

Rice Root Distribution of Four Rice Varieties to Different Depth of Submergence

Arinal H. I. Nurrahma¹⁾, Ahmad Junaedi^{1*)}, Heni Purnamawati¹⁾ and Jun-Ichi Sakagami²⁾

¹⁾Faculty of Agriculture, Bogor Agricultural University
Kampus IPB Dramaga Bogor West Java Indonesia

²⁾Faculty of Agriculture, Tropical Crop Science, Kagoshima University, Kagoshima, Japan

^{*)} Corresponding author e-mail: junaedi_agr@yahoo.com

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ABSTRACT

Rice (*Oryza sativa* L.) is largely cultivated in lowland area that vulnerable to flood occurrence. Root supposed to play an important role on rice tolerance to submergence stress. An experiment which conducted from January to May 2014 in Cikarawang Field Laboratory, Darmaga, Bogor, West Java aimed to study the role of root distribution on rice submergence tolerant. The experiment used Split Plot design replicated three times, with the depth of submergence as main plot and rice variety as subplot. The main plot consisted of water depth of 5 (optimum), 50, 60 and 80 cm from soil surface. The subplot consisted of four rice varieties, namely Ciherang, Inpari 30 (Ciherang Sub1), Indragiri and Margasari. Each plant was planted in a root box (30 cm x 40 cm x 5 cm) which put into container (67 cm x 47 cm x 42 cm) and placed into the pond with water depth as treatment. Submergence started when the plant was 39 Days After Seeding (DAS) for ten days. Observation conducted after the submergence stopped (49 DAS). Result showed that submergence retarded plant growth, root distribution and increase root damage for all rice varieties with the biggest effect on susceptible varieties.

Keywords: flash flooding; *Oryza sativa* L.; root box

INTRODUCTION

Rice (*Oryza sativa* L.) is largely cultivated in the South and South-East Asia which vulnerable to flood occurrence. About 30 % of the total world rice area was covered with rainfed lowland rice which was flooded for at least part of the season and rice was often the only choice as a single crop which grown all year (Haefele, Atlin, Kam, & Johnson, 2004). Lowland rice areas are susceptible to temporary submergence which became the major

constraints to the productivity. Recent decades, global climate change has affected the phenomenon of flood cases due to the combinations of increasing rain intensity and durations, precipitations and soil characters. The frequency of rainfall cases has increased in many parts of Asia (Bates, Kundzewicz, Wu, & Palutikof, 2008) such as increasing 3000 mm of rainfall each year in the period September - November in Vietnam (van Drunen, Lasage, & Dorland, 2006) and increasing heavy rainfall events in Krishna Basin, India (Malano et al., 2015).

The enhancement frequency of flood cases in rice cultivation areas need to be considered from related stakeholders to prevent the food scarcity mainly in the countries where rice is the only source of staple food can be growth. There are two approaches for achieving the high yielding plant in the stress environment; rectifying the cultivation methods and improving physiological characters to creates new tolerant variety. Plant physiological features diverge in various type of flood which required details and depth study to jump on the general conclusion. It has been known that rice develops two contrast strategies to deal with flash flood and stagnant flood (Hattori, Nagai, & Ashikari, 2011; Nagai, Hattori, & Ashikari, 2010; Bailey-Serres, Lee, & Brinton, 2012). The plant will inhibit their growth when water covers the plant and regrowth after the water receded in the case of flash flooding. This mechanism called as conserve strategy where Sub1A gene played a role (Septiningsih et al., 2014). While stagnant flooding requires shoot elongation ability to get the water surface and oxygen access as soon as possible. This contrary mechanism did not support temporary flash flooding because their shoot would wilt and die after the flood (Hattori, Nagai, & Ashikari, 2011; Nagai, Hattori, & Ashikari, 2010).

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Flood is soon followed by the hypoxia or anoxia where the rice swap towards anaerobic metabolism specifically in roots. Since roots are the plant system which associated with water and nutrient uptake, microbial interaction and other signals related to plant and its environment, then the reduction of plant growth was firstly noticed on abiotic stress. The alteration to hypoxia leads the reactive oxygen species (ROS) simultaneously produce H_2O_2 . The unavailability of oxygen generate the anaerobically induced polypeptides (ANPs) including the rise to ACC, the ethylene biosynthesis precursor. This signal transported from the root to the aerial part of the plant in the shoot. ACC in the presence of oxygen and ACC-oxidase configure the ethylene. Conversion ACC to ethylene was assumed as the determining factor of tolerance and facilitating plant regulatory function (Irfan, Hayat, S., Hayat, Q., Afroz, & Ahmad, 2010).

The formation of ethylene induced the auxin mediating the aerenchyma formation below the root tip (Irfan, Hayat, S., Hayat, Q., Afroz, & Ahmad, 2010). Aerenchyma is the space between the cell which develop maximum and commonly found in the stem and root of hydrophytic plants. This tissue was very important to the gas exchanges (Mulyani, 2006). The aerenchyma can be formed through lysigenous or schizogenous. The lysigenous process resulted from the cell death (cell death programmed) while schizogenous occurred through cell separation during tissue development (Drew, He, & Morgan, 2000; Evans, 2003). It was expected that 15-50 % of the shoot carbohydrates production were carried away to maintain the root growth and development (Lynch, Marschner, & Rengel, 2012). As return, water and nutrients were served by the root to the shoot (Shabala, White, Djordjevic, Ruan, & Mathesius, 2015). Plants must precisely partitionate energy for root growth and nutrient uptake due to plant growth limited resources that used for root growth and function (Lynch, Marschner, & Rengel, 2012).

Rice is widely grown by the farmer in Indonesia using the specific variety to obtain the best yield in different location and condition. Ciherang is a newly released variety which dominantly cultivated in Indonesian lowland rice area since 2004 (Nurhati, Ramdhaniati, & Zuraida, 2008) while Inpari 30 (Ciherang Sub1) is the next generation from Ciherang vs IR64-Sub1 with the additional tolerant character to flash flooding (Septiningsih

et al., 2014). The other common varieties of rice are Indragiri and Margasari which are called as Indonesian swampy rice varieties (Suprihatno *et al.*, 2010). These four rice varieties were used in this experiment to compare the response of rice variety with the Sub1 gene in it or not and also to compare to other local swampy varieties. Adaptation mechanisms may vary in different plant growth stage and varieties. Because of the root is firstly affected by the anaerobic condition, it needed deeply information about its tolerance and susceptible role to submergence stress. This experiment was aimed to study the role of rice root distribution to submergence stress.

MATERIALS AND METHODS

Experiment was conducted in January to May 2014 at Cikarawang Field Laboratory, Darmaga, Bogor, West Java, Indonesia. Microscopic observation of root cross section was done in Chromatography Laboratory, Departement of Agronomy and Horticulture, Bogor Agricultural University. Split plot - Randomized Complete Block Design was used in this experiment with depth of submergence as the first factor and rice varieties as the second factor. The first factor as main plot consist of four levels depth of submergence, i.e. 5 cm, 50 cm, 60 cm and 80 cm which measured from the soil surface to the water surface. The second factor as subplot consist of four rice varieties from Indonesian Center for Rice Research (ICRR), including Ciherang, Indragiri, Inpari 30 (Ciherang Sub1) and Margasari. The treatment had 16 combinations with three replications and totaling had 48 experiment units.

Root box methods used in this experiment was modified from Kono, Yamauchi, Nonoyama, Tatsumi, & Kawamura (1987). The root box size is 30 cm x 40 cm x 5 cm which both of side made from glass that covered with black silver mulch and put into a container of size 67 cm x 47 cm x 42 cm and placed into the pond with water depth as their treatment. Rice seeding sow in polybag with the mix media 1:1 of compost and top soil. Rice seedling of 18 DAS were transplanted into the root box then placed in a container with different height level of water as the treatments. NPK fertilizer were applied as much as 2 g per plant before the submergence treatment. The submergence started on 39 DAS for 10 days.

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The observation measured based on growth components as below:

1. Plant height and number of tillers were measured at 32 and 39 DAS (after the submergence stopped).
2. Number of leaf and leaf area measured after the submergence stopped using Portable Leaf Area Meter (LI-3000C, USA).
3. Root cross section was observed by cutting 2 cm of root from the tip. Then the root was stored in the plastic with the water inside to keep the root freshness until the observation using a microscope.
4. Root distribution observed based on its zone in root box. The observation started after the submergence stopped. Glass from one side of root box was opened and the root distribution imaged with gridline to difference the root zone 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm. Root dry weight measured based on depth and radius distribution from the base of clump, they were zona of:

- A : depth of 0-10 cm, with radius 0-5 cm
 A' : depth of 0-10 cm, with radius 5-15 cm
 B : depth of 10-20 cm, with radius 0-5 cm
 B' : depth of 10-20 cm, with radius 5-15 cm
 C : depth of 20-30 cm, with radius 0-5 cm
 C' : depth of 20-30 cm, with radius 5-15 cm
 D : depth of 30-40 cm, with radius 0-5 cm
 D' : depth of 30-40 cm, with radius 5-15 cm

The roots which fused with the soil of each zone separated into pieces. Each piece cleaned in a tub of water to separate the roots, and then the roots were air dried and put into an envelope dried in an oven for 72 hours at 60 °C.

5. Shoot and root biomass measured using the digital scale.

Data were analysed using analysis of variance (ANOVA). If significant, means would be separated by Least Significant Difference (LSD) test at $P < 0.05$. Contrast orthogonal was used to analyse the difference of total root dry weight of each zone.

RESULTS AND DISCUSSION

Plant Growth on Submergence Stress

The depth level of submergence reduced plant growth during the experiment conducted. The deeper level of submergence reduced 27.3 % of additional plant height and 81.7 % of the additional number of tiller (Table 1). Submergence also reduced 77 % of the number of leaves from the optimum condition (5 cm) and reduced 90.3 % of leaf area per plant and 64.0 % of leaf area per leaf, however there were no significant differences between the depth of 50 cm, 60 cm and 80 cm (Table 2). Reducing plant growth occurred due to the changing environment into complete submergence. When the water fully submerged the shoot, delayed of CO₂ entry and light penetration to the leaves tended to limit the photosynthetic process (Colmer & Voesenek, 2009).

Inpari 30 had lower growth rate during submergence compare to Ciherang and Margasari, whilst Indragiri had no significant difference of growth rate with other varieties (Table 1). Tolerant rice developed the conserve strategy which inhibits its growth during fully flash flooding to save the energy and used it to re-grow after the water receded (Hattori, Nagai, & Ashikari, 2011; Nagai, Hattori, & Ashikari, 2010).

Table 1. Plant height and number of tillers observed from rice varieties to various depth of submergence

Treatment	Plant height (cm)			Number of tillers		
	32 DAS	49 DAS	Δ	32 DAS	49 DAS	Δ
Depth of submergence						
5 cm	36.47	75.78 a	39.31 a	2.0	17.9 a	15.9 a
50 cm	34.00	54.00 b	20.00 c	2.0	4.9 b	2.9 b
60 cm	34.29	62.86 b	28.57 b	1.8	4.8 b	3.0 b
80 cm	32.19	55.67 b	23.48 bc	1.7	3.4 b	1.7 b
Rice varieties						
Ciherang	35.04	65.44	30.40 a	1.4	7.8	6.4
Inpari 30	36.51	58.15	21.64 b	2.2	7.9	5.7
Margasari	34.58	66.31	31.73 a	2.1	8.1	6.0
Indragiri	30.92	58.41	27.49 ab	1.8	7.3	5.5

Remarks: Number which followed by the same alphabet in the same column showed no significant difference with LSD (Least Significant Difference) test at $P < 0.05$; DAS = Days after sowing; Δ = additional plant height or number of tillers

Table 2. Number of leaves, leaf area per plant and leaf area per leaf observed at 49 DAS from rice varieties to various depth of submergence

Treatment	Observed parameter		
	Number of leaves	Leaf area per plant (cm ² per plant)	Leaf area per leaf (cm ² per leaf)
Depth of submergence			
5 cm	73.0 a	1256.39 a	17.85 a
50 cm	17.0 b	121.26 b	6.42 b
60 cm	14.7 b	100.15 b	6.23 b
80 cm	9.4 b	61.34 b	6.18 b
Rice varieties			
Ciherang	29.2	409.90	9.79
Inpari 30	28.4	391.52	9.23
Margasari	33.8	410.30	8.40
Indragiri	22.8	327.50	9.26

Remarks: The number which followed by the same alphabet in the same column showed no significant difference with LSD (*Least Significant Difference*) test at $P < 0.05$; DAS = Days after sowing

Table 3. Shoot dry weight, root dry weight and its ratio on rice varieties and different depths of submergence

Observed parameter	Rice variety	Depth of submergence				Average
		5 cm	50 cm	60 cm	80 cm	
Shoot dry weight (g)*	Ciherang	11.99 a	1.58 c	1.59 c	1.20 c	4.09
	Inpari 30	12.39 a	1.25 c	1.52 c	1.40 c	4.14
	Margasari	11.87 a	2.05 c	0.98 c	0.52 c	3.85
	Indragiri	7.68 b	1.22 c	2.64 c	1.21 c	3.18
	Average		10.98	1.53	1.68	1.08
Root dry weight (g)	Ciherang	2.58	0.34	0.23	0.17	0.83
	Inpari 30	3.20	0.37	0.23	0.10	0.96
	Margasari	3.27	0.27	0.20	0.13	0.97
	Indragiri	2.44	0.40	0.49	0.13	0.87
	Average		2.87 A	0.34 B	0.29 B	0.13 B
Ratio of shoot/root dries weight	Ciherang	4.65	4.65	6.91	7.05	5.82
	Inpari 30	3.87	3.38	6.61	14.00	6.97
	Margasari	3.62	7.59	4.90	4.00	5.02
	Indragiri	3.14	3.05	5.39	9.30	5.22
	Average		3.82 B	4.67 B	5.95 AB	8.59 A

Remarks: Value which followed by the upper alphabet in the same row showed no significant difference; (*) Showed the interaction with Least Significant Difference test at $P < 0.05$

Shoot and Root Dry Weight

The submergence reduced the shoot and root dry weight but increased the shoot-root dry weight ratio. Root dry weight in the optimum condition (5 cm) is higher than other submergence conditions. While a ratio of shoot/root dry weight showed a contrast result where the 80 cm depth of submergence had higher ratio of shoot/root dry weight than 5 cm and 50 cm (Table 3). Plants hold their functional of equilibrium between shoot as carbohydrates source and roots as water and nutrients source. Condition

which restrict photosynthesis could be the limitation factor of all the process in shoot and affect in reducing relative allocation at root to maintain the equilibrium function (Lynch, Marschner, & Rengel, 2012).

Ciherang, Inpari 30 and Margasari on optimum condition (5 cm) had the highest shoot dry weight compared to Indragiri in the same condition and compared to all submergence condition (Table 3). It showed that the depth of submergence reducing shoot dry weight in all rice varieties.

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An adequate supply of carbohydrate from the latter photosynthesis or starch reserved from shoot as the origin of energy was relied on the root growth and development (Lynch, Marschner, & Rengel, 2012; Shabala, White, Djordjevic, Ruan, & Mathesius, 2015). In return, roots arranged the shoot growth by serving inorganic nutrients and even signaling distribution (Shabala, White, Djordjevic, Ruan, & Mathesius, 2015). In that case, the environmental conditions which affect photosynthesis may impact on the root growth by affecting carbohydrate supply to the root (Lynch, Marschner, & Rengel, 2012).

Root Dry Weight Distribution

The optimum condition had the biggest root dry weight distribution compared to other submergence treatment. It can be defined that the submergence reduced more than 82 % root dry weight distribution in all zone observed while it showed no significant difference between the depth of submergence 50 cm, 60 cm and 80 cm (Table 4). Rice varieties statistically did not show any difference in root distribution. The root distribution pattern from all of the varieties was quite similar. The root mostly distributed vertically until the zone of C and then starting to be distributed horizontally (zone C' and D').

The root distribution was narrower due to the depth of submergence treatment. The plant which submerged at the depth of 50 cm, the root distributed only to 30 cm vertically (zona C) and

20 cm horizontally (zona B'). While the depth of 60 cm limited the root distribution to 20 cm vertically (zona B) and horizontally (zona B') and the depth of 80 cm had root distribution vertically only to 20 cm (zona B). It is implied that as depth as the submergence, plant would firstly reduce root growth vertically prior to develop root growth horizontally until the submergence depth inhibit the plant from getting the oxygen supply with the compromise of developing root growth vertically in shallow. In the short duration of submergence, adventitious roots in the sediment had a little importance compare to the water (Colmer & Voeselek, 2009).

The Comparison of Total Root Dry Weight in Different Zone

The total root dry weight of zone A and B were significantly different from others, but total root dry weight both of zone C and D had no significant different due to the amount of root dry weight on treatment. These data also showed that the amount of root growth at zone A was the biggest of all zones, followed by zone B that bigger than zone C and D (Table 5).

The root growth was largely distributed on the top zones due to the distance from the soil surface. This data supports the previous statement that the root distribution decreased as far as the distance from the root growth point in all condition. This is assumed because of the availability of nutrients around the soil surface and other factor that influence the root growth.

Table 4. Dry weight root distribution of rice varieties to various depth of submergence

Treatment	Dry weight root zone distribution (g)							
	A	A'	B	B'	C	C'	D	D'
Depth of submergence								
5 cm	1.38 a	0.24 a	0.29 a	0.20 a	0.09 a	0.09 a	0.01 a	0.01 a
50 cm	0.24 b	0.01 b	0.04 b	0.02 b	0.01 b	0.00 b	0.00 b	0.00 b
60 cm	0.18 b	0.02 b	0.03 b	0.01 b	0.00 b	0.00 b	0.00 b	0.00 b
80 cm	0.02 b	0.00 b	0.01 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Rice varieties								
Ciherang	0.35	0.05	0.10	0.07	0.06	0.03	0.00	0.01
Inpari 30	0.57	0.07	0.13	0.07	0.01	0.03	0.00	0.01
Margasari	0.54	0.08	0.08	0.05	0.01	0.02	0.00	0.00
Indragiri	0.43	0.07	0.07	0.05	0.01	0.02	0.00	0.00

Remarks: The number which followed by the same alphabet in the same column showed no significant difference with Least Significant Difference test at $P < 0.05$

Table 5. The comparison of total root dry weight of each different zone

Total root dry weight			
Zone A (0.62 g)	Zone B (0.22 g)	Zone C (0.08 g)	Zone D (0.01 g)
A	b		
A		b	
A			b
	a	b	
	a		b
		a	a

Remarks: The same alphabet in the same row showed no significant difference with contrast orthogonal test at P<0.05

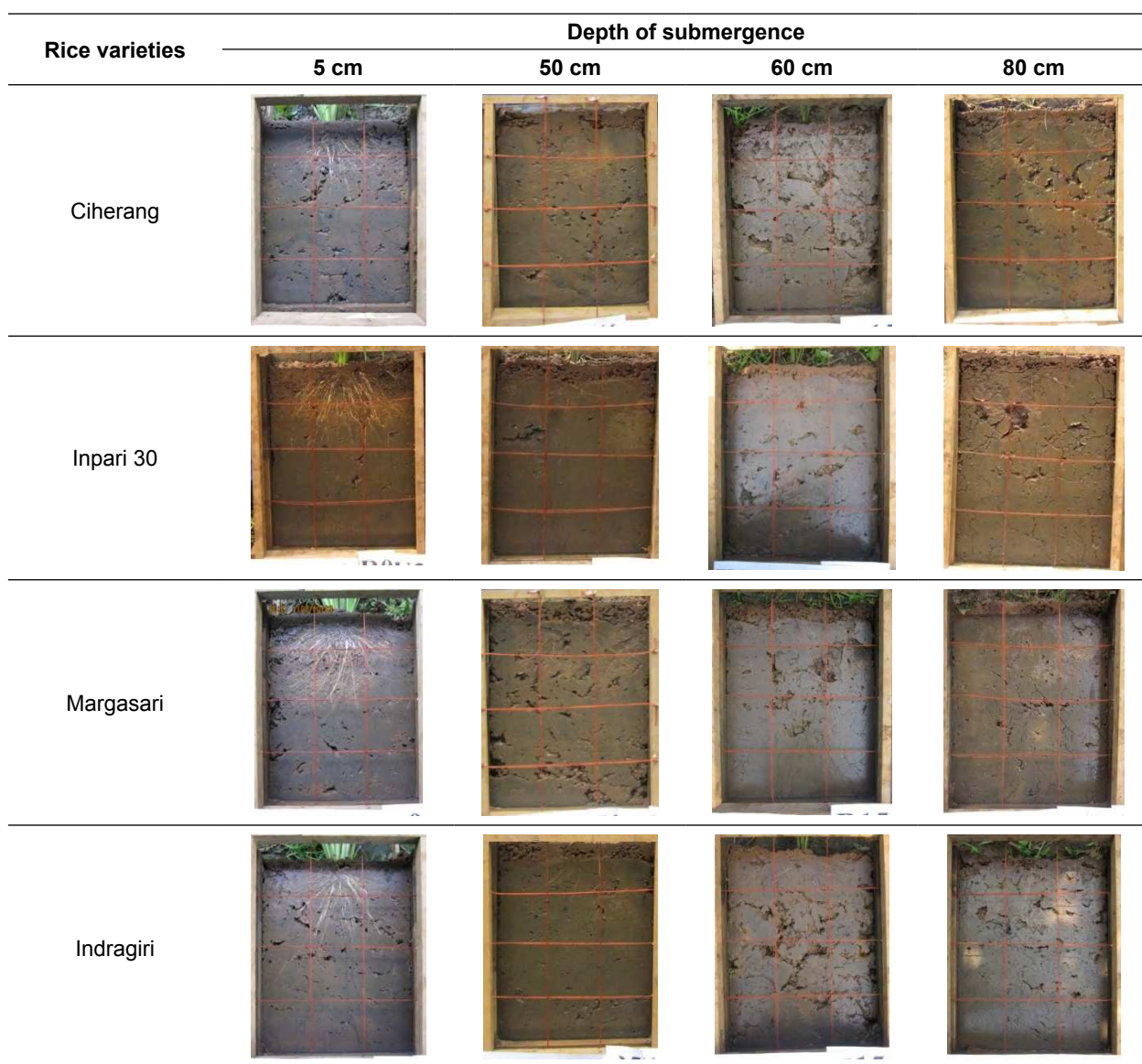


Fig. 1. Root distribution of rice varieties to different depth of submergence

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Imaging of Root Distribution and Root Cross Section of Different Depth of Submergence

The root dispersion showed from the root box after removing the mulch and glass. All rice varieties in optimum condition showed the clear root distribution and the submergence also reduced the visual appearance of root distribution (Fig. 1). The root tissue of four rice varieties in optimum condition did not show the damage and started to enhance the damage as the depth of submergence. The root breakdown occurred in the cortex which the optimum condition (5 cm) showed the compact structure on the root cross section. As the depth of the submergence, the cortex breakdown triggered the channel formation which called as aerenchyma. The root structure of Ciherang and Margasari under the depth of submergence 80 cm showed the

severe cortex degradation in contrast with Indragiri and Inpari 30 which still have cortex covering their endodermis (Fig. 2).

Hypoxia resulted in a various response on plant growth metabolic process which help the continuity of root function during this stress condition. Commonly the gases are diffused slower in submerged condition than aerial condition. The scarcity of oxygen happened during plant tissue respiration, then ethylene trigger various response on aerenchyma formation, adventitious root formation and length of shoot elongation. Roots responded to the scarcity of oxygen supply or the high accumulation of ethylene in plant tissue through increasing aerenchyma formation (Lynch, Marschner, & Rengel, 2012).

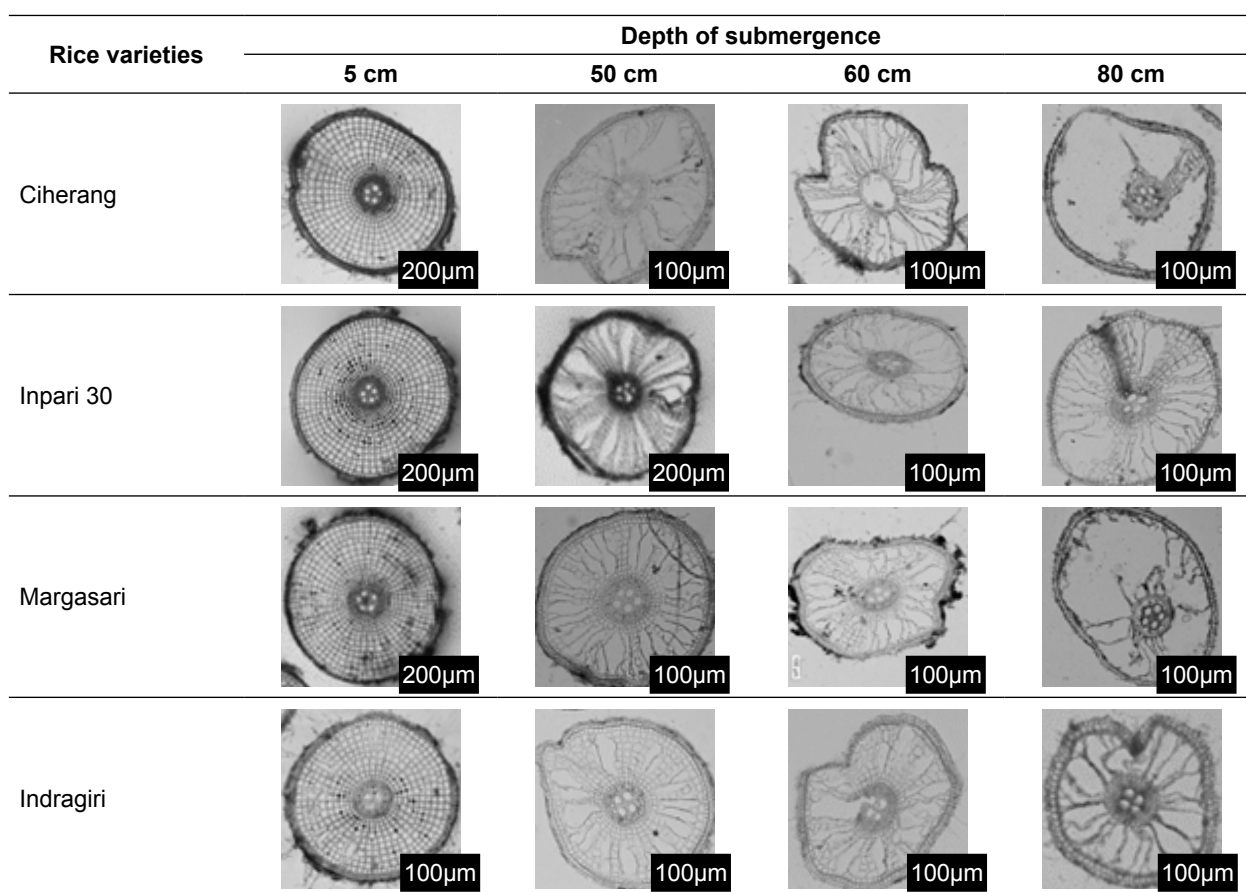


Fig. 2. The differences root crosses-section of rice varieties to different depth of submergence

Table 6. Correlation of the observed parameters

Observed parameters	ANoT	NoL	LAP	LAL	SDR	RDR	RSR
Additional plant height (cm)	0.51**	0.55**	0.56**	0.56**	0.61**	0.56**	0.04ns
Additional number of tillers		0.85**	0.85**	0.79**	0.86**	0.91**	-0.37*
Number of leaves			0.95**	0.77**	0.93**	0.91**	-0.33*
Leaf area per plant (cm ² per plant)				0.89**	0.96**	0.92**	-0.33*
Leaf area per leaf (cm ² per leaf)					0.83**	0.81**	-0.27ns
Shoot dry weight (g)						0.95**	-0.27ns
Root dry weight (g)							-0.41**

Remarks: (ns) = not significantly different; (*) = significantly different at $P < 0.05$; (**) = significantly different at $P < 0.01$; ANoT = Additional number of tiller; NoL = Number of Leaf; LAP = Leaf area per plant; LAL = Leaf area per leaf; SDR = Shoot dry weight; RDR = Root dry weight; RSR = Ratio of Shoot – Root

The aerenchyma formation was the adaptation response to the internal oxygen deficit by facilitating oxygen movement directly from the shoot to the root (George, Horst, & Neumann, 2012). The anaerobic plant commonly has root system which allows the aerenchyma formation, the intercellular channel which facilitated the oxygen and other gases exchange. The living cells separated air space in the cortex which formed by the death cells. Consequently, it formed the tissue structure similar the unstructured wheel which shown on the treatment depth 50 cm of submergence (Fig. 2). The lysigenous process has benefit on the deficit oxygen condition to make the specific channel for facilitating oxygen diffusion, on the other hand the death process reduce the oxygen consumption by the cell and the adaptation respond to the deficit oxygen (Evans, 2003).

Correlation between the Observed Parameters

An additional number of tillers, the number of leaves, leaf area per plant and per leaf, and shoot dry weight had significant strong correlation each others except the ratio of shoot root. It implied that the increasing of one component will affect in the increasing another component. Shoot dry weight and root dry weight had close correlation with all parameters observed except the additional plant height. Shoot dry weight also had close correlation with root dry weight which implied that shoot and root growth were growing in balance. Most of the parameters observed shows a negative weak correlation to shoot – root ratio (Table 6).

CONCLUSION AND SUGGESTION

The study concluded that the submergence retarded plant growth, root distribution and increase root damage for all rice varieties. Submergence

depth under 50 cm was enough to make root damage of all rice varieties, so the further research should be conducted between 0-50 cm to get the critical depth or threshold in which the depth of submergence start giving damage to rice root.

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