partial submergence commenced), 3, 6, and 9 days after treatment (Nio et al. 2018).

Data analysis

Microsoft Office Excel 2016 was used to determine the mean and standard errors. Using SPSS 16.0, an analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) were conducted to find significant differences ($p<0.05$) in the concentration of leaf chlorophylls (total, a, and b) in 10 rice cultivars at 0, 3, 6, and 9 days of treatment.

RESULTS AND DISCUSSION

Rice is the main food crop in Asia. The plants required the appropriate environmental conditions for their growth and development. The changes in the environmental conditions, however, could decrease rice production. Plant response to this changing environment was controlled by limiting variables and tolerance capabilities (Nio et al. 2019). The limiting factor in this study was partial submergence (part of the root system and above-ground shoot were under the water), and the response of rice plants to partial submergence was evaluated based on the content of leaf chlorophyll. The content of leaf chlorophyll (total, a, and b) of rice cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 were measured at 0 (before treatment), 3, 6, and 9 days after partial submergence treatment to explore the changes in the chlorophyll content of ten rice cultivars under partial submergence stress.

The current study found that leaf total chlorophyll content in rice cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 under partial submergence (Figure 1; Table 1) were different amongst the cultivars, and the duration of treatment ($p<0.05$). Rice cv. Superwin had the highest total leaf chlorophyll content (36.99 mgL^{-1}), but it was not significantly different from cv. Pulo Manado and Temo (Table 2). Rice cv. Superwin, Pulo Manado and Temo are North Sulawesi local rice. The total leaf chlorophyll content declined gradually from 0, 3, and 6 to 9 days of partial submergence. The total leaf chlorophyll content at 3, 6, and 9 days after treatment were 34, 42.12, and 76.20% lower, respectively, than before the partial submergence commenced (38.43 mgL^{-1}), as shown in Table 2.

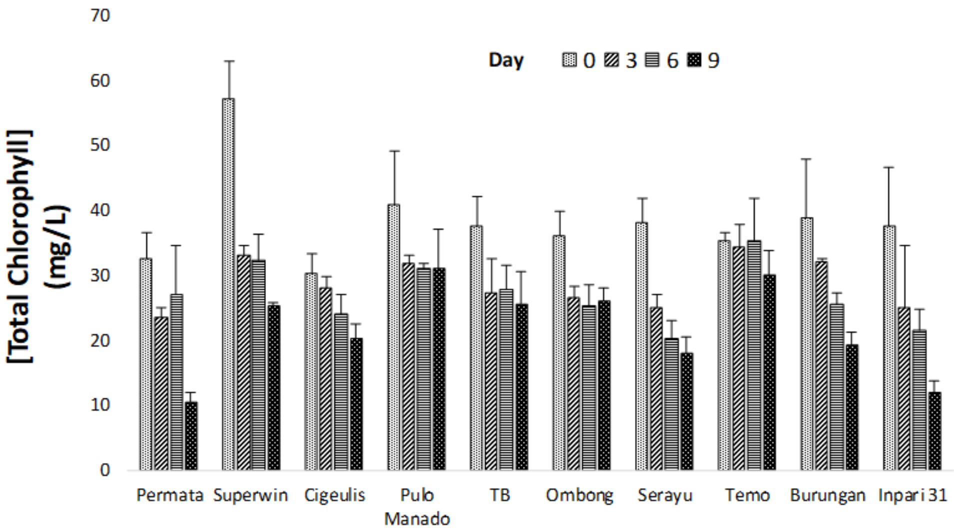

Figure 1. The leaf total chlorophyll content (mean ± SE; n=10) in rice cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 at 0, 3, 6, and 9 days after partial submergence treatment

It was revealed that the content of leaf chlorophyll in ten rice cultivars under partial submergence was different due to the cultivars and the duration of treatment ($p<0.05$) (Figure 2; Table 1). The highest leaf chlorophyll a content (21.93 mgL^{-1}) was observed in rice cv. Superwin, but it was not significantly different from cv. Pulo Manado, Temo, TB and Ombong (Table 2). Rice cv. Superwin, Pulo Manado, Temo, TB and Ombong were North Sulawesi local rice. As shown in Table 2, leaf chlorophyll a content at 3, 6, and 9 days after treatment were 14.30, 23.22, and 49.62% lower, respectively, compared before the partial submergence commenced (22.07 mgL^{-1}). The longer duration of partial submergence resulted in the lower content of leaf chlorophyll a, however, the leaf chlorophyll a content at 3 and 6 days after treatments were not significantly different.

The content of leaf chlorophyll b in rice cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 under partial submergence were significantly different among the cultivars, and the treatment durations (Figure 3; Table 1). The content of chlorophyll b in cv. Superwin (15.10 mgL^{-1}) was the largest, however, it was not significantly different from cv. Pulo Manado (12.40 mgL^{-1}) and cv. Temo (12.23 mgL^{-1}). The content of chlorophyll b at 0, 3, 6, and 9 days of treatment was 16.41, 9.41, 9.16, and 7.08 mgL^{-1}, respectively, however, it was not significantly different at 3, 6, and 9 days of treatment. The content of chlorophyll b at day 0 was 74.39, 79.15, and 131.78%, respectively, higher than at 3, 6, and 9 days of treatment (Table 2).

This present study revealed the pattern of leaf chlorophylls (total, a, and b) content declined during 9 days of partial submergence for rice cultivated in North Sulawesi (cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31) at the vegetative stages (2-week-old rice plant). The highest chlorophylls contents were observed in a North Sulawesi local rice, i.e., cv. Superwin; and the chlorophylls content at day 0 (before partial submergence commenced) were higher than 3, 6, and 9 days after treatment ($p<0.05$). Some previous experiments were also conducted to evaluate the content of chlorophylls under submergence. The chlorophyll a content in tetraploid wheat at 3, 6, and 9 days of submergence declined by 61.50, 46.53, and 55.28%, respectively (Koshravi et al. 2018). The content of chlorophyll b was reduced by 50% in wheat after 7 days of submergence (Todorova et al. 2022). After submergence for 12-14 days, chlorophyll content declined significantly in submergence-tolerant and sensitive rice genotype seedlings (Bui et al. 2019). The content of chlorophyll a, b and total chlorophyll of the second oldest leaves declined by about 90% in both upland and lowland rice seedlings after 10 days of fully submergence (Yang et al. 2017). The chlorophylls (total, a, and b) contents of the above-ground parts in five japonica rice varieties significantly declined under 8-day-full-submergence treatment. The rates of decline in the contents of chlorophylls total, a, and b, were 70-77%, 70-78%, and 68-76%, respectively (Li et al. 2020). Submergence also reduced leaf relative chlorophyll content in maize 26 days after sowing, and this result indicated degradation of chlorophyll (Qi et al. 2021). The chlorophylls contents of cv. Superwin was significantly higher than the other nine varieties, indicating that Superwin's leaf cells were less harmed by partial submergence than others, as reported in rice cv. KH 139 by Li et al. (2020).

Submergence stress limits plant growth in various physiological and metabolic processes. Under submergence stress, crops' required oxygen cannot be exchanged effectively in water, resulting in hypoxic stress. Crops' ability to perform photosynthesis is hampered in a hypoxic environment, resulting in leaf yellowing, slowed root system growth, and decreased metabolism in the mitochondria. Submergence causes an accumulation of excessively activated oxygen groups, such as H_2O_2 and O_2, in plant leaves, causing problems such as oxidative stress and lipid peroxidation (Li et al. 2020). Gaseous diffusion is reduced under submerged conditions, and O_2 depletion can cause anoxia in plant tissues. When rice plants are deprived of oxygen, their metabolism shifts from aerobic to anaerobic, impacting their growth and development since anaerobic respiration consumes a lot of energy. Submergence also has an impact on nutrient and water uptake. Oxygen-dependent pathways, particularly energy-generating systems, are repressed under submergence circumstances, disrupting the functional linkages between organ assimilation and photosynthate usage (Bui et al. 2019).

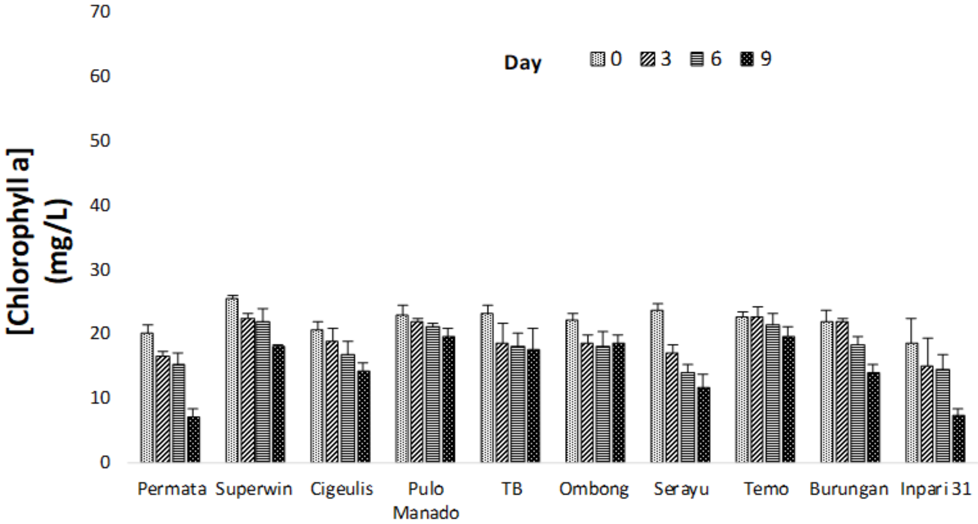

Figure 2. The content of leaf chlorophyll a (mean ± SE; n=10) in rice cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 at 0, 3, 6, and 9 days after partial submergence treatment

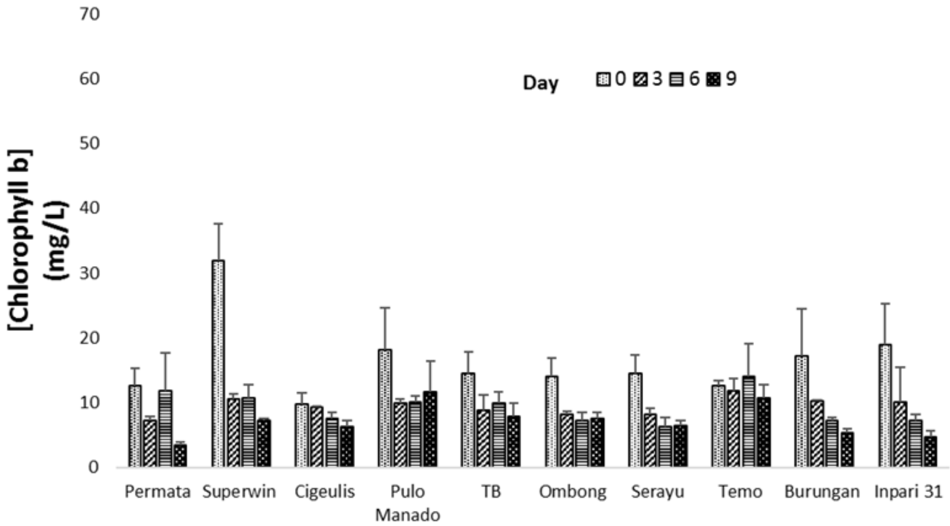

Figure 3. The content of leaf chlorophyll b (mean ± SE; n=10) in rice cv. Permata, Superwin, Cigeulis, Pulo Manado, TB, Ombong, Serayu, Temo, Burungan, and Inpari 31 at 0, 3, 6, and 9 days after partial submergence treatment

Table 1. Combined analysis of variance of chlorophyll content in ten rice cultivars for 0, 3, 6, and 9 days of partial submergence

Source of variance	df	Mean square characters of		
		[Total Chlorophyll]	[Chlorophyll a]	[Chlorophyll b]
Cultivar	9	252.37^{*}	95.52^{*}	54.90^{*}
Duration	3	1445.60^{*}	278.09^{*}	495.34^{*}
Cultivar x Duration	27	56.02^{ns}	10.30^{ns}	33.82^{ns}
Error	80	57.57	9.41	26.93

Note: *significant at 5% level; ^{ns}: non significant at 5% level

Table 2. The chlorophylls content in ten rice cultivars for 0, 3, 6, and 9 days of partial submergence

Factors	Total Chlorophyll (mgL^{-1})	Chlorophyll a (mgL^{-1})	Chlorophyll b (mgL^{-1})
Cultivar			
Permata	23.44 a	14.71 ab	8.75 a
Superwin	36.99 c	21.93 e	15.10 b
Cigeulis	25.69 a	17.56 c	8.17 a
Pulo Manado	33.73 bc	21.37 de	12.40 ab
TB	29.51 ab	19.30 cde	10.25 a
Ombong	28.49 ab	19.27 cde	9.25 a
Serayu	25.36 a	16.58 bc	8.80 a
Temo	33.75 bc	21.56 de	12.23 ab
Burungan	28.93 ab	18.99 cd	9.97 a
Inpari 31	24.01 a	13.82 a	10.22 a
Duration (days)			
0	38.43 c	22.07 c	16.41 b
3	28.68 b	19.31 b	9.41 a
6	27.04 b	17.91 b	9.16 a
9	21.81 a	14.75 a	7.08 a

Note: Different letters in the same column indicated a significant difference ($p<0.05$) based on DMRT 5%

Leaf chlorosis, caused by a decrease in chlorophyll production, is one of the most significant barriers to plant growth. Many annual and perennial plant species exposed to submergence stress have lost chlorophyll (Gan et al. 2020), which indicates the degradation of chlorophyll (Qi et al. 2021). The increase in leaf chlorosis reduced photosynthesis underwater. Plant survival and recovery are dependent on maintaining chlorophyll concentration under submergence circumstances because it ensures photosynthesis underwater and allows for continued plant growth recovery after submergence (Bui et al. 2019). Chloroplasts can cause damage and reduce photosynthesis efficiency in plants when subjected to submergence. The degradation of the chlorophyll content in rice varieties' leaves during submergence resulted from ethylene accumulation (Sone and Sakagami 2017; Li et al. 2020). Ethylene increases the activity of the enzyme chlorophyllase, which causes chlorophyll depletion in submerged plants (Panda and Barik 2021).

The decrease in chlorophyll content under submergence stress could also be linked to the expression of some genes that produce some of the important enzymes responsible for this response. The reduction of chlorophyll content contributed to a decline in rice seedling survival rate following submergence, indicating that the Sub1 gene may be implicated in chlorophyll catabolism under submergence (Bui et al. 2019). The Sub1 genotype of rice cv. Inpari30 was also demonstrated to confer the ability to retain the maximum quantum yield of photosystem II under situations of limited gas exchange for photosynthesis (Nurrahma et al. 2021). The decrease in chlorophyll content was also associated with the downregulation of Chl-biosynthetic genes (genes) and upregulation of Chl-degrading genes. Under abiotic stress, including partial submergence, 5-aminolevulinic acid dehydratase and porphobilinogen deaminase enzymes are involved in chlorophyll synthesis, whereas chlorophyllase and pheophytinase enzymes contribute to chlorophyll degradation (Gan et al. 2020).

Chlorophyll is a significant plant pigment that contributes to photosynthetic ability. Rice suffers from a reduction in chlorophyll content as a result of submergence. Chlorophyll degradation is promoted by submergence in both sensitive and tolerant genotypes, and which can be used to measure submergence tolerance (Panda and Barik 2021). Therefore, leaf chlorophylls (total, a, and b) content, are suitable physiological indicators of partial submergence in some rice cultivated in North Sulawesi at the vegetative phase. Further detailed experiments are still required to screen the submergence-tolerant rice cultivars, including the North Sulawesi local rice cultivars, as potential germplasm sources to meet national food requirements.

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