

Variation of morphology, anatomy and nutrition contents of local cultivar mentik rice based on the altitudes at Ngawi District, East Java, Indonesia

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Abstract. Suranto, Syahidah AT, Mahadjoeno E. 2018. Variation of morphology, anatomy and nutrition contents of local cultivar mentik rice based on the altitudes at Ngawi District, East Java, Indonesia. *Biodiversitas* 19: 652-659. The purposes of this research were to examine the variation in morphology, anatomy and nutrition contents of rice plants grown at three different altitudes at Ngawi District, East Java, Indonesia. The plant samples were collected from three different altitudes (200, 500 and 900 m asl. respectively). The morphological characters of leaf, root and stem were used in this examination, while Paraffin method was employed to look at the anatomical appearances. To look at the content of lipids and proteins, Soxhlet and Kjeldahl methods were used, while the Iodocolorymetre method was chosen to test the amylose content. Randomized Complete Block Design (RCBD) with one factor, namely altitude was used in this research. Morphological and anatomical characters and nutrition contents of rice plant were analyzed using one way of ANOVA. The results showed that the length and diameter of roots, plant height and also stem diameter were declining due to a higher altitude. On the other hand, the length of leaf, the number of roots, the total number of stomata and also the ratio of length per diameter of stomata increased, and so did the number of aerenchyma space roots and stems. In general, there was a tendency of decreasing amylose and protein contents due to higher altitudes. □

Keywords: Altitudes, morphology and anatomy, Ngawi, nutrition content, rice

INTRODUCTION

Indonesia as one of the largest tropical countries in the world which has been recorded to have many varieties of rice. At least 45 varieties of rice have been cultivated in the wet field, meanwhile, more than 150 varieties are usually planted for dry field (Rigg 1862). And more than 40,000 varieties of rice have been documented. These varieties include *Oryza glaberrima* which is quite often called African rice, and also the Canada rice or *Zizania* rice. Both varieties of rice species of *Oryza* have been classified into Poaceae family (Simpson 2008). It is recorded that many varieties of rice could grow not only on low lands but also on a few different high altitudes (Hasanah 2007; Zhang et al. 2017b). The ability of this rice plant to grow and develop under different environmental conditions such as tropical and sub tropical areas, has made many countries around the world possible to be the producers. The diversity of rice varieties in Indonesia is quite easily recognized by the colors of seed pericarps such as white, red and black. In addition, this variety of rice was also noted from the smell. □

One of the very popular kinds of local rice in Java Island is Mentik. This rice has been considered to have a high percentage of carbohydrate with a typical smell like jasmine flower. As a source of energy, this kind of rice has been consumed as edible food not only for Indonesians but also for Asian people and many world population in general (Saragih 2001; Yuan et al. 2017). The nice smell of

this local mentik rice has been believed as the results of 2-acetyl-1 pyrroline (2AP) substance in the mesocarp (Wongpornchai 2003; Tinakorn et al. 2006; Wijaya et al. 2008). Due to the facts that the texture and the flavor of this rice are quite good, many Indonesian people tend to use as a preference in their family for daily consumption. This very nice smell of rice was also found in several countries such as Thailand and UK. Within Asian countries, there have been many kinds of aromatic rice grown widely. In India and Pakistan, the name of Basmati rice is very popular. This rice is usually called the Prince of rice. Meanwhile, in Thailand, the jasmine rice or the Thai fragrant rice is very easy to find. This Jasmine variety of rice could be distinguished by the white and brown pericarp seeds, and the last one is noted to have stronger nice smell like Jasmine flavor. Another rice variety producing nice smell was the American aromatic rice. Such rice is developed using the Basmati and Jasmine rice (Pazuki et al. 2013). The growth and development of all variety of rice would be influenced by their environmental conditions.

Under favorable conditions such as quality and quantity of light, the growth, and development as well as cell differentiation, this plant would eventually determine the quality of rice seed pods (Abidi et al. 2013). Many environmental factors such as temperature and light intensity influence on the plant growth have been studied intensively (Fan et al. 2013; Chen et al. 2016; Li et al. 2017). Under lower environmental temperature, plant could

only produce limited growth, productivity and geographical distribution (Maali-Amiri and Heidarvand 2010). Meanwhile, under high light intensity, the environmental temperature will increase, and this can cause the plant stomata to be opened in order to reduce the water loss (Mildaerizanti et al. 2012; Fukui et al. 2017). Another environmental factor which is considered to have an influence on the plant growth and development is altitude. Higher altitudes of plant habitats would produce lower percentage (%) of primary metabolisms such as lipid or even other nutrition contents (Kadarisman et al. 2011). This phenomenon has been reported by Suranto et al. (2015), although the used sample was pumpkin. Pumpkin plants growing at higher altitudes tended to have lower contents of carbohydrate, lipids, and proteins when they were compared to lower one. This experiment was conducted to examine whether or not the local rice of Mentik which grew on different altitudes at Ngawi District East Java (Indonesia) varied in their morphology, anatomy and nutrition contents as pumpkin plants did. Ngawi district was chosen to be the sample location because this district was recorded as one of rice production centers in East Java (BPS 2015). In addition, we also documented that altitude differences were also shown by morphological and anatomical variations of rice plants.

MATERIALS AND METHODS

Seedling preparation

The seeds of local rice mentik were prepared and planted by the Department of Agriculture of Ngawi District, East Java, Indonesia. Three different locations of rice fields namely at 200, 500 and 900 m asl. (meter above sea level) respectively were used to grow the rice samples (Figure 1). This was conducted from January to June 2016. The above three planting areas were treated similarly under irrigated farming system. These plants were given a fertilizer twice in two weeks time for the first month, after planting and once a month after word. □

Stems and root samples □

Thirty-six (36) rice plants were used in this experiment. The stems were measured for the lengths started from the based internode to the top. Accordingly, the diameter of stem internode was also treated similarly. All the measurements were repeated three times for every single altitude. For the roots, morphology both for the length and diameter were done after washing down with tap water. The total number of root samples was also 36. The length of the roots was collected from the longest root of every single plant, whilst the root diameter was also taken from the widest root of single plant sample used. □

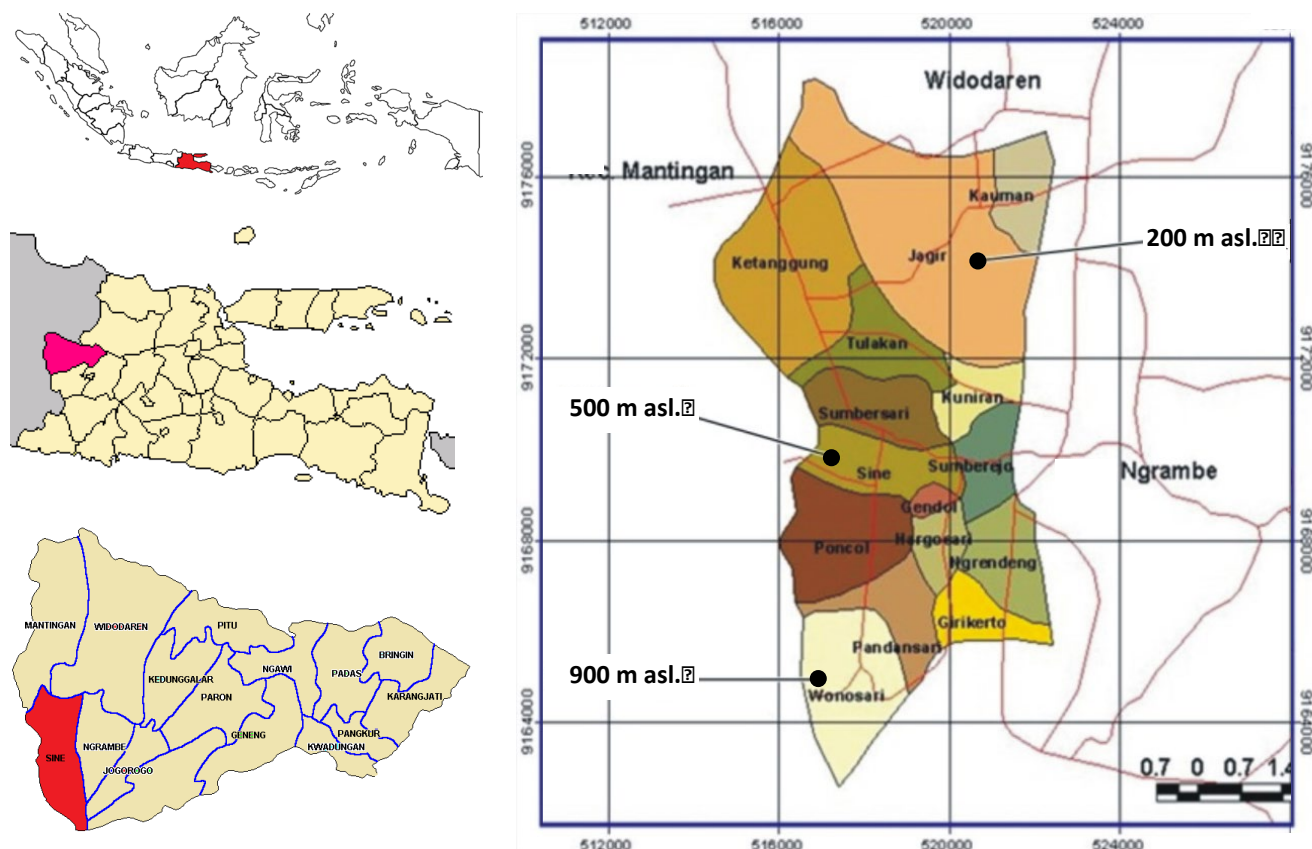


Figure 1. The map showing areas where samples are collected in Sine Subdistrict, Ngawi District, East Java Province, Indonesia

Leaf samples

The observation of leaf characters was conducted using thirty-three (33) individual plants in every single altitude. The measurements of leaves in every single plant were conducted during flowering time. The first, second and third leaves from the top were measured for the length and width. All these data of every single altitude were repeated three times, and the results were presented in Table 3.

The observation of morphological characters such as stem and leaf was conducted using the guidance of evaluation and characterization of rice plants published by Institute of Research and Development, under the Department of Agriculture (2003).

Anatomical observation

Anatomical observation of stem, roots, and leaves was conducted using Paraffin methods (Sass 1958). For observation of leaf stomata, the method of Rompas (2011) was used, while in determining the density of stomata the formula of Lestari (2006) was employed. In order to look at the content of Carbohydrate (amylose), Iodocolorymetre method of Yuliano (1994) was employed. Meanwhile, to examine the lipids and protein contents, Soxhlet and Kjeldahl methods were used respectively (Horwitz 2000). Statistical analysis of one-way ANOVA was used to look at whether or not there was a difference between attitudes and their biochemical contents of this rice. □

RESULTS AND DISCUSSION

Morphological character

As shown in Table 1, the longest root was recorded at 200m asl (11.01 cm), while the shortest was found at 900m asl (8.51 cm). These indicated a reducing root length in accordance with increasing the plant's altitude. Conversely, the total number of root will significantly increase plants growth at the highest altitude (31.41) compared to the lowest one (25.35). At low altitudes, the light intensity and temperature usually become higher, but the rainfall becomes lower. It resulted in a puddle on a land rice plant becoming a bit less productive. The roots of the rice plant that lives on a farm with a little puddle will be longer, as to get water even further into the ground (Talpur et al. 2013). The number of plant roots will increase in more puddles as the plants have to adapt to the water content in the soil which is too high to avoid induction of cell damage (Levitt 1980). It is interesting to note that the ability of rice to the limited water condition may relate to the rice genotype and environmental condition. Zhang et al. (2017a) recorded that under low minimum temperature at higher altitude and rainfall deficit at lower altitude, the new variety PR 23 rice have been able to adapt to the new environmental condition and resulted in high yield. This new variety of perennial rice has been considered to be one of the rice cultivars that could be cultivated under environmental water stress conditions. □

As seen in Table 2, it is predicted that the tallest plant (91.91 cm) is found at the lowest altitude (200 m asl) as

compared to the highest altitudes (85.21 cm). The differences of plant height may be caused by the magnitude of the intensity of the sunlight received by the plants. The increasing altitude could cause the decreasing plant height. At the lower altitude, the sunlight and also temperature usually become higher. The high temperature and light in the reasonable values will induce the process of growth and development of plants (Ndour et al. 1999). It is interesting to note that there is no difference in term of the internode diameters in the number 1, 2, 3 and four at all altitudes. Only internode number five showed real difference at an altitude of 900 m asl (0.20 cm) as compared to 0.38 cm at 200m asl. The low light intensity at a higher altitude may cause the diameter of the plant stem to decrease. This is because plants need light to the power of the stems (Jenabiyan et al. 2014). Plants growing at high altitudes usually have thicker and smaller leaves.

One of the very crucial environmental factors in determining activities of photosynthesis of plants is solar radiation. Light intensity in certain particular habitats is usually varied. This variation of light intensity may have been correlated with altitudes. Under such conditions, many plants have ability in adjusting the different light intensities in order to grow better and develop. The effect of light intensity on the growth of *Salvinia officinalis* has been investigated by Zervoudakis et al. (2012); they recovered that at least, the number of leaves and leaf photosynthetic figments of this plants have a strong positive correlation with the light intensity. On the other hand, under low light intensity, the treated plants showed the increase of their light and leaf photosynthetic pigments. These results suggest that *S. officinalis* is one of the good adaptable species due to the different light intensities.

It was recorded that in low light condition of plant, the activity of the whole plants would reduce the biomass of root, stems, leaves and photosynthetic rate, transpiration as well as water vapor. Conversely, the plant height will be increased under low light intensity. And leaves of plants expanded during high irradiance presented lower photosynthetic pigment content than leaves expanded under low irradiance (Zervoudakis et al. 2012). □

The usefulness of altitudes and seasonal factors on the leaf morphology and anatomical features of *Origanum vulgare* was studied by Kofidis et al. 2013. Increasing the activities resulted in progressive decrease of plant height. Accordingly, plants growing at higher altitudes end to have blade size of leaves reduced. During June, as compared to the October leaves. Meanwhile, at middle on lower altitudes, no difference in size leaves was found. In addition, during the growing period, more or less the thickness of leaves remained stable. Meanwhile, expanded leaves were observed in June and October at low (200 m) altitude. The highest chemical photo-efficiency of this plant appeared in the mid-altitude population (900 m) which were characterized by larger and thicker leaves with highly developing palisade and spongy parenchyma (Kodifis et al. 2003).

Table 1. Morphological measurement of roots of local Mentik cultivar of rice at three different altitudes

Parameters	Altitudes (m asl) ($\bar{x} \pm SD$)		
	200	500	900
Root length (cm)	11.01 ^b ± 0.48	10.68 ^b ± 0.30	8.51 ^a ± 0.50
Root diameters (cm)	0.13 ^a ± 0.02	0.12 ^a ± 0.14	0.11 ^a ± 0.12
Total number of root	25.35 ^a ± 1.30	25.69 ^a ± 0.46	31.41 ^b ± 3.07

Table 2. Morphological measurement of stem of local Mentik cultivar of rice at three different altitudes

Parameters (cm)	Altitudes (m asl) ($\bar{x} \pm SD$)		
	200	500	900
Plant hight	91.91 ^b ± 1.28	88.51 ^{ab} ± 4.00	85.21 ^a ± 1.14
Plant diameter first internode	0.60 ^a ± 0.05	0.54 ^a ± 0.03	0.56 ^a ± 0.02
Second internode	0.59 ^a ± 0.13	0.51 ^a ± 0.04	0.50 ^a ± 0.03
Third internode	0.46 ^a ± 0.05	0.44 ^a ± 0.04	0.43 ^a ± 0.02
Fourth internode	0.38 ^a ± 0.04	0.37 ^a ± 0.03	0.36 ^a ± 0.01
Fifth internode	0.38 ^a ± 0.26	0.26 ^a ± 0.01	0.20 ^a ± 0.01

Table 3. Morphological measurement of leaf local mentik cultivar of rice at three different altitudes

Leaf length (cm)	Altitudes (m asl) ($\bar{x} \pm SD$)		
	200	500	900
First leaf	24.69 ^a ± 0.88	31.69 ^b ± 1.09	32.03 ^b ± 0.29
Second leaf	35.61 ^a ± 1.98	42.39 ^b ± 0.36	41.19 ^b ± 0.48
Third leaf	41.44 ^a ± 0.57	46.89 ^b ± 1.61	50.85 ^c ± 1.19

Table 4. Total number of aerenchyma space of local mentik cultivar of rice at three different altitudes

Parameters (number of aerenchymas) □	Altitudes (m asl) ($\bar{x} \pm SD$)		
	200	500	900
Root	30.90 ^a ± 4.72	48.50 ^b ± 5.87	57.10 ^c ± 3.51
Stem	17.70 ^a ± 1.89	23.40 ^b ± 1.42	29.60 ^c ± 2.63

The increasing leaf length was also recorded at plants grown at higher altitudes as presented in Table 3; and the longest leaf was found at third leaf at 900 m asl (50.85 cm), while the shortest was recorded at 200 m asl (24.96 cm) at first leaf. This occurrence could be related to the ability of plants in capturing light. On the higher altitudes, the intensity of the sunlight becomes lower, so the plants have to adapt to the environmental condition in using the light as much as possible for the photosynthetic purpose. Pantilu et al. (2012) mentioned that the low light intensity produces longer, larger and thinner leaves of plants. Long, wide and thin leaves are needed on the environmental conditions so that the plant can capture as much light as possible with reflected light as low as possible. The increased wide leaves allow increasing wide

areas of the plant to catch the light (Khumaida 2002). A hight factor directly connecting the irradiation of shoot and the elongation of root tops is the local hexose concentration, which correlates very well with growth roots of individual roots of a given species (Nagel et al. 2006). In maize, the length of the growth zone decreased with decreasing light intensity in much the same way as with decreasing water availability. Comparison of maize root growth in decreasing light intensities indicates that a reduction in root elongation rate takes about 4 days. □

Leaf size variation across species may be related to the traits of twig level such as twig size, wood density or internode length (Milla 2009). Leaf size of the individual plants as the very basic unit of foliage display's useful in responding/coping the ecological limitation of harvesting light. Under low light intensity, *Arabidopsis thaliana* showed the pattern of plasticity in increasing either the specific leaf area or leaf number (Pigliucci and Kolodynska 2002). A study in looking at the influence of altitude change in the leaf morphology within three different grass species have been conducted by Zhong et al. 2014. Each species responded differently to altitude change. All three species had significant difference in their leaf traits across an altitudinal gradient. And leaf thickness of all three species increased with the increasing altitude. □

Anatomical observation

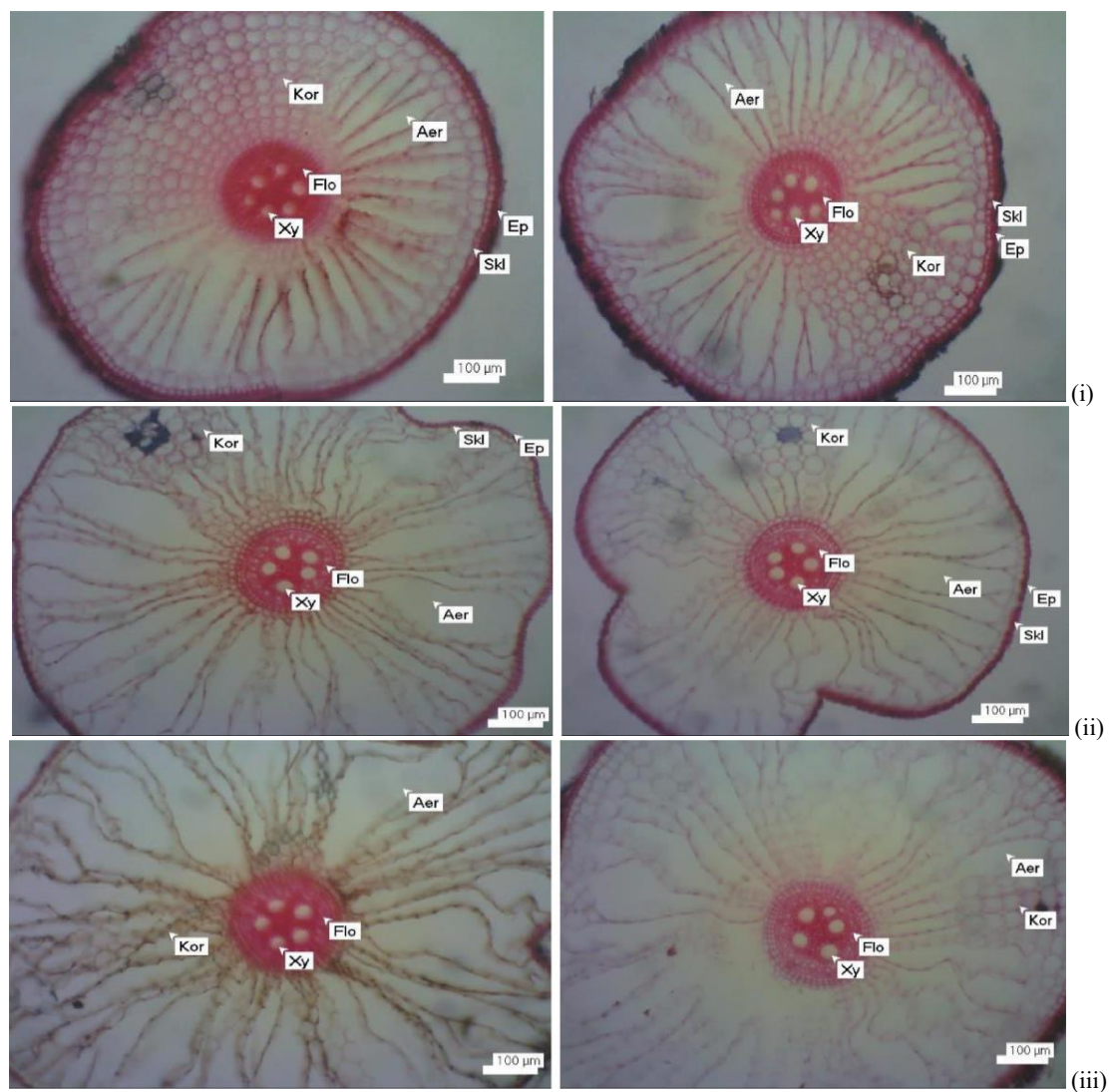
The total number of aerenchyma space at stems and roots significantly increased at higher altitudes (Table 4). Total numbers of aerenchyma space were recorded at root sample rather than stems. The highest number of aerenchyma space on the root was recorded at 900m asl (57.10), and the lowest was only 30.90. Meanwhile, the highest total number of aerenchyma space on the stem at 900m asl was only 29.60 and lowest was 17.70. □

Further treatments on both cross-section stems and leaves at three different levels of altitudes (Figure 2) revealed that the formation of root aerenchyma increased drastically along the higher altitudes of plant habitats. It was recorded that almost all areas of cortex on the roots disappeared and were replaced by these aerenchyma tissues. Presumably, the formed root aerenchyma was due to lysis process of the cortex cell in the middle part and then moved to edge laterally. The light intensity became lower along the increasing altitude, and this could cause the water content in the soil to increase, so the plant would adapt the environment to obtain air to the root zone. In the studies conducted by Kundur et al. (2015) on the observation of the cross-section of the roots of rice plants grown in waterlogged soil, it showed that structure of aerenchyma was found more than rice crops grown in a dry land. Under unfavorable condition such as low nitrogen stress, plants caused to reduce the cell diameter and to increase the amount of aerenchyma, although the number of cell layer in the crown root cortex was unchanged (Gao et al. 2015). Similar experiment to the environmental stress was also done by Abiko et al. (2012) in dealing with waterlogging. Aerenchyma was formed constitutive in the roots of many wetland species. This occurrence also found in dry land of *Zea mays*. This phenomenon showed that the

formation of aerenchyma is induced by waterlogging. This is because the land had a little stagnant air content in the soil, so the plants form more aerenchyma to get air from the shoot. □

In order to look at whether or not different altitudes influence total leaf stomata, the cross-section of fresh leaves was conducted. As seen in Figure 2, the total number of stomata increased drastically from 20/mm² (200 m asl) to 36.9/mm² (500 m asl) and reached a total number of 59.5/mm² at 900 m asl. Similar pictures were also shown for the ratio of length per diameter of stomata. On the altitude of 200m asl, an average number of only 19.87 and 15.55 μm were observed for the length and diameter respectively. In the studies conducted by Holland and Richardson (2009) in which the samples were collected from two broadleaf tree species (*Betula papyrifera* var. *cordifolia* and *Sorbus americana*) and two herbaceous understory species (*Cornus canadensis* and *Dryopteris*

carthusiana), they found that the density of stomata increased along with the increasing level of plant altitudes. Treshow (1970) recorded that the size of stomata usually became larger too. The increasing number of stomata at higher altitude could be interpreted as extraordinary efforts of plants in having the limited conditions such as low light intensity. By having more stomata, the photosynthetic activity would be more efficient as conducted by modifying/ altering leaf anatomy or morphology (Evans and Pooter 2001; Sopandie et al. 2003). Recent studies on the transpiration efficiency in relation to leaf anatomy were conducted by Ouyang et al. (2017) using rice and wheat plants. Under unfavorable condition such as water deficit condition, wheat showed to have higher plasticity in their roots and morphological and anatomical adaptation than rice plants. Meanwhile, rice plants which have smaller stomata responded strongly to drought than the bigger ones. □

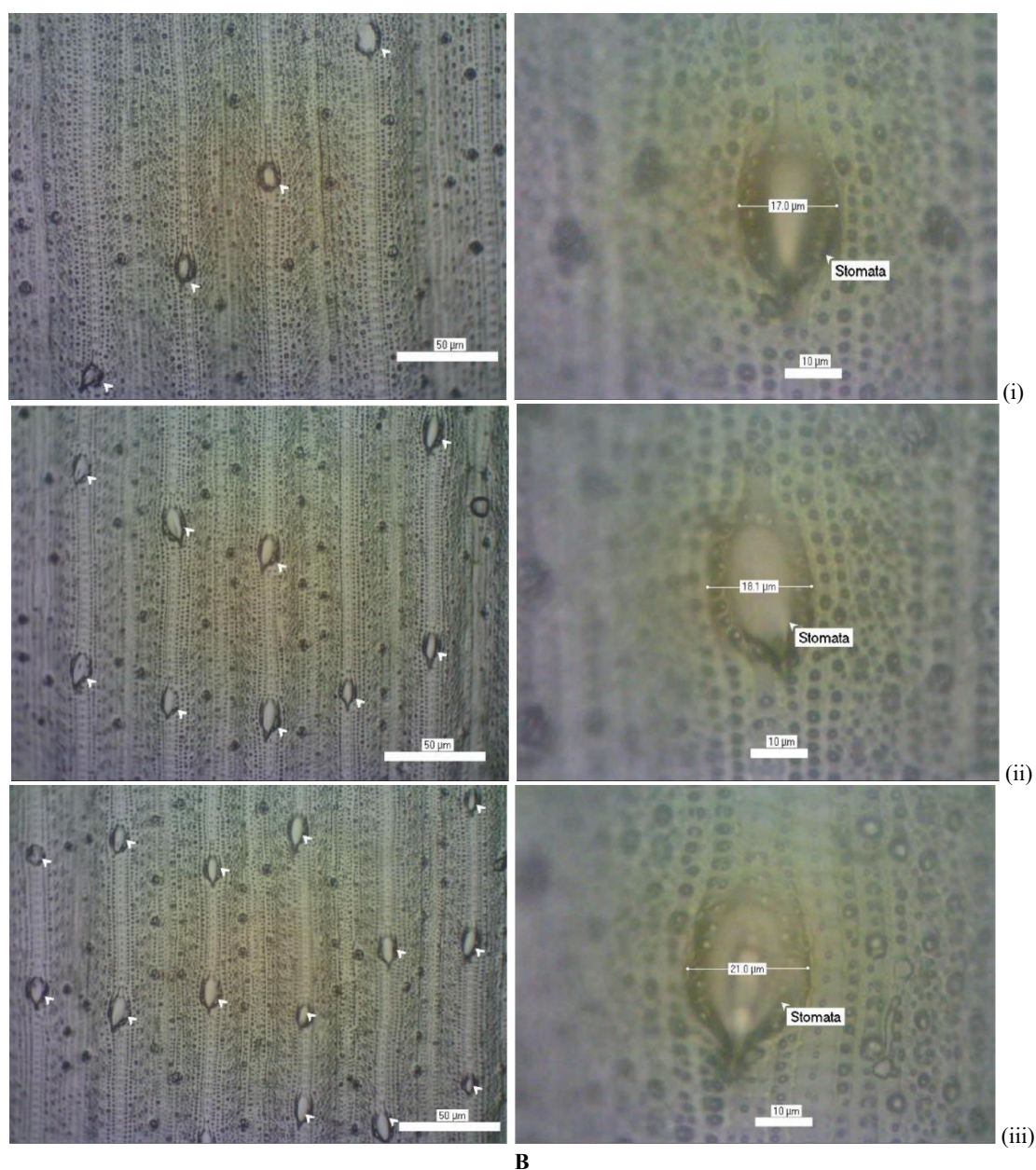


Nutrition contents (carbohydrates, lipids, and protein)

The amylose contents of rice as shown in Table 5 increased drastically along with the higher altitudes, but after 500m asl the contents of amylose decreased drastically. The highest contents of amylose were at 500m asl (18.75%ww) and it decreased drastically to become (15.67%ww) at 900 m asl. The highest percentage of protein contents of rice was recorded at 200m asl (7.37%ww) and the lowest was at 900m asl (5.69%ww). In general, there was a tendency of decreasing amylose and protein contents at plants growing at higher altitudes. This phenomenon has been recorded by Suranto et al. (2015).

Table 5. Value of nutritions content of local cultivar mentik of rice at three different altitudes□

Parameters	Altitudes (m asl) ($\bar{x} \pm SD$)		
	200	500	900
Amilosa (%ww)	17.43 ^b ± 0.28	18.75 ^c ± 0.41	15.67 ^a ± 0.43
Protein (%ww)	7.37 ^b ± 0.59	6.32 ^a ± 0.42	5.69 ^a ± 0.37
Lipid (%ww)	1.85 ^a ± 0.14	1.93 ^a ± 0.11	1.83 ^a ± 0.12

**Figure 2.** Cross section of fresh prepared roots (A) and leaves (B) of local Mentihik rices. Sample were taken at three different altitudes ; (i) 200 m asl (ii)500 m asl and (iii)900m asl. Notes. Ep = Epidermis; Scl = Sclerenchym; Aer = Aerenchym; Cor = Cortex; Flo = Floem; Xy = Xylem

Pumpkin plants grown at higher altitudes tended to have lower contents of carbohydrate, lipids, and proteins when they were compared to lower one. The lower contents of amylase, protein, and lipid will eventually influence the rice physically and chemically (Haryadi 2008). In addition, the quality of rice especially the aromatic rice such as mentik was also influenced by temperature and altitudes (Nagarajan et al. 2010). Although, this experiment was using rice plants, the similar results of reducing percentage (%) of Nutrition Values at pumpkin fruit such as amylose and proteins at plants grown at higher altitudes were real evident which was useful in contributing to the understanding of the plant phenomenon, that plants which grew at high altitudes almost always produce a bit more decreasing in the primary metabolism. High altitude was not the only factor in causing the reduction of plant nutrients including quantity. Another factor such as low temperature and rice cultivar have been considered to have contribution too. Certain cultivars of cold tolerance at booting state (CTB4a) has shown a good adaptation to cold environmental condition (Zang et al. 2017). This finding of relatively new variety of Japonica rice may offer a new strategy to improve cold tolerance in crop plants. And this eventually will be useful in providing the food and nutrition for more than half of the world's population (Chen et al. 2016).

In conclusions, the length and diameter of roots, plant height and also stem diameter were declining due to a higher altitude. On the other hand, the length of leaf, the number of roots, the total number of stomata and also the ratio of length per diameter of stomata increase. Those also occurred on the number of aerenchyma space roots and stems. In general, there was a tendency of decreasing level of amylose and protein contents due to a higher altitude. This rice plants data particularly the biochemical contents were also shown by other plants such as pumpkin fruits. □

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