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Morphological and physiological change of rice (*Oryza sativa* L.) under water stress at early season

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Abstract. Water stress is a major factor affecting rice production. Water stress causes changes in physiology and morphology for plant tolerance to water stress. In this study, six varieties were examined for their tolerance to water stress. The check varieties for comparisons of tolerant, moderate tolerant and sensitivity to water stress, namely Situ Patenggang, Towuti and IR 64; while the test varieties in this study are three Aceh rice traditional varieties. Water stress significantly influences the number of tillers, length of internode, proline, chlorophyll a, b and the total content, leaf rolling, drying score and leaf recovery, in which the changes occur varies among varieties, in vegetative stages. Chlorophyll is the main indicator of rice tolerance to drought in the early season of the rice growing season. Varieties that have good adaptation to water stress are able to maintain their chlorophyll content. Leaf rolling, drying score and three rice traditional varieties of Aceh in vegetative stage. To overcome water stress, it is necessary to change rice cultivation by adjusting planting time, planting season, using water stress tolerant varieties, traditional varieties and national superior varieties; changes in irrigation and fertilization management.

1. Introduction

Rice is the main food crop which supplies 50-80% of calories for more than half of the world's population [1]. The increase in the world's population which is estimated to reach 8 billion people in 2030 must be balanced with an increase in food production [2]. But in reality the increase in food production is constrained by water shortages [3]. Water shortages can occur at the beginning of the growing season, mid and end of the season. Therefore, it is necessary to handle water shortages damages from the beginning by sorting water stress tolerant varieties, adjusting the growing season, planting time, planting patterns, planting systems and agronomic actions to avoid planting failure, failure to grow and harvest failure.

Rice are strongly influenced by environmental factors, especially water shortages [4]. 23 million hectares of rice fields in the world experience water shortages [5]. This situation is getting worse with the increasing drought in rice fields in Asia which reached 130 million hectares [6]. Water stress tolerant varieties are needed to maintain global rice production [7].

Water stress tolerant varieties can be seen from morphological and physiological characters including the ability to maintain canopy and root growth, proline accumulation and chlorophyll content. Water stress tolerant varieties have an increase in chlorophyll a and b [8]. These tolerant varieties have the ability to maintain growth under water stress conditions [9]. At water potential of 0.013MPa rice starts experiencing water stress [10]. Rice experiences stress at 0.015 MPa water potential for lowland rice [11]. Transpiration limits have occurred at -0.07 MPa [12]. At water potential 0.074 MPa an increase in proline in rice leaves. The water potential at PEG 6000, can determine tolerant water stress at early stage [13]

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Lack of water requires changes in water-saving rice planting patterns so that more land can be irrigated. The water-saving rice cultivation can be in the form of alternating wetting and drying (AWD), saturated soil culture (SSC), aerobic systems (AS) which are able to save water respectively SSC 35 to 40% [14], AS can save water 16 to 28% [15]. Besides saving water this system can increase the growth and development of rice roots [16]. However, the water saving method also needs to observe changes in the morphology and physiology of rice in each stage. Various studies have shown rice planting in soil water potential (0.039), (0.048), (0.059) MPa influencing rice morphology and physiology [17]. The minimum limit for testing the tolerance of genotypes to plant water stress is at least 0.05 MPa [18].

Rice plants change physiological and morphological character in the water potential of 0.013 MPa. Changes in morphology and physiology of rice under water stress are dependent on the duration of stress, the time of stress, the condition of rice growth [17]. Changes in morphology and physiology can be compared to tested varieties with comparative varieties of IR 64 standard for sensitive water stress [21] Situ Patenggang as a comparison of water stress tolerance [23] and Towuti as the comparison is moderate tolerant of water stress. Changes in morphology and physiology of the rice at vegetative stage determine the growth and development of the reproductive and maturation phases. The assimilates used for the formation of yield components also come from the vegetative stage determine rice condition in the reproductive and maturation stage and yield.

2. Materials and methods

2.1 Plant materials, growth conditions and water stress treatment

The study was conducted from November 2015 to January 2016 in a plastic house, in the experimental farm of faculty of agriculture at the Syiah Kuala University Darussalam, Banda Aceh, Indonesia, minimum/maximum temperature of 23/31. The experiment was arranged in a randomized block design with 3 replications, water stress as the main plot consisting of: well watered (WW), moderate water stress with alternating wetting and drying (AWD1), severe water stress with alternating wetting and drying (AWD2). Six varieties/genotypes of Situ Patenggang rice (V1), Towuti (V2), IR 64 (V3), Sipulo (V4), Sanbei (V5), Bo Santeut (V6) as subplot. Drought treatment starts from the age of 2 weeks after planting (WAP) to 5 WAP. Positive control of water stress tolerant variety (V1), moderate water stress tolerant variety (V2), negative control of water stress tolerant variety V3 and V4, V5 and V6 are tested varieties.

PVC tube with diameter of 10 cm and height of 110 cm is filled with podzolic soil which has been sieve with 8 MESH sieve, 10 kg PVC tube⁻¹. Soil in a PVC tube is saturated with water and becomes muddy and keeps saturated for 2 weeks. After 2 weeks the soil was rewatered and planted 1 rice seedling 12 day old, in each PVC tube. Fertilized with 1g urea, 0.5g KCl and 0.5 g single super phosphate as a basic fertilizer. Fertilization is done again at the age of 4 WAP with 0.5 g urea.

The water stress treatment is done by saturating the water until flooded 2 cm above soil surface for 2 weeks after planting for all treatments. After 2 weeks, the treatment, WW remained flooded 2 cm until the age of 5 weeks. AWD1 was flooded by 2 cm and then stopped irrigated and watered when the soil water potential reached 0.035 MPa, then the rewatered until 2 cm flooded again. AWD2 was flooded 2 cm and then stopped irrigating and re-watered when the soil water potential reached 0.070 MPa, and flooded 2 cm above soil surface again. Soil Water potential was observed with tensiometer jet fill 2725, soil water content was observed by installing a global water logger version 2.10 until age 5 WAP.

2.2 Plant growth

Plant height measure from the bottom of hill until the topest tip of leaf. Length of internode measures from the bottom of the stem until uppermost of the stem and divides the number of the internode. *Relative growth Rate* (RGR) use formula described by [24].

RGR = (In L2-In L1)/(T2 - TI)RGR : relative growth rate (cm d⁻¹).

L1 : Length of Shoot at 4 WAP (cm)

- L2 : Length of Shoot at 5 WAP (cm)
- T1 : time (4 WAP)
- T2 : time (5 WAP)

2.3 Proline content

Proline content measure according [11] modified [17] 0.1 g of leaf was homogenized with 5 ml of 3% aqueous sulfosalicylic acid. Two ml of extract reacted with 2 ml of acid ninhydrin 100 °C for I hour. The reaction was stopped by placing 2 ml of glacial acetic acid and boiled in a water bath at tubes on ice. The solution was extracted with 4 ml toluene and the absorbance of the toluene fraction was measured at 520 nm. The amount of free proline was evaluated using a standard curve and expressed as mg g-1 tissue fresh weight and converted to μ mol g⁻¹ FW sampling were taken of the 4th leaf from above when the soil water content reached 0.035 and 0.070 MPa.

2.4. Chlorophyll content

Chlorophyll concentrations were calculated using aquation discribe by [17]. Chlorophyll were measured at 5 WAP, using a UV 1800 spectrometer, the Fujitsu brand made in Japan with the formula proposed by [17];[31]; [4].(Bushan et al, 2007).

2.5. Leaf rolling, Drying score and recovery

Measurement of leaf rolling, drying score and recovery after water stress is done by observing every day from 2 to 5 WAP. Using the standard evaluation system for rice (SES 2014), a score of 1-9. The number of tillers and the length of the internodes were measured 5 WAP.

2.6. Data Analysis

Analysis of varian (ANOVA) was performed using microsoft excel window 10 and mean separation was done with HSD at P = 0.05 to compare the variation in caracter measure to tolerated ability between the variety and water stress treatment.

3. Results and discussion

3.1. Effect of water stress

Water stress shows significant differences in decreasing and increasing the number of tiller, plant height, relative growth rate, length of internode, proline content, chlorophyll a, b, and total content under water stress at vegetative stage (see table 1).

Table 1. Average value number of tiller, plant height, relative growth rate, length of internode, proline content, chlorophyll a, b, total content under water stress at vegetative stage

Plant Characters	WW	AWD1	AWD2	HSD 0,05
Number of tiller at 5 WAP (tiller hill ⁻¹)	24,03c	20,76b	18,76a	2,65
Plant height at 5 WAP (cm)	71,50c	64,00b	63,44a	0,98
Length of internode at 5 WAP (cm)	1,62 c	1,40 b	1,28 a	0,11
Relative growth rate at WAP (cm d ⁻¹)	1,77b	1,25a	1,49a	0,33
Proline content (μ mol g ⁻¹ FW)	11,04a	16,41c	13,82b	0,45
Chlorophyll a content (mg g ⁻¹ FW)	2.97b	1.03a	3.48c	0.47
Chlorophyll b content (mg g ⁻¹ FW)	3.86b	1.59a	4.27b	1.58
Chlorophyll total content (mg g ⁻¹ FW)	6.83b	2.62a	7.58c	0,72

Note: Values followed by the same letter in the same row are not significantly different according to HSD analysis at P<0.05

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Water stress significantly influences the morphology of rice on the number of tillers plant height and length of internode (table 1). Water stress changing leaf rolling, drying score and recovery after drying. There are differences in the rolling, drying score and recovery in AWD1 and AWD2. Changes in physiology of water stress character can be seen from changes in RGR, Proline, chlorophyll a, b and total at AWD1 and AWD 2. This is because the Vegetative stage of rice plants prioritizes the formation of tillers so that the RGR based on plant height is also significantly different. Also due to the use of photosynthates for the formation of tillers is higher than the height of plants [10]. An increase in proline in AWD1 and AWD2, in line with the results of the study [17]. Increased proline is the initial response of plants due to water stress. Chlorophyll a, b, and total decrease in AWD1, but in AWD2 an increase. This is in line with the results of research [21]; [22]. Increased chlorophyll due to active enzymes and compatible compounds to increase photosynthate and translocate to roots and maintain chlorophyll levels.

3.2. Effect of variety

Variety shows significant difference in decreasing and increasing number of tiller, plant height, relative growth rate, length of internode, proline content, chlorophyll a, b, and total content under water stress at vegetative stage (see table 2).

Table 2. Average value of number of tiller, plant height, relative growth rate, length of internode, proline content, chlorophyll a, b, total content of six varieties under water stress at vegetative stage

Plant Characters	Varieties						HSD _{0,05}
	V1	V2	V3	V4	V5	V6	
NT at 5 WAP (tiller hill ⁻¹)	11,56a	20,11c	17,22b	21,22c	18,00b	23,11d	1,79
PH at 5 WAP (cm)	<mark>71,89d</mark>	<mark>60,33c</mark>	<mark>56,00a</mark>	<mark>80,22e</mark>	<mark>71,33d</mark>	<mark>58,11b</mark>	1,83
LI at 5 WAP (cm)	1,53 c	1,20 b	1,01 a	1,93 e	1,75 d	1,19 b	0,11
RGR at 5 WAP (cm d ⁻¹)	<mark>2,05d</mark>	<mark>1,25 b</mark>	<mark>1,13b</mark>	<mark>2,22e</mark>	<mark>1,41c</mark>	<mark>0,97a</mark>	0,14
PC (µmol g ⁻¹ FW)	15,74d	13,73c	11,72b	16,77e	13,72c	10,88a	0.65
Chl a (mg g^{-1} FW)	2.87c	2.41b	1.77a	2.34b	3.36d	2.21b	0.24
Chl b (mg	3.76b	2.93a	2,82a	2,67a	4,52c	2,75a	0.85
Chl total (mg g ⁻¹ FW)	6.63d	5.34c	4.14a	5.23b	7.77e	4.96b	0,43

Note: Values followed by the same letter in the same row and column are not significantly different according to LSD analysis at P<5%

There were differences in morphological characters between varieties, where the highest number of tillers in V4, the highest plants in V4 as well as the length of internode. Differences in morphological characters are more genetically influenced by varieties [22]. Morphological differences need to be considered to get high yields, usually high-tillering varieties will have high productivity [23]. Differences in physiological properties among varieties, seen from RGR highest in V1. Proline is highest on V4, lowest on V3. Chlorophyll a, b are highest at V5 as well as total chlorophyll. Chlorophyll content is an important characteristic of tolerance to water stress. Chlorophyll a and total the lowest in V3. The ability of plants to maintain chlorophyll content is important for maintaining tillering and growth rate in the Vegetative stage [17].

3.3. Interaction Effect of water stress and Variety

Effect of water stress and variety in decreasing and increasing on number of tiller, plant height, relative growth rate, length of internode, proline content, chlorophyll a, b, and total content under water stress at vegetative stage (see table 3).

Plant	Water	Varieties H						
Characters	Stress	V1	V2	V3	V4	V5	V6	0,05
Number of tiller at 5	WW	15,33b	26,00d c	16,00b	22,33bc	19,00b	29,67d	
WAP (tiller	AWD1	10,33a	18,67b	19,33bc	19,00b	19,33bc	22,00bc	3,85
hill ⁻¹)	AWD2	9,00a	15,67b	16,33b	22,33bc	15,67b	17,67b	
D1	WW	77,33d	66,00b	62,33b	86,67f	71,33c	65,33b	
5 WAP (cm)	AWD1	70,33c	56,33b	53,00a	79,33e	73,00c	52,00a	3,75
5 WAI (elli)	AWD2	68,00c	58,67b	52,67a	74,67d	69,67b	57,00	
Length of	WW	1,67b	1,65 b	0,98a	2,04c	1,76b	1,61bc	
internode at 5	AWD1	1,69b	0,76 a	1,06 a	2,11b	1,87bc	0,93a	0,32
WAP (cm)	AWD2	1,24a	1,19 a	0,98 a	1,63b	1,61 b	1,02a	
Relative	WW	2,48i	0,90b	2,10h	2,86j	1,38d	0,90b	
growth rate at	AWD1	1,43e	1,81g	0,57a	1,62f	1,33d	0,76b	0,17
$\frac{5 \text{ WAP}}{1}$	AWD2	2,24h	1,05c	0,71a	2,19h	1,52e	1,24d	
Proline	WW	11,92c	14,21e	12,56c	14,23e	9,95b	3,38a	
content (µmol	AWD1	22,06i	16,92f	13,21d	19,85h	12,19c	14,23d	0,81
g ⁻¹ FW)	AWD2	13,24d	10,05b	9,38b	16,22f	19,01g	15,02e	
Chlorophyll a	WW	6.37h	1.36b	2.35d	2.39d	3.38e	1.99c	
content (mg g	AWD1	0.80a	0.61a	1.22b	0.85a	1.78c	0.91a	0,3
¹ FW)	AWD2	1.45b	5.26g	1.73c	3.77e	4.93f	3.73e	
Chlorophyll b	WW	7.57f	1.26a	4.14d	3.90d	4.27d	2.03ab	
content (mg g	AWD1	1.44a	1,26a	2.02ab	1.52a	1.36a	1.96ab	0,51
¹ FW)	AWD2	2.28b	6.28e	2,28b	2,60bc	7,93f	4.26d	
Chlorophyll	WW	13.93j	2.62ab	6.49e	6.29e	7.64g	4.01cd	
total content	AWD1	2.23a	1.87a	3.24bc	2.37 a	3.14b	2.87ab	0,54
$(mg g^{-1} FW)$	AWD2	3.73c	11.53h	2,68ab	7.03 ef	12,52i	7.99g	

Table 3. Average value of relative growth rate, length of internode, proline content, chlorophyll a, b, total content of six varieties under water stress

Note: Values followed by the same letter in the same column are not significantly different according to HSD analysis at P<5%

Different varieties and levels of water stress affect the morphology and physiology of rice. Changes in the number of tillers, plant height and length of internode. The highest number of tillers in AWD1 is at V6, the lowest at V1. While the highest at AWD2 on V4, the lowest on V1. V4 is capable of forming tillers in severe water stress. Tiller formation in severe water stress at vegetative stages due to the ability to maintain assimilates which can be used for tillering [21].

Plant height decreased in AWD1 and AWD2, significantly different. Plants prioritizes the formation of tillering and roots at this stage [20]. The length of the internode has decreased in AWD1 and AWD2 except in V4 there has been an increase in AWD1. While the highest reduction in the length of internode on V2. At AWD2 the length of internode with the lowest decrease is at V5. V5 being able to maintain the length of the internode in AWD2. The morphological characteristics of tillers, plant height and length of internode are important properties that show the proportion of assimilated partitions in the Vegetative stage. While the plant height and length of internode will influence the development of the next stage [24].

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Changes in physiological character are indicated by changes in the rate of relative growth (RGR), proline levels, chlorophyll a, b and total levels. RGR generally decreases with increasing water stress except in V4 there is an increase in growth in AWD1 as well as V2. In AWD2 an increase in RGR on V4 and V6. This shows that V4, V5 and V6 have a tolerance mechanism to water stress and are able to keep the growth rate in the vegetative stage. Growth rates keep height due to accumulation of compatible compounds and growth enzymes [19].

The highest proline in AWD1, in V1. Whereas in AWD2 the highest in V5, the lowest in V3. Proline can maintain cell turgidity and membrane stability from damage. Proline can directly or indirectly influence growth and protect cells from oxidative stress [21]; [22]; [19]. Proline influences osmotic adjustment and increases water uptake under water stress [25]; [26].

Chlorophyll a content decrease in AWD1, but in AWD2 increase in chlorophyll a in V2, V4, V5 and V6. Increasing chlorophyll a need keep photosynthates rate to maintain growth rates [21], [22]. The increase in chlorophyll is due to the active nutrient uptake under water stress to support chlorophyll formation. Chlorophyll b decrease in all varieties except V2 in AWD1. However, in AWD2 there was an increase in chlorophyll b in V2, V5 and V6. This is in line with the results of research [21] and [22]. An increase in chlorophyll b under water stress conditions is need to keep photosynthate rate due to chlorophyll b function [19] in line [21]. Total chlorophyll levels decreased in all varieties in AWD 1 but in AWD 2 there was an increase except in V3. The highest content of chlorophyll total are found in V5. Total chlorophyll determine tolerance to water stress [22]. Chlorophyll content in plants depends on availability of Nitrogen, Magnesium, Ferum and Potassium to accelerate forms of chlorophyll [28]. So it need to increase N, Mg, Fe and K in fertilizer management in water stress condition. Scores of leaf rolling, drying and recovery were carried out with daily observations (see table 4).

Plant Characters	Varieties						
		V1	V2	V3	V4	V5	V6
Leaf rolling (score)	AWD1	1,1	1,5	2,88	1,47	1,2	1,35
Leaf drying (score)	AWD1	1	1	3	1	1	1
recovery (d)	AWD1	1	1,5	2	1,5	1,7	1,7
Leaf rolling (score)	AWD2	1,9	2,1	3,87	2,16	2,2	2,23
Drying (score)	AWD2	3	3	5	3	3	3
recovery (d)	AWD2	2,1	2,5	3,1	2,6	2,5	2,6

Table 4. Average value of leaf rolling, drying score and recovery of six varieties under water stress.

Changes in leaf rolling, drying and recovery scores, check and tested varieties in vegetative stages are tolerant to water stress except V3 (a water stress sensitive variety) [22]. In vegetative stage metabolism is faster than other stages. Rice can anticipated water stress through the mechanism of escape, avoidance and tolerance. Avoidance is done by rolling the leaves to minimize the area of sun exposure to reduce transpiration. The rolling and drying scores of varieties V1, V2, V4, V5 and V6 are tolerant to water stress based on scores below 4. In line with research of [27] and [21] (V3) is sensitive to water stress.

4. Conclusions

Based on the comparison of morphology and physiology of 6 varieties, the levels of chlorophyll a, b and total can be used as the main criteria of tolerance to water stress in the vegetative stage. Three traditional varieties showed morphological changes in the number of tillers, lengths of internode, leaf rolling, drying score and recovery which showed variations the same as the tolerant varieties of water stress, V1. The traditional varieties V4, V5 and V6 are tolerant to water stress in vegetative stage. Changes in

the technical culture of rice are needed to increase tolerance to water stress by adjusting planting time, planting season, increasing fertilizer, water-saving irrigation to avoid failure growth and development in the early season of rice cultivation.

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