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The Effect of Low pH on Physiological Characters in Vegetatif Phase of Kalimantan Local Swamp Rice (*Oryza sativa* L.)

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Abstract. Indonesia is a country with high rice consumption and also potential to develop local rice plants with adaptation to tidal swamp. This study aimed to analyze physiological characters of local Kalimantan swamp rice cultivars in low pH condition. To achieve this purpose, plants were grown hydroponically with two pH variation, 4 (low pH) and 6.8 (normal). This study used 6 cultivars namely 'Amas', 'Pandan Ungu', 'Kambang', 'Sikin Merah', 'Argo Pawan', 'Suatek Merah', and 2 comparative cultivars 'Inpara 5' and 'Ciherang'. Growth characters were observed by measuring plant height, leaf length and width, and leaf number up to 21 DAT (Day After Treatment). Physiological characters observed including chlorophyll, carotenoid and proline content of leaves as measured on 21 DAT. Under low pH condition, the physiological characters of some swamp rice cultivars were better than that under pH 6.8. This can be seen from plant performance showing higher plant, longer and wider leaves, and more of leaf number in 'Amas', 'Pandan Ungu', and 'Kambang'. In addition, at pH 4 treatment, there was a higher in total chlorophyll content in 'Pandan Ungu' and 'Kambang' and higher carotenoid content in 'Kambang'. Meanwhile, proline levels of swamp rice cultivars increased at pH 6.8 indicated that these cultivars are more tolerant to low pH. Based on the results it can be concluded that pH 4 treatment provides high stability in growth (plant height, leaf length, and width, leaf number), chlorophyll, carotenoid and proline content in 'Amas', 'Pandan Ungu', and 'Kambang'.

INTRODUCTION

Rice (*Oryza sativa* L.) is the main food crop in Asia used as a staple food by more than half of the population in the world. Indonesia is one country where the population consume rice as a staple food. Rice production in 2014 was in the first of 10 important food commodities [1]. One way that can be done to increase rice productivity is by utilizing suboptimal land as rice production areas.

Suboptimal land that can be utilized for rice production is tidal swampland. Tidal swampland is widespread in Indonesia, and is very prospective as a substitute for fertile land which is limited in Java and Bali. Indonesian tidal swamp area is estimated to be around 20.11 million ha spread across the east coast of Sumatra (Lampung, South Sumatra, Jambi, Riau), the southern coast of South Kalimantan and the southern coast of Irian Jaya. Tidal swampland is characterized by an irrigation system that relies on tides, low soil pH, pyrite (FeS_2) layer, nutrient deficiency, deep inundation, accumulation of Fe^{2+} and Al^{3+} and low organic matter content [2]. The characteristics of tidal swampland pose obstacles to the growth and productivity of rice, mainly due to the low soil pH and presence of pyrite. In aerobic condition, pyrite will react with oxygen to form Fe^{3+} and SO_4^{2-} caused decreasing soil pH and toxic to plants [3].

Soil pH is an important chemical property, which determines iron solubility and its uptake by plants. In addition, soil pH is often highly changeable property because of the dynamic nature of various soil processes and the interactions of these processes with plants and microorganisms [4]. The low pH condition causes very high Fe and Al concentration in soil. High concentrations of Al^{3+} and Fe^{2+} in the soil environment can be toxic to plants. The presence of excess Al

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and Fe can affect plant growth and hamper soil nutrient availability. Al^{3+} can cause the inhibition of root development as well as root retardation [5]. At pH <4.8, dissolved Al^{3+}in the soil solution can reach the critical level of 30 µM, which can damage rice plants. Al toxicity is often related to phosphorus deficiency because soil with high Al concentration will decrease the availability of P due to Al-Fe-phosphate interaction [6]. That is why the most recognized symptom of Al toxicity is P-related inhibition of root growth. However, without enough Fe, chlorophyll cannot be sufficiently produced. Under low pH condition, the availability of Fe is increased, causing excessive accumulation of Fe in the leaves of rice. The visual symptom of Fe toxicity on rice is bronzing [7]. Besides, Fe toxicity also increase panicle sterility that reduces plant growth [8]. Along with decreasing crop productivity caused by low pH levels, the common causes of yields reduction included aluminum, manganese, and hydrogen (H^+) ions toxicities, and deficiencies in nutrients such as phosphorus, molybdenum, calcium, and magnesium. Among these constraints, proton toxicity (low-pH stress) is considered to be one of the major stresses limiting plant growth in acid soils [9].

Low pH levels directly inhibited plant growth via high H^+ activity [10]. A high concentration of H^+ triggers typical oxidative stress on plants by inducing the accumulation of excess reactive oxygen species (ROS), such as superoxide radicals ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) in plant tissues [11,12]. To counteract oxidative damage, plants have evolved complex antioxidant systems including antioxidant enzyme,s such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DR), and antioxidants such as a-tocopherol, ascorbate, reduced glutathione [13]. Studies have indicated that higher activity levels of antioxidant enzymes may contribute to better H^+ tolerance by increasing the protective capacity against oxidative damage [14,15].

Crop tolerance of low soil pH has become extremely important in the agricultural development of the humid tropics because so many of those soils have low pH. In Indonesia, this tolerance can be important because there are extensive areas of soils with low calcium (Ca) and considerable areas with soil toxicity due to high levels of iron (Fe), aluminum (Al) and manganese (Mn). One strategy that can be done to improve crop yields in tidal swamps is to obtain rice genotypes that are tolerant of low pH stress and high pyrite. Through an effective method, it is hoped that rice germplasm will be obtained which has a tolerance gene for low pH and iron stress (pyrite) and will be a quick method to test the results of crosses to obtain a rice genotype tolerant to low pH and iron stress. In this approach, the initial stages that need to be available are rice lines with diversity that control stress tolerance of low pH and high pyrite. Available both of these will facilitate evaluation and decision making on the results of the study.

Thus, by observing changes in the morphological and physiological characteristics of rice and evaluating their photosynthetic performance during the low pH-treatment phase of local Kalimantan swamp rice during the vegetative period, we can determine the low pH-resistant rice genotype. The purpose of this study was to analyze physiological characters of local Kalimantan swamp rice cultivars in low pH condition.

METHODS

This research was conducted at Plant Physiology Laboratory, Faculty of Biology, UGM. The materials on the research used were 6 local swamp rice (*Oryza sativa* L.) cultivars from Kalimantan consisting of 'Amas', 'Pandan Ungu', 'Kambang', 'Sikin Merah', 'Argo Pawan', 'Suatek Merah', and 2 comparative cultivars Inpara 5' and 'Ciherang') as control and susceptible cultivars. Planting was carried out with a floating rafts hydroponic method with Yoshida growth solutions in 2 different pH treatment, pH 4 and pH 6.8.

The procedure in the research started with sterilizing the seeds using 10% Clorox for 15 minutes, washed with distilled water and soaked for 24 hours. Germination was carried out for 3–4 days at room temperature and in the darkroom. Uniform germinating seeds were chosen, then transferred to hydroponic styrofoam sheets measuring 33 × 26 × 2 cm consisting of 80 holes of 8 mm in diameter with a distance of 3 × 3 cm and have been coated with plastic gauze on the bottom and installed in a plastic container containing 8 L of full concentration of Yoshida nutrient solution. Each hole was used to grow one seed. The germination of those 8 cultivars were done randomly in one tray to minimize data error. Hydroponic Yoshida's solution in a plastic container was replaced once a week.

Three to four days old seedlings were acclimatized hydroponically in Yoshida's solution full concentration until 14 DAP (days after planting). The treatment of pH was carried out during the vegetative period from the age of 14 DAP to 35 DAP. The pH treatment used were pH 4 (low pH) and pH 6.8 (normal). Observation on morphological and phenotype changes was started from the germination stage (the measurement of germination percentage) then continued with growth characters including plant height, leaf length and width, and number of leaves. Plant height, leaf length and width, and number of leaves were measured every 7 days. Plant height was measured as the distance between the longest leaf tip and the root base above the rockwool surface. The leaf length and width were measured on the second leaf from the tip of every plant. The number of leaves was determined by counting the number of whole leaves in every plant. Root length was measured as the distance between the longest root tip and the root base in every plant cultivar.

Physiological characters were measured on chlorophyll and carotenoid content according to the Harborne method [16] with some modifications at 21 DAT. A leaf sample of 0.3 g was grinded with a mortar and homogenized with 3 ml of 80% cold acetone solution. Chlorophyll content was determined spectrophotometricaly (GENESYS 10 UV Scanning, Thermo Scientific) at multiwavelength of 470, 645, and 664 nm. Proline content was measured according to the Bates method [17] at 21 DAT. Proline content was determined by comparing the absorbance of the solution at wavelength 520 nm and compared with the proline standard curve.

Significance and interaction values of cultivars and pH treatment on plant growth parameters (plant height, leaf length and width, number of leaves) and plant physiological characteristics at the end of this research were tested with One-Way ANOVA and continued with Duncan Multiple Range Test (DMRT) conducted at 95% confidence level ($P<0.05$) with IBM-SPSS Ver. 23.0 (US).

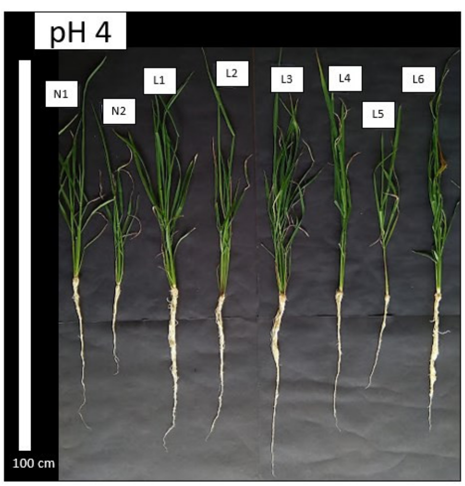
RESULT AND DISCUSSION

With a limited agricultural area, improving the fertility of marginal soils (such as tidal swamp land that has acid sulfate) is one of the ways to increase rice production worldwide [18]. Under normal condition, rice growing on this soil performs poorly due to Al^{3+}, Fe^{2+} or H^+ stress [19,20]. It was reported that low pH and high Al and/or Fe concentrations are the result of pyrite (FeS_2) oxidation when the soils are utilized for rice cultivation [21].

Local rice was directly obtained through exploration in the Kalimantan Province. The effect of low pH treatment was conducted by the measurement of physiological and biochemical characters in 2 variations of pH conditions, pH 6,8 (normal) and pH 4 (acidic/low pH). Local rice plants were compared to control rice plants of Inpara-5 (N1) as a tolerant rice and Ciherang (N2) as an intolerant/susceptible cultivars.

Root Morphology

The ability of a plant to optimize water absorption and nutrients from the soil/media are influenced by the root character. The morphology of root system is influenced by many factors included amount of fertilizer, temperature, growing media, and also environmental stress factors included high temperature, low pH, drought, salinity or heavy metal [22]. Generally, the longer of root, the greater the ability to absorb water from the growing media. In normal condition, variation among the root system of each cultivar represented different genetic diversity [23].



(a)

(b)

FIGURE 1. Root morphology of rice plants after 3 weeks of treatment (a) pH 4 and (b) pH 6.8. Code:N1= Inpara 5; N2= Ciherang; L1= Amas; L2= Pandan Ungu; L3= Kambang; L4= Sikin Merah; L5= Argo Pawan; and L6= Suatek Merah.

Based on the data in Figure 1, the longest root length was observed in 'Kambang' (in pH 4). When compared to control tolerant plants (Inpara-5) in acid condition (pH 4), almost all of these local cultivars have longer root. These results are different when compared with the research on lowland rice. Generally, it was found that high H^+ concentrations significantly suppressed root growth, reduced the development of fine (small diameter) roots and decreased the biomass of rice seedlings [24]. This research showed that 3 of 6 local Kalimantan swamp rice cultivars ('Amas', 'Pandan Ungu', and 'Kambang') have longer root in pH 4 compared to that of control tolerant 'Inpara 5'. It is indicated that some local swamp rice cultivars have a great ability to adapt and grow well in acidity condition (tidal swampland) (Fig. 1).

Physiological Characters

The growth ability of a rice plant can be observed through changes in plant height, increase in the number of leaves and tillers. The ability to grow to a certain height depends on the genetic character of a particular cultivar that it varies between cultivars. In addition, environmental factors that influence changes in plant height, number of leaves and number of tillers include light, nutrient availability in media/soil, water and other physicochemical factors including pH of planting media, soil temperature, CEC (soil exchange capacity) and moisture.

TABLE 1. Plant height, leaf length and width, and leaf number of Local Kalimantan and Comparative Cultivars aged 21 DAT

Code	Cultivar	Plant Height (cm)		Leaf length (cm)		Leaf width (cm)		Number of leaves (sheets)	
		pH 4	pH 6.8	pH 4	pH 6.8	pH 4	pH 6.8	pH 4	pH 6.8
N1	Inpara 5	17.97 a-e	12.56 a	13.11 abc	9.85 ab	0.43 abc	0.35 a	3.43 abc	3.04 abc
N2	Ciherang	21.96 cde	11.67 a	15.88 bc	9.46 a	0.51 bc	0.33 a	4.05 bc	2.87 ab
L1	Amas	23.00 de	13.78 ab	17.93 c	10.78 ab	0.53 c	0.32 a	4.07 bc	3.01 abc
L2	Pandan Ungu	24.68 e	13.36 ab	18.19 c	10.07 ab	0.52 c	0.33 a	4.18 c	3.14 abc
L3	Kambang	21.40 b-e	15.19 a-d	15.61 abc	12.26 abc	0.50 bc	0.36 ab	4.03 bc	3.29 abc
L4	Sikin Merah	17.30 a-e	14.30 abc	12.39 abc	10.45 ab	0.36 ab	0.34 a	3.43 abc	2.86 ab
L5	Argo Pawan	17.79 a-e	15.79 a-d	11.46 ab	11.45 ab	0.30 a	0.35 a	2.74 a	3.20 abc
L6	Suatek Merah	19.52 a-e	12.74 a	15.20 abc	10.43 ab	0.44 abc	0.33 a	3.27 abc	2.75 a

*Mean value Followed by the same latter in a column of each parameter shows no significance different at $p<0.005$

Low pH affected plant water uptake by causing a large and rapid decrease in water flow rate and the hydraulic conductivity in seedling roots of paper birch (*Betula papyrifera*) [25]. Due to this under unfavorable pH (stress environment), plants undergo lower growth and physiological performance.

In this study, observation on plant growth was carried out at 2 different levels of pH. In general, lowland rice cultivars showed a decrease in plant height in line with a decrease in the root lenght. Acidic condition can inhibit the process of nutrient dissolution, ion exchange, and further to the metabolism and photolysis in photosynthesis process. Based on the data in Table 1, it is known that several cultivars showed an increase in plant height in line with the condition of low pH which can be observed in the 'Amas', 'Kambang', and 'Pandan Ungu'. The increase in plant height and root could be due to the limited H^+ from the acid of water absorbed to carry out metabolic processes (respiration and photosynthesis) that inhibit a decrease in plant height.

In line with the character of plant height, the number of leaves increased in line with the condition of low pH. When compared to control tolerant 'Inpara-5' in acid condition (pH 4), only 'Argo Pawan' which has decreased in the number of leaves. The same results were also found in the leaf length and width. In low pH conditions, 3 of 6 local rice cultivars namely 'Amas', 'Pandan Ungu', and 'Kambang' have significantly increase in growth characteristics. Plants with the highest number of leaves in low pH conditions were 'Amas', 'Kambang', and 'Pandan Ungu' (Table 1). This indication reinforces that some local swamp rice cultivars have a high ability to adapt and grow well in acidic conditions (tidal swamps).

Chlorophyll Content

Chlorophyll and carotenoids are pigments that function to collect the energy of photons (light) and transfer them to the reaction center (photosystem) in the process of photosynthesis. By knowing the chlorophyll content in leaf tissue of a plant, it can also be known the influence of pH stress treatment on plant metabolic processes, especially photosynthesis. This character is indirectly related to the character of plant productivity. The results of measuring chlorophyll content are presented in Figure 2.

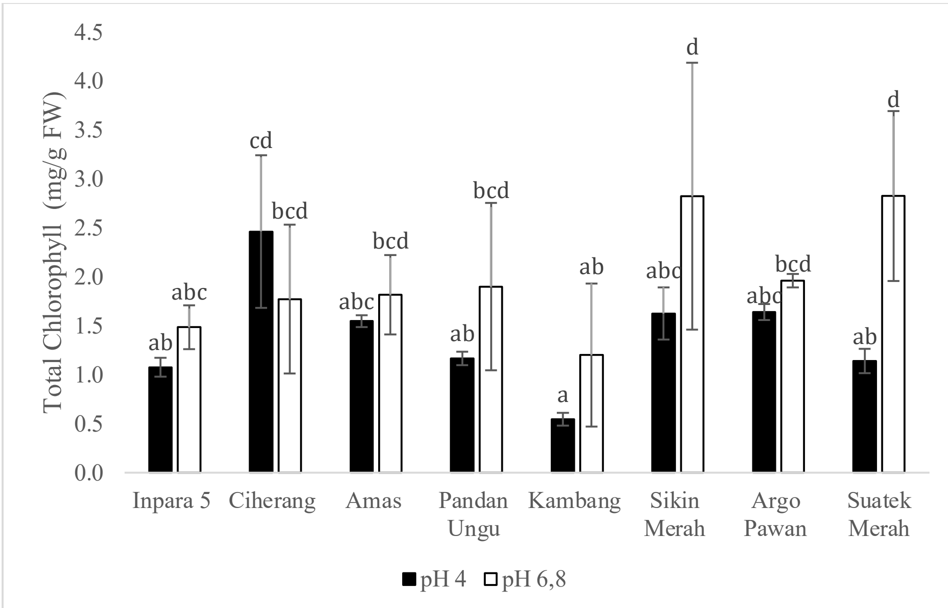


FIGURE 2. Total Chlorophyll content; in rice aged 21 DAP (Day after planting) treatment of low pH stress.

Based on the analysis of total chlorophyll content, there are differences in chlorophyll content in some rice cultivars. Total chlorophyll content in the treatment of acidic/low pH (pH 4) results were higher in 'Pandan Ungu' and 'Suatek Merah' (> 2000 mg / l) local types of rice, so that in this condition plants are assumed to be in normal condition to support photosynthetic processes. Meanwhile, 'Kambang' rice showed low results (<1500 mg / l). Treatment of pH stress can affect the content of chlorophyll pigments in plants. The effect of pH treatment on the total chlorophyll observed was not significantly different ($p > 0.05$). So, the low pH treatment did not significantly influence the total chlorophyll in rice.

Low pH in *C.sinensis* and *C.grandis* resulted in a decrease of CO_2 assimilation, by reducing stomatal conductance due to lower water uptake [26] and decrease of chlorophyll content of *Eucalyptus* leaves [27]. Specialized in rice of tidal swamp environment, most of the cultivars showed increasing in chlorophyll content as the photosynthetic pigment because tidal swamp rice was already adapted to acid environment and low oxygen level of root area. Photosynthetic pigments decrease with increasing pH. The local rice also has high potential to adapt to local condition with changing environments and system [28].

Carotenoid Level

Carotenoids are potential scavengers of reactive oxygen species (ROS) protecting pigments and unsaturated fatty acids of lipids from oxidative damage during pH stress [29]. Previous studies show that carotenoid also protects plant tissue by modulating photosynthetic compartments which involve xanthophyll cycle [30].

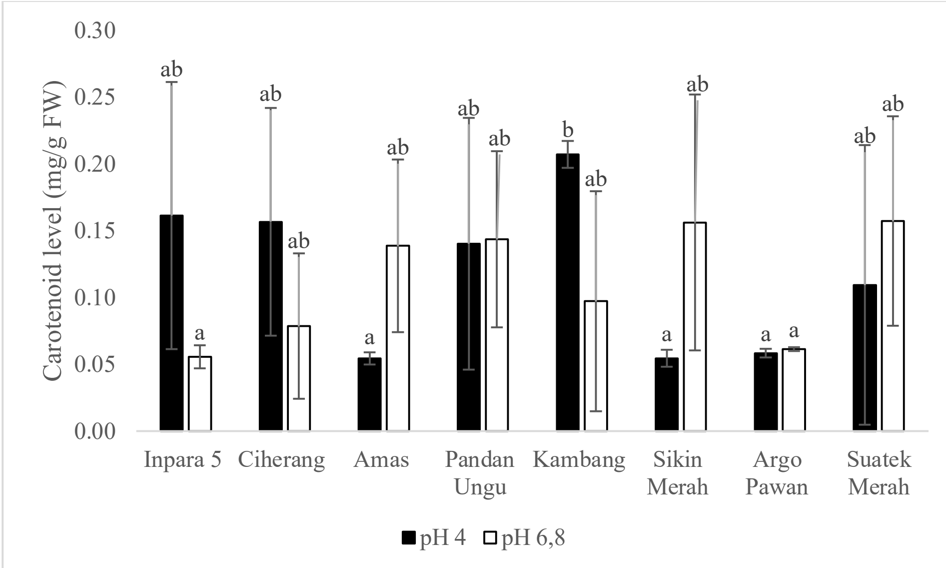


FIGURE 3. Carotenoid Level; in rice aged 21 DAP (Day after planting) treatment of low pH stress.

In the analysis of carotenoids, some rice cultivars showed an increase in carotenoid levels under conditions of acidic pH (pH 4). This is one form of the plant's response to stress. In stress conditions, plants will form self-defense and reduce the metabolic rate to survive. For this reason, a major photosynthetic pigment (chlorophyll) will be transformed into carotenoids. The effect of pH treatment on the carotenoid level observed was significantly different ($p < 0.05$). So, the low pH treatment significantly influences the carotenoid level in rice. In this study, the highest cultivar of carotenoids was 'Kambang' rice (Figure 3).

Proline Level

In the process of defense against acidity or low pH stress conditions (especially through osmosis adjustments), plants accumulate osmoprotectants in the tissues. Generally, osmoprotectants (dissolved compounds) produced belong to the group of sugars and amino acids including the highest levels of proline. Proline acts as an osmoregulator so that the higher the level, the greater the tolerance to low pH stress.

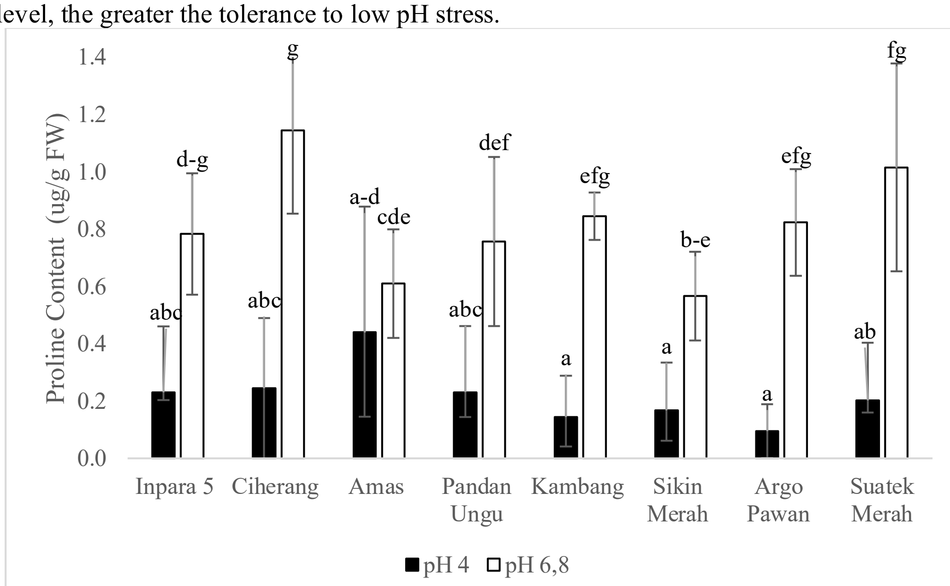


FIGURE 4. Proline Level Analysis in rice aged 21 DAP (Day after planting) treatment of low pH stress.

In general, plants that accumulate proline in stress conditions have better morphological features with higher survival compared to non-accumulator plants. Proline works through the mechanism of osmoregulation by regulating the osmotic potential in cells is proportional to the cell's external potential. Plants with higher levels of increased osmoticum are assumed to be plants that are more tolerant of pH stress. From figure 4, it can be seen that plants with the highest proline levels in acidity or low pH conditions is Amas cultivar. The effect of pH treatment on the proline level observed was significantly different ($p < 0.05$). So, the low pH treatment has a highly significant effect on increasing levels of proline in rice.

CONCLUSION

Acidity or low pH stress condition can affect the phenotypic character and the internal physiology of plants. Some Kalimantan local rice cultivars showed a tolerant character to low pH stress which is characterized by increased levels of carotenoid and proline and decreased levels of chlorophyll content. Based on physiological characters, 'Argo Pawan' is an intolerant cultivar while 'Amas', 'Kambang' and 'Pandan Ungu' are tolerant cultivars to low pH.

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