

The effect of shade on chlorophyll and anthocyanin content of upland red rice

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Abstract. Upland red rice (*Oryza sativa*) is a staple food and contains anthocyanin, which can act as antioxidants, plays an important role both for the plant itself and for human health. Levels of antioxidants in rice can be affected by the availability of light. The results showed that the difference of shade, cultivar, and interaction both significantly affect the content of chlorophyll *a*, chlorophyll *b* and total chlorophyll. The results also showed that shade could increase chlorophyll in all cultivars tested. The highest levels of chlorophyll *a* were present in the moderate shade level (n_2), then decreased at the shelter level (n_3) and increased again at high levels (n_4). While on chlorophyll content *b*, it appears that shade increased chlorophyll *b* in all cultivars tested and this increase was linear to the increase of shade. The shade treatment may increase the anthocyanin content and the increase depending on the type of cultivar. Increased levels of anthocyanin highest due to shade occurred on Jangkobembe cultivar. The original level of anthocyanin on Jangkobembe cultivar averaged 0.096 mg g^{-1} increased to 2.487 mg g^{-1} or increased 26 fold. It is concluded that the shade had a significant effect on the chlorophyll and anthocyanin content.

1. Introduction

Red rice (*Oryza sativa*) is a staple food of high health value. In addition to carbohydrates, fats, proteins, fiber, and minerals, brown rice also contains anthocyanins [1]. Anthocyanins are phenolic compounds that enter the flavonoid group and act as antioxidants, plays an important role both for the plant itself and for human health. The role of antioxidants for human health to prevent liver disease (*hepatitis*), colon cancer, stroke, diabetes, is essential for brain function and reduce the effects of brain aging.

Increased red rice production has associated with efforts to increase rice production, especially the upland rice production. Red rice derived from upland rice and the efforts to increase upland rice production with local cultivars are therefore always associated with increase red rice production [2]. Indonesia actually is major rice producing countries together with China, India, Bangladesh and Vietnam [3-4], but at the same time, Indonesia also the main consumer of rice [5]. The Indonesian government has two policy to reach rice self-sufficiency [4,6], through the increasing rice production and trying to curb rice consumption, while promoting consumption of other source staple foods, such as sago [7].



One of the alternatives to increase the national rice production is through the development of upland rice [8] as an insertion plant under stands of plantation crops/forestry or crops intercropping in agroforestry patterns [2,5]. Upland rice cultivation can be planted between the rows of plantation or young forestry crops [9] when the leaf canopy has not shaded each other [10]. The plantation area in 2013 reaches 22 million hectares [11] and every year there is approximately two million ha of plantation reforestation land that can be cultivated upland rice as intercrops.

The potential of the forestry sector to support food security are also very large by making the forest for food production [2,5]. Development of upland rice production in forest areas can be done through the provision of food forests in forest areas, community forests and other types of forest exploitation such as social forestry. Until 2013 there are potentials of more than 10 million hectares of forest land, which can be used for the development of upland rice, both as insertions in agroforestry and food reserves in forest areas.

The main ecological constraints of land use under stands of plantation crops and forestry are the low intensity of solar radiation, and the plants will be light stress, which will result in the process of growth and production. The decrease in the intensity of solar radiation will affect other micro climate elements such as air temperature, relative humidity, and soil temperature.

Shade can affect on growth and production, and reduce the efficiency of solar radiation [12-14]. Some research results showed that at a shade level of more than 50%, there is a drastic reduction in production. The magnitude of the decline in rice production due to shade, is depending on the level of tolerance and growth phase of each strain. Improved and moderate upland rice cultivar is recommended for planting upland rice between plantation stands and forestry. However, the shade or low light intensity, can affect the increase of anthocyanin levels. Therefore, although shade can reduce the production of upland rice but the anthocyanin content has increased.

Development of red rice will have a broad economic impact for farmers because the types or varieties of rice planted can produce rice with high selling value and the pattern of planting has integrated with existing farming, especially mixed farming in the form of plantation or forestry. Under these conditions, the choice is to plant shade of red rice that is tolerant to shade, so that it can be planted under the stands of a plantation tree or a young forestry.

2. Material and Method

The research design using split plot design. The main plot was the different of shade treatment consisting of 4 levels of shading and the second factor is different of cultivar. The main plot is the difference of shading as follows: n_1 =shade level < 25 %, n_2 =shade level 25-50 %, n_3 = shade level 50-75 % and n_4 = shade level > 75%. While in subplot is the difference of upland red rice cultivar i.e., (V_1) = *La Bandiri*, (V_2) = *Jangkobembe*, (V_3) = *Ranggohitam*, (V_4) = *Paedara*.

3. Results and Discussion

3.1. Chlorophyll content

The result showed that the difference of shade, cultivar, and interaction both have a significant effect on the content of chlorophyll a, chlorophyll b and total chlorophyll of upland red rice. Based on the cross-tabulation between cultivars and shade as in Table 1 shows that shade can increase chlorophyll content in all cultivars tested.

The highest levels of chlorophyll a are present at moderate shade level (n_2), then decrease at the medium shade (n_3) and increase again at heavy shade levels (n_4). While on chlorophyll b content, it appears that shade increases chlorophyll b in all cultivars tested and this increase is linear, where the higher the shade level, the higher the chlorophyll b content.

A similar pattern is also found in total chlorophyll content, which increases as a result of shade. At low shade (n_2) and medium shade (n_3) levels, total chlorophyll content increases, but at a heavy shade (n_4) the total chlorophyll content decreases. Based on this pattern it appears that plants in the shaded condition will adapt by increasing the content of chlorophyll, both for the content of chlorophyll a, chlorophyll b and total chlorophyll. In general, chlorophyll content increases with

increasing shade. Further test results of shade and cultivar interaction on chlorophyll a content as shown in Table 2, it appears that in Labandiri cultivars, there is no significant difference in the content of chlorophyll a due to shade.

Table 1. Leaf chlorophyll content of various rice cultivars of upland red rice at different levels of shade [2]

Shade Treatment	Chlorophyll Content (mg 100g ⁻¹)				
	Chlorophyll a				Average
	v ₁	v ₂	v ₃	v ₄	
n ₁	4.12	4.12	4.12	4.12	4.12
n ₂	4.56	4.56	4.56	4.56	4.56
n ₃	4.18	4.18	4.18	4.18	4.18
n ₄	4.46	4.46	4.46	4.46	4.46
Average	4.33	4.33	4.33	4.33	4.33
	Chlorophyll b				Average
	v ₁	v ₂	v ₃	v ₄	
	v ₁	v ₂	v ₃	v ₄	Average
n ₁	44.22	44.22	44.22	44.22	44.22
n ₂	51.47	51.47	51.47	51.47	51.47
n ₃	54.30	54.30	54.30	54.30	54.30
n ₄	59.19	59.19	59.19	59.19	59.19
Average	9.1	9.1	10.1	9.4	9.4
	Total Chlorophyll				Average
	v ₁	v ₂	v ₃	v ₄	
	v ₁	v ₂	v ₃	v ₄	Average
n ₁	21.96	21.96	21.96	21.96	21.96
n ₂	32.68	32.68	32.68	32.68	32.68
n ₃	33.71	33.71	33.71	33.71	33.71
n ₄	32.34	32.34	32.34	32.34	32.34
Average	30.17	30.17	30.17	30.17	30.17

Rem : n₁ = Shade level < 25 %, v₁ = Cultivar *Labandiri*
n₂ = Shade level 25-50 % v₂ = Cultivar *Jangkobembe*
n₃ = Shade level 50-75 % v₃ = Cultivar *Ranggohitam*
n₄ = Shade level > 75% v₄ = Cultivar *Paedara*

3.2 Anthocyanin content

Shade treatment may increase anthocyanin levels of upland red rice, and the increasing depends on the type of cultivars tested as shown in Table 3. The highest increase of anthocyanin levels due to shade stress occurs in Jangkobembe cultivar.

The anthocyanin content of the original *Jangkobembe* cultivar averaged 0.096 mg g⁻¹ increased to 2.487 mg g⁻¹ or increased 26 fold. In the other three cultivars also increased levels of anthocyanin from the shade, but the increase was not as large as the *Jangkobembe* cultivar. The subsequent increase in the highest levels of anthocyanin occurred in the *Labandiri* cultivars, followed by *Ranggohitam* and *Paedara* cultivars.

In general, shade stress plants have an increased amount of chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll). This increase in chlorophyll content is in line with the higher level of shade (Table 1) and occurs for the four cultivars tested. Increasing the chlorophyll content is one of the plant's efforts to adjust to low light intensity conditions. The decrease in light intensity to some extent

can increase the chlorophyll content. If the available light is no longer able to support the plant for photosynthesis, the rate of chlorophyll increase also decreases. Adaptation mechanism for low light intensity in soybean through increasing the chlorophyll content occurring in all shading-treated genotypes [15]. The content of chlorophyll a and b are both increased, but the proportion of increase occurs more in chlorophyll b.

Table 2. Interaction between shade and cultivar on chlorophyll a content [2]

Shade Treatment	Chlorophyll a content (mg 100g ⁻¹)								DMRT 0.05 Between v
	v ₁		v ₂		v ₃		v ₄		
n ₁	4.12	a	1.40	c	2.30	d	1.36	b	
	p		r		q		r		2= 0.71
n ₂	4.57	a	2.91	ab	3.40	c	1.00	b	
	p		q		q		r		3= 0.75
n ₃	4.19	a	2.47	b	4.45	b	3.01	a	
	p		Q		P		q		4= 0.78
n ₄	4.46	a	3.37	a	5.69	a	2.63	a	
	q		r		p		s		
Remarks :	Number followed by the same index in the same row, are not significantly different at Duncan's multiple Range Test (DMRT).								

Table 3. Effect of shade on anthocyanin content of upland red rice [2]

Shading treatment	Anthocyanin content (mg g ⁻¹)							
	v ₁		v ₂		v ₃		v ₄	
Low shade (n ₁)	0.127	a	0.096	a	3.832	a	0.153	a
	r		P		Q		p	
Heavy shade (n ₃)	0.336	b	2.487	b	6.226	b	0.218	b
	q		R		Q		p	
Increasing level	2.64		26.03		1.62		1.42	

Remark : Number followed by the same index in the same row, are not significantly different at Duncan's multiple Range Test (DMRT).

The results showed that shade could increase the anthocyanin level of red rice, even can reach 26-fold as happened in Ranggohitam cultivars. While in the other three varieties (Labandiri, Jangkobembe and Paedara) there was also an increase in anthocyanin levels up to nearly threefold (Table 3).

Anthocyanins are phenolic compounds belonging to the flavonoid group. Flavonoids are potent antioxidants that prevent the formation of free radicals. Light has a very significant effect on anthocyanin content of red rice gogo rice. An increase in anthocyanin content is due to the leaves exposed to full light, anthocyanins found only in epidermal cell vacuoles (Generally treated plants generally have increased chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll)).

This increase in chlorophyll content is in line with the higher level of shade and occurs for the four cultivars tested. Increasing the chlorophyll content is one of the plant's efforts to adjust to low light intensity conditions. Increasing the formation of anthocyanins in low light will reduce the assimilates formed to reduce the efficiency of light use [16], but the increase of anthocyanin formation at low light intensity is a consequence of decreasing carotene content at low light intensity [17].

4. Conclusion

The results also showed that shade could increase chlorophyll content in all cultivars tested. The shade treatment may increase the anthocyanin content and the increase depending on the type of cultivar. It is concluded that the shade had a significant effect on the chlorophyll and anthocyanin content.

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