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Morpho-Physiological and Anatomical Character Changes of Rice Under Waterlogged and Water-Saturated Acidic and High Fe Content Soil

(Morfo-Fisiologi dan Perubahan Ciri Anatomi Padi di bawah Pengelogan Air dan Tanah Air Tepu Berasid serta Tinggi Kandungan Fe)

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ABSTRACT

Waterlogging is one of the limiting factors in crop cultivation. Moreover, high iron (Fe) content in acidic soils could also disturb plant growth. However, there is limited scientific information of morpho-physiological and anatomical responses of rice grown in waterlogged acidic soils with high Fe. Therefore, the objective of the research was to investigate the morpho-physiological and anatomical responses of rice to waterlogged and water-saturated soil condition in acidic soil with high Fe. Morpho-physiological and anatomical characters of rice were evaluated. The results showed that the waterlogging in acidic and high Fe content soil disturbed the rice growth as indicated by the change of morpho-physiological and anatomical characters. The water-saturated soil showed better condition for rice cultivation than that of waterlogging. The plant biomass, root anatomical, lipid peroxidation level, Fe absorption, and leaf gas exchange parameter could be evidences of changes in rice under both conditions. Based on the waterlogging tolerance coefficient (WTC), we proposed shoot and root dry weight, cortex thickness, and Fe content in shoot as screening tools for waterlogging tolerance of rice in acidic and high Fe content soil. The finding offers insight about waterlogged condition in acidic and high Fe soil could be restored in crop cultivation.

Keywords: Leaf gas exchange; root anatomical; waterlogging; water-saturated

ABSTRAK

Pengelogan air merupakan salah satu faktor pembatasan dalam penuaian tanaman. Selain itu, kandungan besi (Fe) yang tinggi dalam tanah yang berasid juga boleh mengganggu pertumbuhan tanaman. Namun, terdapat maklumat saintifik yang terhad berkenaan morfo-fisiologi dan tindak balas anatomi terhadap pertumbuhan padi di kawasan tanah berasid pengelogan air dengan kandungan Fe yang tinggi. Oleh itu, objektif kajian ini adalah untuk mengkaji morfo-fisiologi dan tindak balas anatomi padi terhadap pengelogan air dan tanah tepu air dalam keadaan tanah berasid dengan kandungan Fe yang tinggi. Morfo-fisiologi dan ciri anatomi dinilai. Keputusan kajian menunjukkan bahawa pengelogan air berasid dan kandungan Fe yang tinggi di dalam tanah mengganggu pertumbuhan padi seperti yang ditunjukkan daripada perubahan morfo-fisiologi dan ciri anotomi. Tanah tepu air menunjukkan keadaan yang lebih baik untuk penuaian padi berbanding kawasan pengelogan air. Biojisim tumbuhan, anatomi akar, tahap pemperoksidaan lipid, penyerapan Fe dan parameter pertukaran gas daun boleh menjadi bukti untuk mengkaji perubahan padi di bawah keadaan yang ditetapkan. Berdasarkan pekali toleransi pengelogan air (WTC), kami mencadangkan berat kering pucuk dan akar, ketebalan korteks serta kandungan Fe pada pucuk sebagai alat saringan untuk toleransi pengelogan air padi di kawasan berasid dan kandungan Fe yang tinggi dalam tanah. Hasil kajian memberikan pandangan berkenaan keadaan pengelogan air berasid dan kandungan Fe yang tinggi dalam tanah dapat dipulihkan dalam penuaian tanaman.

Kata kunci: Air tepu; anatomi akar; pertukaran gas daun; takung air

INTRODUCTION

Roots as the plant organ play an important role in the absorption of mineral nutrition. The ability of the roots to absorb mineral nutrition and support plant growth depends on their soil environment. Soils with available and adequate mineral nutrition, sufficient organic matter, and good drainage will support the root ability to absorb mineral nutrition. Conversely, soils with less nutrient availability, highly soluble toxic metal ion, low pH, less organic matter, and bad drainage will decrease the ability of root to absorb mineral nutrition as well as to support plant growth.

The waterlogged condition, which is commonly found in tidal and swampy land, creates low oxygen level in the rhizosphere. The condition could inhibit root respiration, decrease soil pH, and increase metal ion solubility, i.e.: Iron (Fe^{3+}), Aluminum (Al^{3+}), and Manganese (Mn^{4+}) (Grzesiak et al. 1999; Matin & Jalali 2017), which consecutively will inhibit plant growth and decrease yield. Many previous studies have been reported that the waterlogging impairs morphological and physiological characteristics of several grass species, such as barley (*Hordeum vulgare* L.) (Garthwaite et al. 2003), tropical forage grass (*Brachiaria* spp.) (Cardoso et al. 2013), corn (*Zea maize* L.) (Boonlertniruna et al. 2010; Grzesiak et al. 1999; Iu et al. 1982; Yamauchi et al. 2019), wheat (*Triticum aestivum* L.) (Bramley et al. 2011; Malik et al. 2001; Singh & Setter 2015; Sundgren et al. 2018; Yamauchi et al. 2019; Yavas et al. 2012), millets (Matsuura et al. 2016), and rice (Nishiuchi et al. 2012; Suralta & Yamauchi 2008; Yamauchi et al. 2019).

Administration of water supply management while maintaining good drainage is an effective strategy to solve the problem in waterlogged acid soils. Management of water supply through saturation of soils with water provides beneficial condition for plants grown in tidal or swampy soils. Water-saturated soil condition increased the soil pH and reduced the toxic effect Fe and Al (Noya 2014; Sagala et al. 2019). As it has been reported in several previous studies, water-saturated soil offers evidence of increasing crop productivity in tidal and swampy soils. The water-saturated condition of tidal acidic soil increased the growth and yield of soybean (Ghulamahdi et al. 2016; Sagala et al. 2019), bean (*Phaseolus vulgaris* L.) (White & Molano 1994) and rice (Ghulamahdi et al. 2012; Harahap 2014; Nguyen et al. 2009). However, there was poor scientific information about the morphological, anatomical, and physiological changes of rice responses to the change of waterlogging to water-saturated condition of soils, especially in acidic and high Fe content soils. In the present study, we evaluated the morphological, anatomical, and physiological responses of rice plant to waterlogged and water-saturated condition in acidic soil with high Fe content. The findings could be useful knowledge basis for improving sub-optimal lands (tidal and swampy acidic soil) productivity for rice cultivation.

MATERIALS AND METHODS

EXPERIMENTAL SETUP

The experiment used four rice varieties with different tolerance levels to Fe toxicity based on our previous study (Turhadi et al. 2018), i.e.: IR64 (Fe-sensitive), Inpara 5 (Fe-sensitive), Inpara 2 (Fe-moderate tolerant), and Pokkali (Fe-tolerant).

The experiment was conducted from late March to early May 2018 in the Taman Bogo Experimental Station, Lampung Province, Indonesia (5˚00’18”S; 105˚29’13”E) with an altitude of 27 m a.s.l. The weather of the station during the experiment as described as follows: temperature (26.0-27.0 °C), humidity (85.0-87.0%), duration of sunshine (60.9-65.2%), and rainfall (148-274 mm) according to Statistics Indonesia (2019a, 2019b). The soil type used for the experiment is categorized as low fertile sandy clay loam (ultisol) with very high Fe content (Table 1).

TABLE 1. Physical and chemical soil characteristics of Taman Bogo Experimental Station, Lampung Province, Indonesia

Characteristics	Values
Sand (%)	50.5
Silt (%)	24.7
Clay (%)	24.8
pH	5.1
Organic carbon (%)	1.4
N-total (%)	0.1
C/N ratio	11.4
K^+ (cmol kg^{-1})	0.1
Na^+ (cmol kg^{-1})	0.3
Ca^{2+} (cmol kg^{-1})	1.8
Mg^{2+} (cmol kg^{-1})	0.3
CEC (cmol kg^{-1})	3.9
Fe total (ppm)	12 209.7

The experiment was arranged as a split block design with three replications. There were 3 blocks in this experiment which consisted of two main plots in every block with two different treatments, i.e.: waterlogging and water-saturated soil condition (Figure 1). Water level in the waterlogged plots were kept at 5 - 10 cm above soil surface, while the saturated soil plots were kept at 5 - 10 cm below soil surface by checking regularly every day using a ruler. If water was reduced, the experimental plots were watered using local irrigation water until reached the required water level. In order to prevent the experimental plots from entering or exiting water and to avoid metal ion leaching due to water flow, the water channels were closed. There were four rice varieties as a subplot in each main plot, therefore, each block consisted of 8 experimental units with 96 individual rice planted in 3.5 × 2.5 m area each unit.

Rice seeds from each variety were soaked in distilled water for 48 h before sowing to the seedling beds until they reach 3 week-old seedlings. The uniform seedlings (20 - 30 cm) were transplanted in the experimental plots with one individual plant for each planting hole. The waterlogging condition was carried out by adjusting the water level at 5 - 10 cm above the soil surface, while the water-saturated treatment was carried out by adjusting the water level at 5 -10 cm below the soil surface.

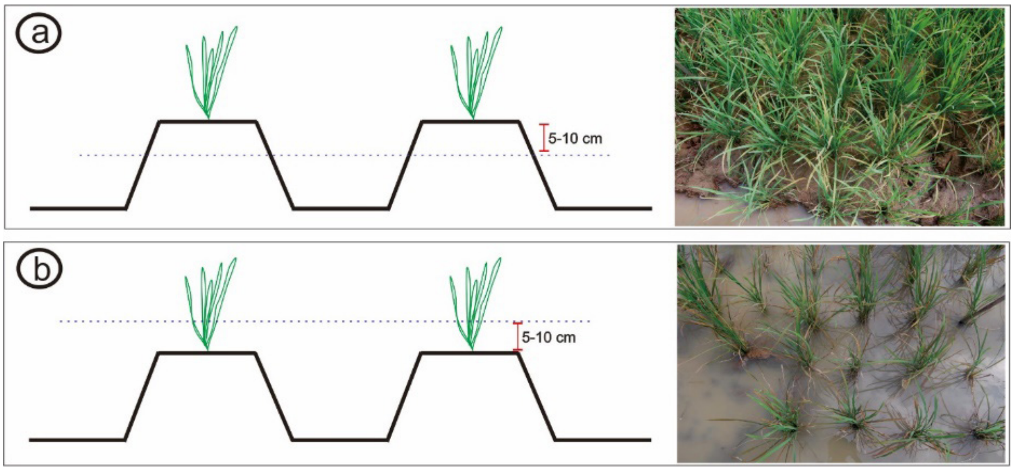

FIGURE 1. Condition of water surface for water-saturated soil (a) and waterlogging (b) treatments during the experiment. The 5 - 10 cm indicated as water surface height from the soil surface

MEASUREMENT OF PLANT GROWTH

The plant growth characters observed in this study were shoot and root fresh and dry weight. Shoot and root biomass were harvested from each individual plant at 40 days after transplanting. The shoots fresh weight was measured after it was washed with water to clean from soil residues, while roots fresh weight was measured after the plaque dissolved into 2 M HCl for 2 h using a shaker at 150 rpm. The shoots and roots dry weight were measured after being dried at 80°C for 72 h.

MEASUREMENT OF ROOT ANATOMICAL CHARACTERS

Forty-days after transplanting, roots were harvested, washed with water to clean from soil residues, then preserved in 96% ethanol. In each sample, three intact crown roots were selected and used for root anatomical observation. Root section samples of 1 - 1.5 cm from the root tips, where the aerenchyma formation begins, were prepared for analysis (Zhu et al. 2015). Cross-section of the roots were prepared by hand-sectioning using a razor blade according to Yamauchi et al. (2016). The sections were then observed under light microscope Olympus CX21 (Olympus Optical Co. Ltd., Tokyo, Japan) and photographed using the Optilab camera (Miconos, Indonesia). Root anatomical features were measured using ImageJ software 1.48v (Schneider et al. 2012).

MEASUREMENT OF FE CONTENT IN SHOOT TISSUES

Shoot samples of 40 days after transplanting were incubated in an oven 80 °C for 72 h. Fe content of shoot tissues were extracted according to Association of Analytical Communities (AOAC) (2012) using HNO_3-

$HClO_4$ digestion procedures (HNO_3:$HClO_4$ = 3:1, v:v). The Fe content was determined using atomic absorption spectrophotometer (AAS) Agilent 200 Series AA Systems (Agilent Technologies, Inc, USA).

DETERMINATION OF LIPID PEROXIDATION LEVEL

The lipid peroxidation level was quantified based on malondialdehyde (MDA) concentration. The tissue extracts were prepared according to Wang et al. (2013) using trichloroacetic acid (TCA) and thiobarbituric acid (TBA). About 500 mg of samples were ground in 5 mL of 0.1% (g/v) TCA. The extracts were then centrifuged at 3000 g for 25 min at 4 °C. After centrifugation, 2 mL of 0.5% TBA : 20% TCA solution was added to 3 mL supernatant. The mixture was heated at a water bath (85 °C) for 30 min and then cooled at room temperature. The absorbance of samples were measured at 450, 532, and 600 nm using a spectrophotometer. The MDA content was calculated according to Wang's formula (Wang et al. 2013).

MEASUREMENT OF CHLOROPHYLL CONTENT

The chlorophyll content was extracted from leaves according to Quinet et al. (2012) using cold 80% (v/v) acetone. Briefly, 100 mg of samples were ground in 10 mL of 80% (v/v) acetone. The extracts were then centrifuged at 3000 g for 25 min at 4 °C. The absorbance of the supernatant was measured at 663 and 646 nm using a spectrophotometer. The chlorophyll content was calculated according to Lichtenthaler's formula (Lichtenthaler 1987).

MEASUREMENT OF PHOTOSYNTHESIS RATE, TRANSPIRATION RATE, AND STOMATAL CONDUCTANCE

Photosynthesis rate (A), transpiration rate (E), and stomatal conductance (gs) were measured at day 40 after transplanting plants using a LI-COR 6400XT Portable Photosynthesis System (LI-COR Bioscience Inc., Lincoln, Nebraska, USA). The measurements were carried out at 09:00-11:30 am under constant photosynthetically active radiation (PAR) of 1200 μmol m^{-2} s^{-1} on the 3rd and 4th leaves in each plant (Hidayati et al. 2016).

DETERMINATION OF WTC

In order to measure the tolerance level of varieties to waterlogging, the WTC was used in this study. The WTC was determined using the Liu's formula equation (1) (Liu et al. 2010) and expressed as percentage:

$$\text{WTC (\%)} = \frac{\text{Value under waterlogging}}{\text{Value under water-saturated}} \times 100\% \qquad (1)$$

where values under waterlogging or water-saturated are the measurement values of shoot dry weight (SDW), root dry weight (RDW), root diameter, cortex thickness, stele width, photosynthesis rate (A), Fe content of shoot, MDA content of shoot, and MDA content of root of waterlogged or water-saturated plants, respectively.

STATISTICAL ANALYSIS

The collected data were analyzed using analysis of variance (ANOVA) and *post hoc* Duncan Multiple Range Test (DMRT) with a 5% significance level using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The graphs and images were prepared using Microsoft excel and CorelDraw X6 (Corel Corporation).

RESULTS AND DISCUSSION

THE PH LEVEL OF WATERLOGGED AND WATER-SATURATED SOIL CONDITION

The soil pH before the experiment was conducted showed 5.1, which is categorized as an acid soil (Table 1). The different water supply treatments affected the soil pH during the experiment. In the present study, we showed that the water-saturated condition increases the soil pH. We observed that the soil pH increased 4th week after the treatments. The pH level of water-saturated soil showed significantly ($p<0.05$) higher than that of waterlogged soil, especially at 6th weeks after treatment (Table 2). An increase of soil pH of 5.6 under water-saturated soil in all rice variety plots was suggested due to the decreasing of metal ions solubility. Waterlogging condition in acid soils have been well reported to increase certain potentially oxidized metals (Fe, Al, and Mn) (Bjerre & Schierup 1985; Bojórquez-Quinta et al. 2017; Khabaz-Saberi & Rengel 2010; Khabaz-Saberi et al. 2006) and could potentially be toxic for plants and affect to the plant growth. In addition, Audebert and Sahrawat (2000) stated that Fe ion is more soluble in the waterlogged soils. Harahap (2014) also showed that rice grown under the waterlogged-soils is easier to experience Fe toxicity than that of the water-saturated soils. The result was consistent with previous study that found a decreasing of soil pH at 2 - 4 weeks after waterlogging (Matin & Jalali 2017).

RICE GROWTH IN THE WATERLOGGED AND WATER-SATURATED SOIL CONDITION

The plant biomass of all rice varieties in water-saturated treatment showed higher than that of the waterlogged treatment. The waterlogged condition significantly ($p<0.05$) decreased shoot and root fresh and dry weight in all rice varieties (Table 3). Pokkali showed the lowest reduction percentage of their shoot biomass under waterlogging in compared to three other tested varieties. All of four rice varieties showed better growth in the water-saturated soil than waterlogged condition (Table 3). The result indicates that water-saturated condition effective to support rice cultivation in high-Fe soils. We suggested that the poor rice growth under waterlogging or paddy-field system in the high-Fe soils due to the high solubility of some metals, such as Fe, aluminum (Al), mangan (Mn), and other toxic metal ions. Previous studies reported that the waterlogging in the acid soils also reduced the dry weight of oat (*Avena sativa* L. cv. Selma) (Bjerre & Schierup 1985) and wheat (*Triticum aestivum* L.) (Khabaz-Saberi et al. 2006; Khabaz-Saberi & Rengel 2010). The poor growth was also exhibited in spring barley (*Hordeum vulgare* L. cv. Ingrid) and spring wheat (*T. aestivum* L. cv. Thassos) within 15 days waterlogging condition (Steffens et al. 2005).

TABLE 2. The pH level of waterlogged and water-saturated soil condition during the experiment

Varieties	Treatments	pH		
		2nd week	4th week	6th week
IR64	Waterlogging	5.1^{a}	5.1^{b}	5.4^{bc}
	Water-saturated	5.2^{a}	5.3^{ab}	5.6^{ab}
Inpara 5	Waterlogging	5.1^{a}	5.4^{a}	5.3^{cd}
	Water-saturated	5.1^{a}	5.3^{ab}	5.6^{ab}
Inpara 2	Waterlogging	5.1^{a}	5.1^{b}	5.4^{bc}
	Water-saturated	5.2^{a}	5.4^{a}	5.6^{ab}
Pokkali	Waterlogging	5.1^{a}	5.4^{a}	5.2^{d}
	Water-saturated	5.1^{a}	5.4^{a}	5.6^{a}

$^{a\text{-}d}$uppercase letters in the same column indicate significant differences based on Duncan's multiple range test (α=5%).

TABLE 3. Shoot and root fresh and dry weight of four rice varieties in waterlogging and water-saturated soil condition

Varieties	Treatments	SFW (g hill^{-1})	%	SDW (g hill^{-1})	%	RFW (g hill^{-1})	%	RDW (g hill^{-1})	%
IR64	Waterlogging	3.2^{b}	81	0.4^{b}	56	1.1^{b}	73	0.1^{c}	65
	Water-saturated	16.8^{a}		0.9^{b}		4.1^{ab}		0.3^{bc}	
Inpara 5	Waterlogging	4.5^{b}	74	0.8^{b}	77	6.2^{ab}	20	0.7$^{a\text{-}c}$	22
	Water-saturated	17.4^{a}		3.4^{a}		7.7^{ab}		0.9^{ab}	
Inpara 2	Waterlogging	2.7^{b}	80	0.5^{b}	86	1.3^{b}	86	0.1^{c}	90
	Water-saturated	14.0^{ab}		3.7^{a}		9.0^{b}		1.2^{a}	
Pokkali	Waterlogging	16.1^{a}	2	3.2^{a}	13	6.6^{ab}	6	0.7$^{a\text{-}c}$	31
	Water-saturated	16.5^{b}		3.7^{b}		7.0^{ab}		1.1^{ab}	

SFW = shoot fresh weight; SDW = shoot dry weight; RFW = root fresh weight; RDW = root dry weight; % (percentage of reduction) = (water-saturated – waterlogging)/ water-saturated; $^{a\text{-}c}$uppercase letters in the same column indicate significant differences based on Duncan's multiple range test (α=5%)

Root diameter, cortex thickness, stele width, aerenchyma number, and aerenchyma width were measured in this study to show its variation of the root anatomical features between two treatments, waterlogged and water-saturated soil condition. In the water-saturated soil, root diameter significantly ($p<0.05$) higher than that of waterlogged condition. The water-saturated soil treatment increased the root diameter of all four rice varieties (Table 4). A large increase (more than 40%) in root diameter occurred in Fe-sensitive varieties (IR64 and Inpara 5). The cortex thickness in all rice varieties significantly ($p<0.05$) increased under water-saturated soil but the percentage of cortex thickness in this treatment showed smaller than that of waterlogged condition (Table 4). As a part of the transport system, the size of the rice stele under water-saturated soil significantly ($p<0.05$) larger than that of under waterlogged soil condition. However, the percentage of stele width under water-saturated condition showed significantly ($p<0.05$) lower than that of under waterlogged soil condition, except in rice var. Inpara 2 (Table 4). The variation of aerenchyma number and width were showed among all four rice varieties (Table 4). The aerenchyma number in the water-saturated soil showed higher than that of waterlogged condition, except for rice var. Inpara 5. In addition, the waterlogged condition increased the aerenchyma width, except for rice var. Pokkali.

By comparing the rice responses to the waterlogged and water-saturated soil condition, it showed that there were some modification responses. As reported by Bai et al. (2010), the rhizosphere with low oxygen supply due to waterlogging caused negative impact on the plant root systems. Previous studies showed that waterlogging impaired the morphological and physiological of many plants (Bai et al. 2010; Boonlertniruna et al. 2010; Bramley et al. 2011; Cardoso et al. 2013; Fu et al. 2012; Garthwaite et al. 2003; Grzesiak et al. 1999; Horchani & Aschi-Smiti 2010; Iu et al. 1982; Malik et al. 2001; Nishiuchi et al. 2012; Singh & Setter 2015; Sundgren et al. 2018; Suralta & Yamauchi 2008; Yamauchi et al. 2019; Yavas et al. 2012; Zhou et al. 2011). As shown in our results, there were differences in root anatomical of the plants grown under waterlogged- and water-saturated soil condition (Table 4). The lack of oxygen in the soil induced aerenchyma formation in maize under waterlogged condition (Drew et al. 2000). Conversely, well-drained condition reduced tropical root aerenchyma percentage of forage grass (*Brachiaria* spp.) compared to the root grown under waterlogged condition. Other root characters of rice in this study also responded to the waterlogged condition similarly with that of other previous studies in other plants (Cardoso et al. 2013; Grzesiak et al. 1999). The root diameter, stele, and the cortex thickness of maize under waterlogging showed lower than that of control (soil moisture was maintained to 65% of field water capacity) and drought treatments (soil moisture was maintained to 35% of field water capacity) (Grzesiak et al. 1999). In addition, the waterlogging also reduced the root stele percentage of the tropical forage grass (Cardoso et al. 2013).

Waterlogging significantly ($p<0.05$) increased the ratio of aerenchyma width to cortex thickness (ae/cx) and the ratio of aerenchyma width to root diameter (ae/rd) (Figure 2(a)-2(b)). The ae/cx and ae/rd ratio in sensitive varieties under waterlogged condition was much higher than that of tolerance varieties (Figure 2(a)-2(b)). The increasing of ae/cx ratio in sensitive rice varieties under waterlogged in comparing to water-saturated soil condition was 1.9 - 2.1-fold, while only 1.0 - 1.1-fold increasing of ae/cx ratio in tolerance varieties (Figure 2(a)). In addition, the increasing of ae/rd ratio in sensitive rice varieties under waterlogged in comparing to water-saturated soil condition was 2.0 - 2.2-fold, while only 1.0 - 1.1-fold increasing of ae/rd ratio in tolerance varieties (Figure 2(b)). These results indicated that sensitive rice varieties under waterlogging have more aerenchyma in comparing to their cortex and root diameter than that of water-saturated soil condition. A similar ratio of cx/st was showed between water-saturated soil and waterlogged condition in rice vars. IR64, Inpara 5, and Inpara 2 (Figure 2(c)). Those varieties showed 0.8 - 1.0-fold in their root cx/st ratio. Only rice var. Pokkali showed the clearly different in their cx/st ratio between water-saturated soil and waterlogged condition. These results indicate that the cx/st ratio in rice roots varied among genetic backgrounds.

The change of metal solubility and soil pH between waterlogged and water-saturated condition as reported in this present study (Table 2) and in several previous studies (Bjerre & Schierup 1985; Bojórquez-Quinta et al. 2017; Khabaz-Saberi & Rengel 2010; Khabaz-Saberi et al. 2006) suggested as a cause of morpho-physiological and anatomical modifications of rice in response to the two different soil water condition. Our study showed that the cortex to stele ratio (cx/st) in rice var. Pokkali, which is a tolerant variety to Fe toxicity, was higher in waterlogged condition than that of other rice varieties in the same water condition. It suggested that the cx/st ratio was a tolerance strategy under waterlogged acidic and high Fe content soil. Yamauchi et al. (2019) reported that cx/st ratio and aerenchyma to cortex (ae/cx) ratio in rice were associated with the areas of gas spaces and essential for high capacity of oxygen transport along with roots. The high cx/st ratio and large root diameter are traits that promote oxygen transport from shoot base to root tips and contributes to waterlogging tolerance in plants. Furthermore, Nishiuchi et al. (2012) also reported that the adaptation strategies to waterlogging in rice could be through lysigenous aerenchyma mechanism and development of barrier to radial O_2 loss (ROL) formation. Root anatomical features were suggested to influence the internal oxygen deficiency and root hydraulic properties in wheat (*Triticum aestivum* L.), narrow-leafed lupin (*Lupinus angustifolius* L.) and yellow lupin (*Lupinus luteus* L.) under waterlogging and determine survival and recovery level of the plants to the waterlogged condition (Bramley et al. 2011).

Interestingly, rice var. Inpara 5, which is developed from rice var. IR64 containing submergence tolerant *Sub1* gene (IR64-*Sub1*) (Hairmansis et al. 2012), showed different responses to water condition in compared IR64. The previous studies reported that these two rice varieties categorized as sensitive-type to Fe toxicity (Nugraha & Rumanti 2017; Turhadi et al. 2018). The difference of genetic background between IR64 and Inpara 5 might contribute to the different responses of the two varieties to waterlogged and water-saturated soil condition. The responses of the two varieties under submergence and or flooding have been reported by Rachmawati et al. (2019), Singh et al. (2014), and Sitaresmi et al. (2019). Submergence decreased plant height, biomass, chlorophyll content, and yield (Rachmawati et al. 2019; Sitaresmi et al. 2019). In addition, submergence increased aerenchyma diameter (Rachmawati et al. 2019). Our results showed that less reduction on biomass of Inpara 5 than that of IR64 under waterlogged condition (Table 3). This result was similar to the study by Singh et al. (2014) who reported that IR64-*Sub1* (Inpara 5) showed lower reduction of stem, leaf, and root dry weight under submergence condition than that of IR64. The greater reduction occurred on biomass accumulation in the lacking *Sub1* genotype (IR64) under submergence (Singh et al. 2014). The results suggested that modification of soil water status from waterlogged to water-saturated give more benefits to rice var. IR64 or other sensitive varieties than that of tolerant and moderate varieties, such as Pokkali and Inpara 5, respectively.

TABLE 4. Root anatomical characters of rice under waterlogging and water-saturated soil condition

Traits	Treatments	Varieties			
		IR64	Inpara 5	Inpara 2	Pokkali
Root diameter (mm^2)	Waterlogging	0.59^{a}	0.63^{c}	0.77^{bc}	0.67^{bc}
	Water-saturated	1.10^{c}	1.10^{bc}	0.84^{bc}	0.88^{bc}
Cortex thickness (mm^2)	Waterlogging	0.231^{b}	0.267^{b}	0.376^{b}	0.315^{b}
	Water-saturated	0.756^{a}	0.760^{a}	0.486^{ab}	0.491^{ab}
Cortex thickness (%)*	Waterlogging	84.34^{ab}	86.45^{ab}	83.59^{a-c}	87.89^{a}
	Water-saturated	75.76^{cd}	78.65^{b-d}	89.48^{a}	73.39^{d}
Stele width (mm^2)	Waterlogging	0.020^{b}	0.019^{b}	0.017^{b}	0.021^{b}
	Water-saturated	0.054^{a}	0.054^{a}	0.026^{b}	0.044^{ab}
Stele width (%)*	Waterlogging	6.72^{a}	6.25^{a}	3.54^{c}	5.90^{a}
	Water-saturated	5.34^{ab}	5.59^{a}	3.89^{bc}	5.34^{ab}
Aerenchyma number (n)	Waterlogging	32.67^{a}	43.67^{a}	28.33^{a}	28.33^{a}
	Water-saturated	35.33^{a}	38.00^{a}	37.33^{a}	69.33^{b}
Aerenchyma width (%)	Waterlogging	57.83^{c}	57.83^{bc}	49.43^{b}	37.80^{bc}
	Water-saturated	25.97^{a}	23.23^{a}	42.80^{b}	43.67^{b}

*% means cortex thickness or stele width in mm^2 divided root diameter. $^{a-d}$uppercase letters in each trait indicate significant differences based on Duncan's multiple range test (α=5%).

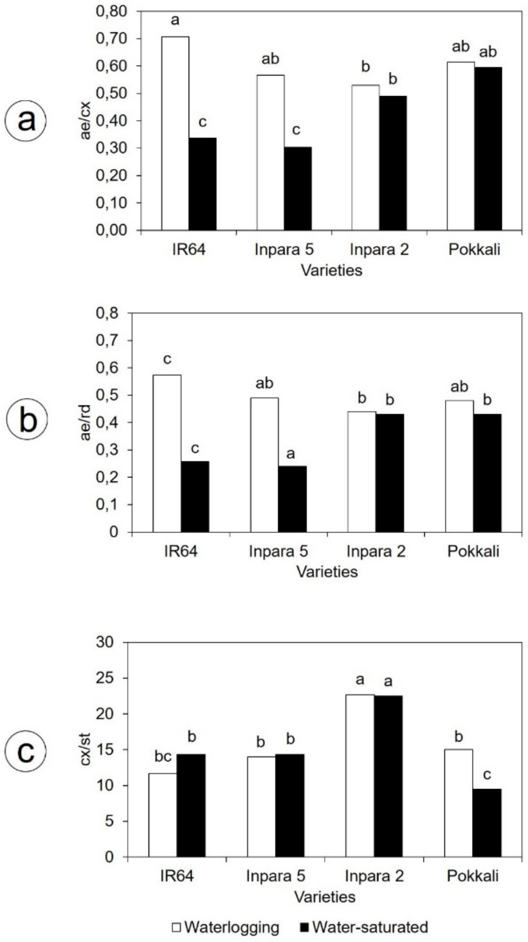

FIGURE 2. Ratio of aerenchyma width to cortex thickness (ae/cx) (a), aerenchyma width to root diameter (ae/rd) (b), and cortex thickness to stele width (cx/st) (c) at ~1 - 1.5 cm from the root tip of rice. Three-week-old rice seedlings were grown under waterlogged and water-saturated soil condition for 40 days. Letters above bar charts indicate significant differences based on Duncan's multiple range test (α=5%)

ACTIVITY OF FE ABSORPTION OF RICE IN THE WATERLOGGED AND WATER-SATURATED SOIL CONDITION

To investigate the activity of Fe absorption in rice during the waterlogging and water-saturated condition, we measured Fe content in the shoot tissues. Fe content in the shoot tissues showed significantly ($p<0.05$) different between water-saturated soil and waterlogged condition (Figure 3). Water-saturated condition decreased the Fe accumulation of the shoot tissues compared to waterlogged soil condition (Figure 3). These results indicated that the difference in water surface height in the soils with high Fe content affected the activity of Fe absorption in rice.

The uptake of nutrient in plants was also affected by soil water status. Waterlogging not only led increasing the uptake of certain nutrients, but also decreasing the uptake of other nutrients. Singh and Setter (2015) reported that waterlogging increased Fe, Al, and Na uptake, but decreased K, P, and S uptake as compared to drained soils. The increase of Fe solubility was attributed from the dissolution of Fe oxide under reduced condition, such as waterlogging (Matin & Jalali 2017), which increase Fe availability to the toxic level for plant. Khabaz-Saberi et al. (2006) showed that high accumulation of Fe ion in shoot of wheat after 49 days of waterlogged acidic soil. Uptake Fe and Mn in French bean and maize also increased under waterlogged soil (Iu et al. 1982). Those reports were consistent with our findings in this research study. The high accumulation of Fe occurred in all rice varieties under waterlogging (Figure 3). Previous research studies demonstrated the poor growth of spring wheat and spring barley under waterlogging were not induced by nutrient toxicity (Fe and Mn) but it caused by sub-optimum nutrient supply of N, P, K, Mg, Cu, and Zn (Steffens et al. 2005). This imbalance of nutrient uptake under waterlogging could be solved using water-saturated condition. The previous studies reported that water-saturated condition increased nutrient uptake of N, P, and K elements in soybean and increased their productivity (Ghulamahdi et al. 2018, 2006; Sagala et al. 2019). The water-saturated condition also increased the photosynthesis activity and its yield in bean (*Phaseolus vulgaris* L.) (White & Molano 1994) and in rice (Ghulamahdi et al. 2012; Nguyen et al. 2009).

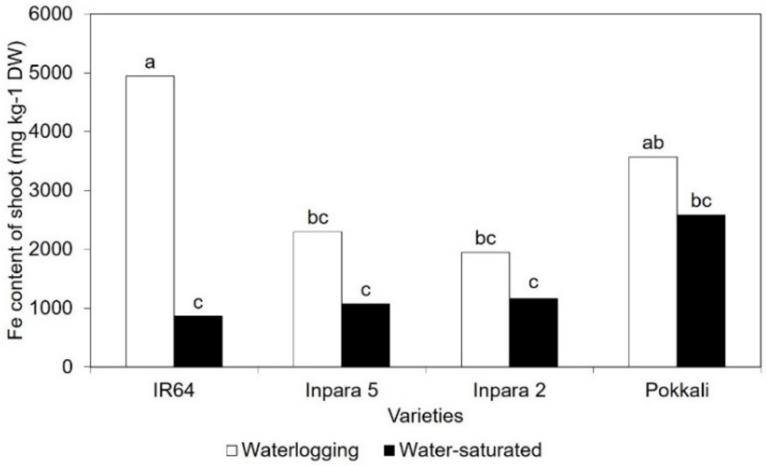

FIGURE 3. Fe content in shoots of four rice varieties on waterlogging and water-saturated soil condition. Letters above bar charts indicate significant differences based on Duncan's multiple range test (α=5%)

LIPID PEROXIDATION ACTIVITY, CHLOROPHYLL CONTENT AND LEAF GAS EXCHANGE PARAMETERS OF RICE IN THE WATERLOGGED AND WATER-SATURATED SOIL CONDITION

To assess the stress level of rice under waterlogging and water-saturated soil condition, lipid peroxidation activity as represented as malondialdehyde (MDA) and chlorophyll content were measured in this study. The treatments had a significantly ($p<0.05$) effect on the lipid peroxidation level of rice shoots and roots (Figure 4(a)-4(b)). In addition, the chlorophyll content was also affected by the treatments. The MDA content in both shoots and roots and in all of the rice varieties was decreased under water-saturated condition (Figure 4(a)-4(b)). Furthermore, water-saturated condition was also significantly ($p<0.05$) increased the chlorophyll content in all of the rice varieties (Figure 5). These results indicated that the water-saturated condition in the soils with high Fe content could restore the plant from waterlogged stress.

To investigate the effect of different soil condition on gas exchange activities of rice, the photosynthesis rate, transpiration rate, and stomatal conductance were also measured in this study. Water-saturated condition significantly ($p<0.05$) increased the photosynthesis rate, transpiration rate, and stomatal conductance (Table 5). The results indicated that the waterlogged condition in the soils with high Fe content disturb the vital processes in the cells due to experienced stress.

In this present study, the physiological responses of rice showed better in water-saturated than that of waterlogged soil condition as indicated by gas exchange activities (Table 5), MDA (Figure 4), and chlorophyll content (Figure 5). The MDA content increased and the chlorophyll content decreased under waterlogged condition. The waterlogging treatment of six maize genotypes for 6 days also increased MDA content (Liu et al. 2010). The results indicated that the waterlogging could increase the level of stress in plant. Interestingly, only rice var. IR64 showed greater reduction of chlorophyll content under waterlogging compared other rice varieties (Figure 5). Based on our results, rice var. IR64, which is categorized as a sensitive variety to waterlogging condition in acidic and high Fe content soil is suggested due to the lack of *Sub1* gene. Singh et al. (2014) suggested that *Sub1* lines, such as Inpara 5, maintained higher chlorophyll content during submergence. The reduction of chlorophyll content might also be as a consequence of the damage in chloroplasts during waterlogged condition. Waterlogging damaged chloroplast, mitochondria, nucleus, and cell wall of *Kosteletzkya virginica* (Zhou et al. 2011). Further impact of those breakage was reduction of the photosynthesis rate (Table 5). Waterlogging for 24 h also reduced the photosynthesis rate of wheat and its stomatal conductance (Malik et al. 2001).

TABLE 5. Photosynthesis rate (A), transpiration rate (E), and stomatal conductance (g_s) of four rice varieties on waterlogging and water-saturated soil

Varieties	Treatments	A (μmol CO$_2$ m^{-2} s^{-1})	E (mmol CO$_2$ m^{-2} s^{-1})	g_s (mol H$_2$O m^{-2} s^{-1})
IR64	Waterlogging	13.59^{c}	6.93^{cd}	0.54^{b}
	Water-saturated	14.58^{bc}	7.32^{bc}	0.67^{ab}
Inpara 5	Waterlogging	15.69^{b}	6.21^{d}	0.50^{b}
	Water-saturated	16.05^{b}	8.03^{ab}	0.65^{ab}
Inpara 2	Waterlogging	15.18^{bc}	6.09^{d}	0.23^{c}
	Water-saturated	15.75^{b}	8.35^{a}	0.77^{a}
Pokkali	Waterlogging	15.27^{bc}	6.59^{cd}	0.55^{b}
	Water-saturated	18.56^{a}	8.82^{a}	0.76^{a}

$^{a-d}$uppercase letters in single column indicate significant differences based on Duncan's multiple range test (α=5%)

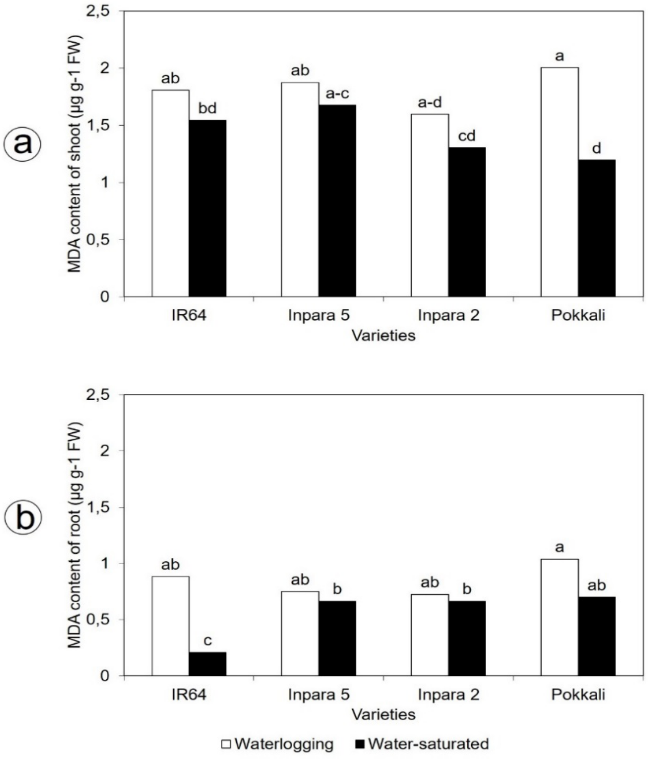

FIGURE 4. Malondialdehyde (MDA) content of shoot (a) and root (b) of four rice varieties on waterlogging and water-saturated soil condition. Letters above bar charts indicate significant differences based on Duncan's multiple range test (α=5%)

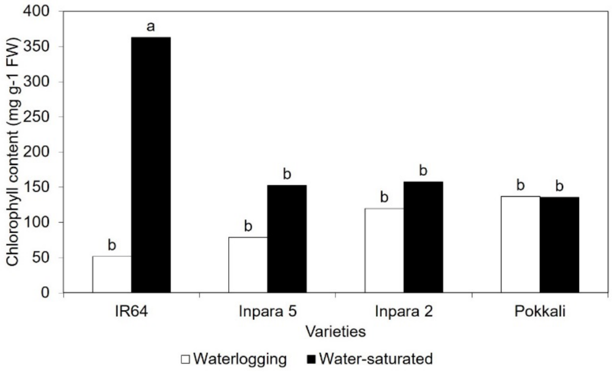

FIGURE 5. Chlorophyll content of four rice varieties on waterlogging and water-saturated soil condition. Letters above bar charts indicate significant differences based on Duncan's multiple range test (α=5%)

THE WTC OF MORPHO-PHYSIOLOGICAL AND ANATOMICAL CHARACTERS OF FOUR RICE VARIETIES

Various patterns were showed in WTC of nine morpho-physiological and anatomical characters (Figure 6). Interestingly, the WTC of SDW, RDW, CX, and FES also showed a variation among rice varieties. In this case, the tolerance level of rice under waterlogging as well as Fe toxicity stress were suggested could be distinguish based on the WTC of those four characters. The WTC pattern of RD, ST, A, MDAS, and MDAR were similar among four rice varieties (Figure 6).

The difference among the WTC in morpho-physiological and anatomical characters of each variety could be used as a screening tool to the waterlogging tolerance of rice in acidic and high Fe content soil. The WTC showed as effective screening methods for waterlogging tolerance in maize. WTC of dry weight of shoot in maize can be used for a screening character to waterlogged condition (Liu et al. 2010). The high WTC was also showed in waterlogged-tolerant *Brachiaria humidicola* under 15 days waterlogging, whereas the low WTC was showed in waterlogged-sensitive *B. ruziziensis* under 15 days waterlogging (Jiménez et al. 2015). The less value of WTC means the more susceptible to waterlogging for the plants (Liu et al. 2010). In our results, The WTC values of the characters SDW, RDW, CX, and FES indicated as good characters for screening tool of rice under waterlogging in acidic and high Fe content soil. In addition, the WTC values of the characters RD, ST, A, MDAS, and MDAR could be used as evidences that water-saturated soil was a better soil environment for rice cultivation than that of waterlogged condition in acidic and high Fe content soil.

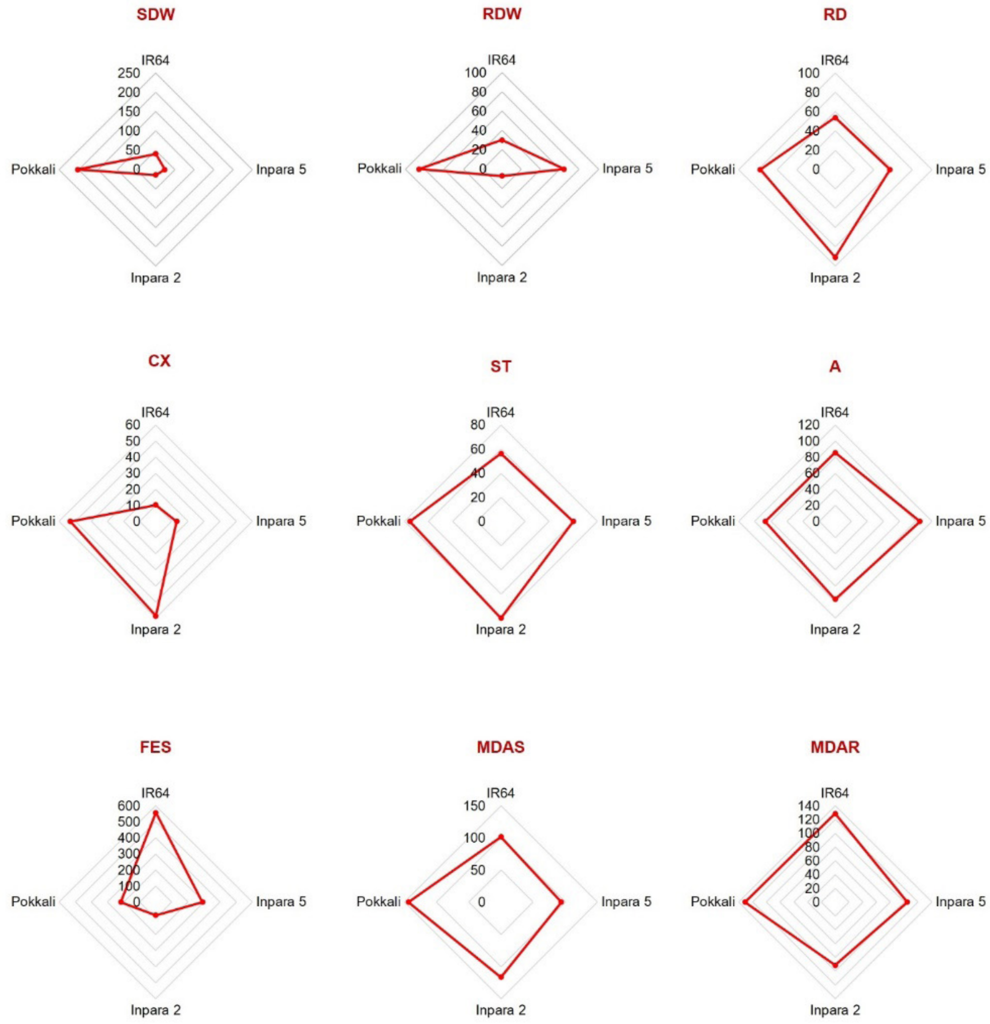

FIGURE 6. WTC based on the morpho-physiological and anatomical characters in four rice varieties. SDW = shoot dry weight; RDW = root dry weight; RD = root diameter; CX = cortex thickness; ST = stele width; A = photosynthesis rate; FES = Fe content of shoot; MDAS = Malondialdehyde content of shoot; MDAR = Malondialdehyde content of root

CONCLUSION

The present study demonstrated that the rice performance in term of morpho-physiological and anatomical characters in the acidic and high Fe content soil was better when it grown in the water-saturated than that of waterlogged condition. Water-saturated condition decreased Fe uptake and increased the root diameter, stele width, and cortex thickness. In addition, photosynthesis and transpiration activities increased under water-saturated condition. In general, water-saturated condition was able to alleviate negative effect of waterlogged condition in acidic and high Fe content soil. Shoot and root dry weight, cortex thickness, and Fe content in the shoot were proposed to be used as screening characters for waterlogging tolerance of rice in acidic and high Fe content soil based on its waterlogging tolerance coefficient. The study highlighted the importance of the soil water status controlling especially in the acidic and high Fe content soil, such as swampy and tidal land, as a fundamental basis rice cultivation in such marginal land.

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