http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1666.1675

Research Article
Physiological Responses to Drought in Six Rice (*Oryza sativa* L.) Cultivars Cultivated in North Sulawesi, Indonesia

[1]Daniel Peter Mantilen Ludong, [2]Fanny Nella Nanlohy and [3]Song Ai Nio

[1]Department of Agricultural Technology, Faculty of Agriculture, University of Sam Ratulangi, Kampus Unsrat, Manado 95115, North Sulawesi, Indonesia
[2]Biochemistry and Biomolecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Manado State University, Tondano 95618, North Sulawesi, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi, Kampus Unsrat, Manado 95115, North Sulawesi, Indonesia

Abstract

Background and Objective: The drought-tolerant crop plants, including rice, are required for fulfilling food requirements when drought occurs in Indonesia. The response to drought stress in rice cultivars could be studied based on the morphological, anatomical and physiological characteristics. This study evaluated the drought tolerance based on the physiological characteristics at the vegetative phase in six rice cultivars cultivated in North Sulawesi, Indonesia. **Materials and Methods:** The Completely Randomized Design experiment was conducted in the greenhouse by using six rice cultivars (cv. Superwin, Sultan, Ciherang, Serayu, Cigeulis and IR 64) grown in the soil mixture at the vegetative phase. The treatments in this experiment were water deficit (without water for up to 22 days) and well-watered (watering until field capacity). The evaluated physiological characteristics consisted of leaf water content, leaf relative water content, concentrations of chlorophylls (total, a and b) at 0, 7, 14, 17 and 22 days after treatment. **Results:** Withholding water for 22 days at the vegetative phase resulted in a decrease of water content and the increase of concentration of chlorophylls (total, a and b) in leaf. There were two categories of drought tolerance in rice observed in this study, i.e. semi tolerant for Cigeulis, Superwin, Serayu, IR 64, Sultan and non-tolerant for Ciherang. **Conclusion:** Rice cv. Cigeulis, Superwin, Serayu, IR 64 and Sultan were semi tolerant, whereas cv. Ciherang was non-tolerant rice cultivar. Leaf water content was a potential physiological indicator for drought tolerance in rice.

Key words: chlorophyll, drought, relative water content, vegetative, water content

Citation: Ludong, D.P.M., F.N. Nanlohy and S.A. Nio, 2020. Physiological responses to drought in six rice (*Oryza sativa* L.) cultivars cultivated in North Sulawesi, Indonesia. Pak. J. Biol. Sci., 23: 1666-1675.

Corresponding Author: Song Ai Nio, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi, Kampus Unsrat, Manado 95115, North Sulawesi, Indonesia Tel: +62 (431) 525502 Fax +62 (431) 853715, 62.25517

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water availability is a primary limiting factor for plant productivity including rice[1]. The quantity and quality of crop plants are constrained by temporary or continuous drought stress. Drought induces a broad range of plant responses from the cellular level to whole plant levels such as growth rate and crop yield. Understanding the morphological, anatomical, physiological and biochemical mechanisms as a response to drought is required for increasing agricultural production[2].

Developing economical corridor in Indonesia is one of the prominent strategies for economic development in Indonesia. The development theme of economical corridor in the Sulawesi-North Moluccas is "The Centre of Production and Processing of National Product in Agriculture, Plantation and Fishery". Increasing the ability of North Sulawesi Province to be the pillar of national food security by the enhancement of food production is the strategic objective because Sulawesi is the third biggest food producer that contributed 10% of national rice production[3]. The appropriate selection method, therefore, is required to contribute to the achievement of this objective.

The increase in atmospheric carbon dioxide and water deficit threatens the global productivity and sustainability of rice. Water shortage has appeared as one of the biggest restrictions for maintaining rice production. Rice is the most important staple food crop that serves about 35-80% of total calorie intake in Asia and the second most important staple food in the world with an annual production of 740 million tons on 160 million ha rice area[4]. The dry season resulted in a decrease in agricultural production, including rice production in North Sulawesi. Rice production in Indonesia was 71.29 million ton in 2013 and decreased by about 0.63% to be 70.83 million tons in[5] 2014. Rice production in North Sulawesi was 4.793 t ha^{-1} in 2017 and then reduced to 4.402 t ha^{-1} in 2018 (https://www.pertanian.go.id/Data5tahun/TPATAP-2017(pdf)/30-ProdtvPadi.pdf). One reason for the reduction of rice production was a drought stress, resulting in crop failure. Developing drought-resistant rice cultivars is required to elevate rice productivity and reduce food insecurity and poverty[4].

The mechanism of plant response to drought is plant drought resistance. There are three types of drought resistance, i.e. drought escape, drought avoidance and drought tolerance. Drought tolerance enables the plants to survive under drought with the low water potential in plant tissues by turgor maintenance and/or dehydration tolerance. The drought-tolerant plants can be alive and functional in the suboptimal condition when the plant tissues are under drought conditions or the water potential of plant tissues declined. The drought tolerance in crop plants can be evaluated by identifying morphological, anatomical and physiological characteristics that were closely related to crop production under drought condition[6].

The morphological, anatomical and physiological characteristics should be evaluated in some rice cultivars cultivated in North Sulawesi as a response to drought stress. The morphological characteristics (root dry mass, shoot dry mass, total dry mass and root : shoot ratio) had been studied in six rice cultivars cultivated in North Sulawesi. This previous study indicated that root: shoot ratio was the potential indicator for drought tolerance in rice[7]. Some other previous studies reported that drought stress or water deficit decreased leaf water content in wheat[8], leaf relative water content in wheat[8] as well as chlorophylls concentration in sorghum[9]. The present study aimed to evaluate the drought tolerance based on the physiological characteristics (water content, relative water content, the concentrations of total chlorophyll, chlorophyll a and b) and determine the physiological characteristics as drought tolerance indicators in six rice cultivars cultivated in North Sulawesi i.e. Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang. This information will be useful for the breeding program of drought-tolerant rice cultivars that supported the achievement of the rice-self-sufficiency program in North Sulawesi.

MATERIALS AND METHODS

Location and duration of study: The study was carried out in Manado, North Sulawesi, Indonesia from April to November 2012.

Experimental design and treatment: This study was carried out in the greenhouse using rice plants grown in the soil in the polybag at the vegetative phase. The treatments in this experiment were watering until field capacity (control) and withholding water for up to 22 days. Six rice cultivars cultivated in North Sulawesi Province, i.e. Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang were used in this experiment. The mixture of soil, compost and hull of rice (5:1:1) was used as the media in the polybag. Seeds of rice were submerged in the water and the sown seeds were grown on the filter paper that was saturated with water in a petri dish under dark condition. After 2 days, the germinated seeds were sown one cm depth in the media that had been watered until filed capacity in the polybags. All polybags were placed in the greenhouse that was covered with a shading net (55%) above the roof to reduce the light intensity during the experiment.

The plants were grown for five weeks and watered with fertilizer solution (5 g fertilizer/10 L water) until field capacity every second day before the treatments commenced. The fertilizer contained 20% N total, 15% P_2O_5, 15% K_2O, 1% $MgSO_4$, Mn, B, Cu, Co, Zn, aneurine, lactoflavin, nicotinic acid amide[7]. This experiment consisted of two treatments (well-watered as control and water deficit), six cultivars, five sampling times and three replicates. Water deficit treatment commenced when the plants were at the 5-fully expanded leaf stage (35 days after sowing), whereas well-watered plants were watered (water only without fertilizer) every second day. The sampling time was conducted when the water deficit treatment commenced (day 0), 7, 14, 17 and 22 days after treatment.

Data collection: Water usage was measured by weighing polybags with the soil and plants every 2-3 days. Cumulative transpiration was calculated by adding the amount of lost water at every measurement. Soil water content was calculated using this following equation[8]:

$$100\times\frac{\text{Fresh weight-dry weight}}{\text{Dry weight}}$$

The fresh weight of each soil sample was weighed and then the soil was dried at 105°C for 24 hrs to determine the dry weight[8]. The water content, relative water content, the concentrations of chlorophylls (total, a and b) of rice leaves were measured on cv. Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang. The leaf water content and relative water content were determined by weighing the fresh weight of cut leaves, submerging in 0.5 mM $CaSO_4$ for 24 hrs under dark condition, weighing the turgid weight, drying in the oven for 24 hrs at 70°C and weighing the dry weight. The leaf water content (mL g^{-1} dry weight) was calculated as[8]:

$$\frac{\text{Fresh weight-dry weight}}{\text{Dry weight}}$$

The leaf water relative content (%) was calculated as[8]:

$$100\times\frac{\text{Fresh weight-dry weight}}{\text{Turgid weight-dry weight}}$$

Leaf chlorophyll was extracted using 95% ethanol and the concentration of concentrations of chlorophylls (total, a and b) were measured using spectrophotometer SP-3000 nano Optima® at λ 649 and 665 nm[10].

Statistical analysis: Microsoft Office Excel 2010 was used to calculate the mean and standard errors of the data. Data were analyzed using Analysis of Variance (ANOVA) followed by the Least Significant Difference (LSD) 1% to identify the significant differences and interactions among treatments (where $p<0.01$). Pearson correlation coefficients of physiological characteristics were calculated by using Microsoft Office Excel 2010. The levels of drought tolerance in six rice cultivars were determined using deviation standard of each physiological characteristics and then was categorized in three groups: (1) Tolerant (T) if $xi>x+SD$, (2) Semi tolerant (ST) if $x-SD\leq xi\leq x+SD$ and (3) Non-tolerant (NT) if $xi<x-SD$ where xi is mean of certain physiological characteristics in each cultivar, x is mean of certain physiological characteristics in all cultivars and SD is deviation standard[11,12].

RESULTS

Stress response by rice crops is a complex phenomenon including morphological, anatomical and physiological aspects. The study on six rice cultivars (Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang) showed that the leaves of rice cultivar Superwin started to be rolled at 10 days after water deficit treatment. The rolling leaves were observed in all rice cultivars at 14 days after treatment, except in Ciherang at 17 days after treatment. The water deficit, therefore, was stopped at 22 days after treatment. The leaves were sampled at 0 (the treatments commenced), 7, 14 and 22 days after treatment for evaluating physiological characteristics. The water content, relative water content, the concentration of chlorophylls (total, a and b) in leaf were measured as the response of rice plants to water deficit. The cumulative transpiration and soil water content were also measured to indicate the plant water usage and lost water by evapotranspiration.

Cumulative transpiration and soil water content: The cumulative transpiration in six rice cultivars cultivated in North Sulawesi Province, Indonesia, under well-watered (WW) condition (Fig. 1a) were larger than under water deficit (WD, Fig. 1b). The cumulative transpiration under WW condition increased exponentially from day 0 and reached 1300 mm at day 21 (Fig. 1a), but it slightly increased to 200 mm from day 0-day 5 and was stable at 500 mm-towards 21 days after WD treatment (Fig. 1b).

Soil water content under water deficit (WD) was smaller than under well-watered (WW) condition from 7 until 22 days of treatment. Soil water content under WD declined gradually and depleted to 9% at 22 days after treatment, whereas soil water content under WW was about 40% (Fig. 2).

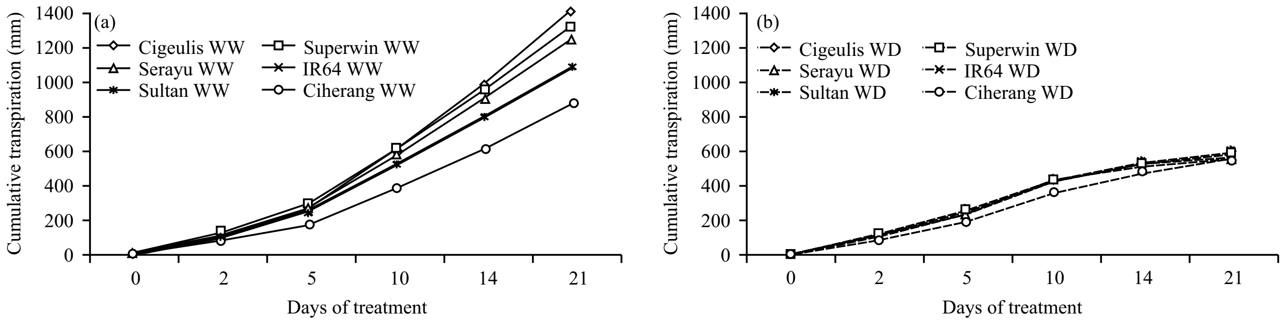

Fig. 1(a-b): Cumulative transpiration of rice cultivar Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang under well-watered or watering until (a) field capacity and (b) water deficit for up to 22 days of treatment. WW: Well-watered, WD: Water deficit

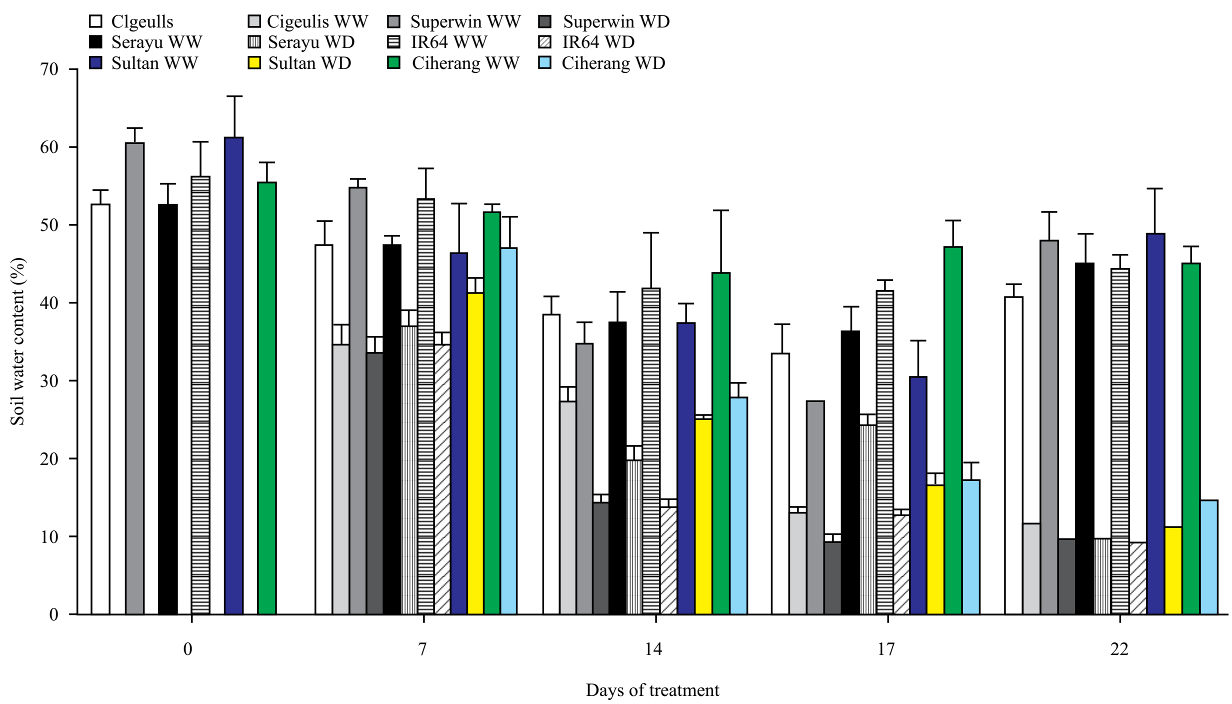

Fig. 2: Soil water content (Mean$\pm$SE; n = 3) in rice cultivar Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang at 0, 7, 14, 17 and 22 days after well-watered (WW) and water deficit (WD) treatments

The values are significantly different among six rice cultivars at 0, 7, 14, 17 and 22 days after treatment (p<0.01)

Leaf water content and leaf relative water content: Leaf water content in six rice cultivars under WW and WD conditions decreased during 22 days of treatment. The leaf water content in WW reduced slightly from 3.5 mL g^{-1} dry weight at day 0 until 2.2 mL g^{-1} dry weight after 22 days of treatment. The leaf water content in WD also decreased from 3.5 mL g^{-1} dry weight at day 0 to about 1.2 mL g^{-1} dry weight at 22 days after withholding water (Fig. 3). The difference of leaf water content between WW and WD treatments (p<0.01) was observed at 14 days after treatment and more significantly at 22 days after treatment. The significant differences of leaf water content between WW and WD were observed in cv. Cigeulis, Serayu, IR 64 and Sultan at 22 days after treatment. Leaf water content in WD was about 50% lower than in WW. The differences of leaf water content between WW and WD in cv. Superwin and Ciherang were smaller than those in four other cultivars, i.e. 2.1 mL g^{-1} dry weight in WW and 1.5 mL g^{-1} dry weight in WD for cv.

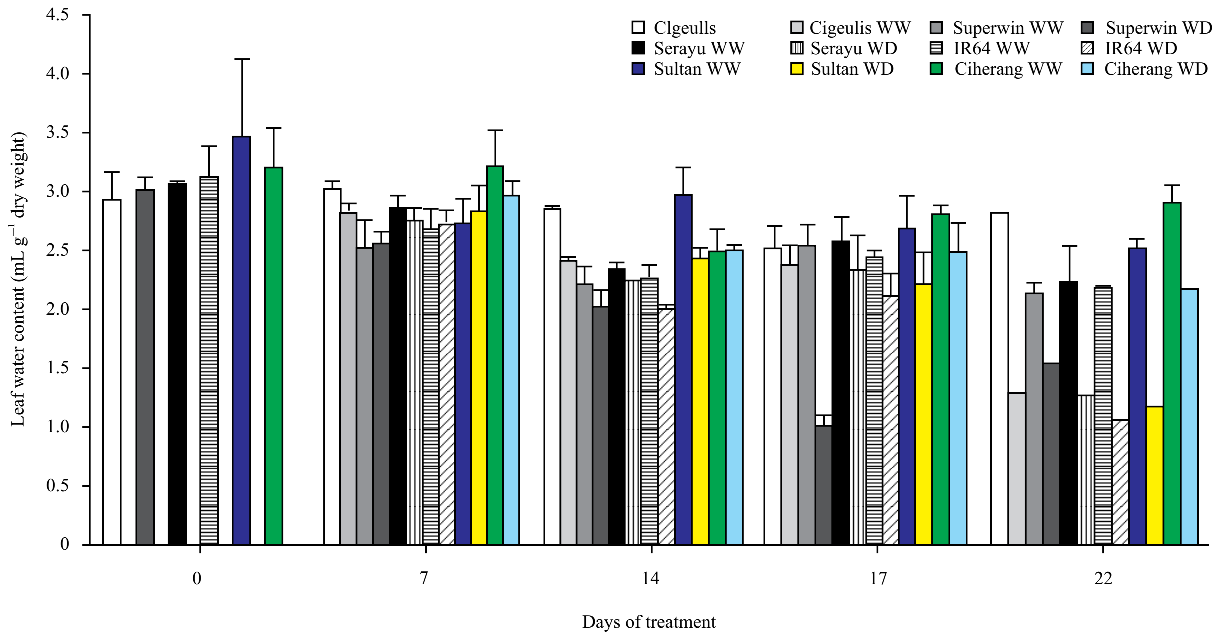

Fig. 3: Leaf water content (Mean±SE; n = 3) in rice cultivar Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang at 0, 7, 14, 17 and 22 days after well-watered (WW) and water deficit (WD) treatments

The values are significantly different among six rice cultivars at 0, 7, 14, 17 and 22 days after treatment (p<0.01)

Superwin as well as 2.9 mL g^{-1} dry weight in WW and 2.2 mL g^{-1} dry weight in WD for cv. Ciherang. Under WD leaf water content in cv. Superwin and Ciherang were 54% higher than four other rice cultivars. The ANOVA results in this study, however, showed that there were no significant differences (p>0.01) of leaf relative water content between WW and WD, among the cultivars and among the treatment periods (Fig. 4).

Leaf chlorophylls (total, a and b): The concentration of leaf total chlorophylls in cv. Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang were significantly different (p<0.01) at 22 days after withholding water, i.e. leaf total chlorophylls in WD were 25% larger than in WW (Table 1). The significant differences were observed in cv. IR 64 (26 mg L^{-1} in WW and 47 mg L^{-1} in WD) and Sultan (26 mg L^{-1} in WW and 40 mg L^{-1} in WD). Similar to the concentration of total chlorophyll, the concentration of chlorophyll a under WD was generally 15.72% higher than under WW at 22 days after treatment (Table 1). The big differences were observed in cv. IR 64 (18 mg L^{-1} in WW and 26 mg L^{-1} in WD) and Sultan (18 in WW and 25 mg L^{-1} in WD), but the differences in cv. Serayu and Superwin were very small.

The effect of water deficit treatment on the concentration of chlorophyll b during 22 days in six rice cultivars was inconsistent. In general, the concentration of chlorophyll b in WD was higher than in WW, except in cv. Ciherang (Table 1). The significant differences between the WW and WD treatments were observed in cv. IR 64 and Sultan. The concentrations of leaf chlorophyll b in WD were two and three times higher than in WW for cv. Sultan and IR 64, respectively.

Correlation between the physiological characteristics at 22 days after water deficit treatment: The correlation between physiological characteristics in six North Sulawesi cultivated rice cultivars at 22 days after water deficit treatment at the vegetative stage was determined using Pearson correlation coefficient (Table 2). Leaf water content and relative water content had a strong and positive correlation with soil water content, but these physiological characteristics had a strong and negative correlation with the concentration of chlorophylls (total, a and b). There were a positive strong correlation between the concentration of chlorophylls (total, a and b). It implied that leaf water content and relative water content decreased (Fig. 2 and 3) when the concentration of chlorophylls (total, a and b) increased (Table 1) under WD condition.

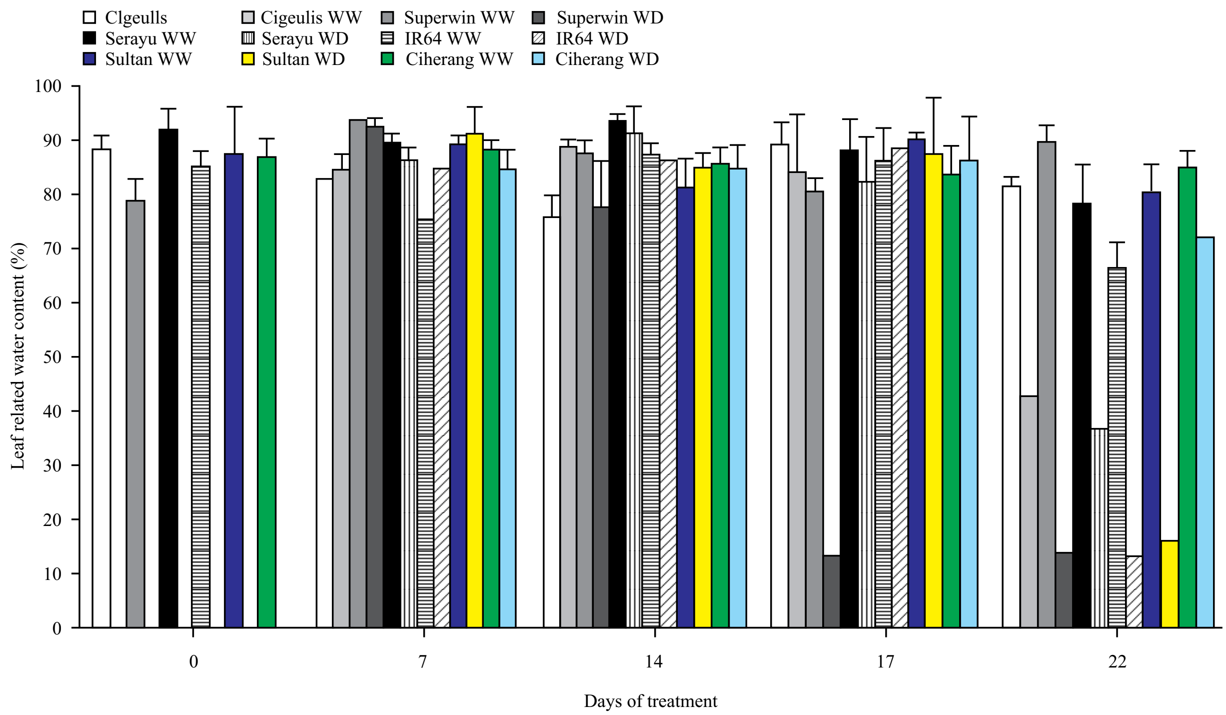

Fig. 4: Leaf relative water content (Mean±SE; n = 3) in rice cultivar Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang at 0, 7, 14, 17 and 22 days after well-watered (WW) and water deficit (WD) treatments

The level of drought tolerance based on the physiological characteristics: The level of drought tolerance in six rice cultivars cultivated in North Sulawesi under water deficit for 22 days based on the physiological characteristics were categorized based on deviation standard. Leaf water content, relative water content, the concentration of chlorophylls (total, a and b) were used in categorizing the level of drought tolerance (Table 3). The total level of drought tolerance based on five physiological characteristics that were observed in each cultivar showed that cv. Cigeulis, Superwin, Serayu, IR 64 and Sultan were semi tolerant rice cultivars, whereas cv. Ciherang was a nontolerant rice cultivar.

DISCUSSION

This study showed that cumulative transpiration in six rice cultivars cultivated in North Sulawesi under WD was smaller than under WW. Similar to this study, the difference of cumulative transpiration between WW and WD plants in wheat (*Triticum aestivum* L.) cv. Hartog and Sunco were observed at 10 days after treatment and this condition could be related to the leaf rolling and the inhibition of leaf growth[8]. The declined water usage that was measured as cumulative transpiration was also reported in rice[7] and this phenomenon was an adaptive mechanism to reduce the negative effect of water deficit.

In this study, soil water content under WD decreased gradually and reached 9% at 22 days after treatment, whereas soil water content under WW was about 40%. This low soil water content could be related to the leaf rolling from 10-22 days after withholding water. It was also reported that soil water content in rice commenced to be decreased in WD plants since 7 days of treatment and reached 12% compared with 47% in WW treatment[7].

Water content is considered as a measurement of water status that reflected the metabolic activity in plant tissues and used as the most meaningful indicator for drought tolerance[2]. Leaf water content in plants under WD condition was commonly smaller than in WW plants as observed in wheat (*Triticum aestivum* L.) cv. Hartog and Sunco[8] as well as in North Sulawesi local rice (cv. Superwin, Ombong, Temo and Burungan) for 14 days of treatment at the vegetative phase[13]. The leaf water content in WD was lower than in WW and this condition could be related to the significant difference in soil

Table 1: The concentrations of leaf total chlorophyll, chlorophyll a and chlorophyll b (Mean±SE; n = 3) in rice cv. Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang at 0, 7, 14, 17 and 22 days after well-watered (WW) and water deficit (WD) treatment

Cultivar	Treatments	Day	Chlorophyll total (mg L^{-1})	Chlorophyll a (mg L^{-1})	Chlorophyll b (mg L^{-1})
Cigeulis	WW	0	39.48±3.57^{a-i}	23.420±1.02^{a-g}	15.88±3.54^{a-h}
		7	34.87±4.55^{a-m}	22.27±2.31^{a-i}	12.430±2.25^{f-p}
		14	21.10±7.72^{no}	14.76±5.00^{jk}	6.240±2.68^{p}
		17	36.54±0.95^{a-k}	23.44±0.46^{a-g}	12.930±0.53^{d-o}
		22	37.30±2.91^{a-k}	24.47±1.04^{a-g}	12.650±1.91^{e-p}
	WD	0	39.48±3.57^{a-i}	23.420±1.02^{a-g}	15.88±3.54^{a-h}
		7	22.17±3.61^{l-o}	14.94±2.49^{jk}	7.110±1.11^{n-p}
		14	33.42±0.53^{b-o}	23.16±0.06^{a-g}	10.090±0.57^{f-p}
		17	39.77±3.99^{a-h}	24.28±1.48^{a-g}	15.310±2.50^{a-i}
		22	42.90±0.63^{a-e}	26.08±0.20^{ab}	16.630±0.58^{a-f}
Superwin	WW	0	34.11±4.19^{a-n}	21.730±2.09^{a-j}	12.22±2.08^{f-p}
		7	29.47±6.28^{f-o}	18.73±3.87^{a-j}	10.600±2.39^{f-p}
		14	36.17±4.09^{a-k}	24.11±1.98^{a-g}	11.890±2.10^{f-p}
		17	29.98±1.95^{e-o}	19.99±1.09^{a-k}	9.850±0.88^{g-p}
		22	41.93±3.12^{a-f}	25.56±0.74^{a-d}	16.180±2.51^{a-g}
	WD	0	34.11±4.19^{a-n}	21.730±2.09^{a-j}	12.22±2.08^{f-p}
		7	29.11±3.89^{f-o}	19.64±2.59^{c-k}	9.330±1.28^{h-p}
		14	29.30±3.55^{f-o}	20.60±2.19^{a-k}	8.550±1.41^{i-p}
		17	44.72±5.51^{abc}	24.93±0.78^{a-f}	19.610±4.78^{a-c}
		22	46.05±3.49^{ab}	25.92±0.67^{a-c}	19.940±2.84^{ab}
Serayu	WW	0	29.65±6.44^{f-o}	19.310±3.47^{b-k}	10.20±2.95^{f-p}
		7	31.01±5.54^{e-o}	19.53±3.33^{b-k}	11.340±2.21^{f-p}
		14	28.47±6.17^{g-o}	19.66±3.83^{a-k}	8.660±2.43^{i-p}
		17	37.01±2.10^{a-k}	23.51±0.79^{a-g}	13.320±1.31^{b-n}
		22	35.15±2.13^{a-l}	23.92±0.99^{a-g}	11.050±1.13^{f-p}
	WD	0	29.65±6.44^{f-o}	19.310±3.47^{b-k}	10.20±2.95^{f-p}
		7	30.87±2.63^{o}	19.87±1.61^{a-k}	10.860±1.01^{f-p}
		14	33.45±3.85^{b-o}	22.84±1.82^{a-h}	10.450±2.03^{f-p}
		17	34.73±2.09^{a-m}	22.41±1.17^{a-i}	12.160±0.91^{f-p}
		22	38.13±2.75^{a-k}	24.52±1.07^{a-g}	13.420±1.70^{b-n}
IR 64	WW	0	38.66±4.41^{a-j}	23.840±1.86^{a-g}	14.64±2.55^{a-k}
		7	23.82±7.25^{l-o}	15.60±4.47^{h-k}	8.100±2.75^{k-p}
		14	34.41±3.18^{a-m}	23.10±1.57^{a-g}	11.140±1.62^{f-p}
		17	34.75±5.67^{a-m}	21.63±2.42^{a-j}	12.950±3.29^{c-o}
		22	26.12±4.31^{j-o}	18.70±2.72^{c-k}	7.280±1.57^{m-p}
	WD	0	38.67±4.41^{a-j}	23.840±1.86^{a-g}	14.64±2.55^{a-k}
		7	46.28±3.73^{ab}	26.87±0.36^{a}	19.220±3.38^{a-e}
		14	34.73±4.21^{a-m}	23.43±2.19^{a-g}	11.130±2.01^{f-p}
		17	37.62±1.50^{a-k}	23.60±0.47^{a-g}	13.850±1.10^{b-m}
		22	47.25±4.53^{a}	26.02±0.52^{ab}	21.040±4.09^{a}
Sultan	WW	0	44.38±6.37^{a-d}	24.640±1.61^{a-g}	19.56±5.01^{a-d}
		7	27.86±9.16^{g-o}	17.34±4.86^{g-k}	10.400±4.27^{f-p}
		14	21.77±4.52^{mno}	15.13±3.13^{ijk}	6.521±1.374^{op}
		17	27.28±0.82^{g-o}	18.18±0.31^{e-k}	8.960±0.54^{i-p}
		22	26.30±7.14^{i-o}	18.25±4.74^{d-k}	7.910±2.37^{l-p}
	WD	0	44.38±6.37^{a-d}	24.640±1.61^{a-g}	19.56±5.01^{a-d}
		7	28.68±6.74^{g-o}	18.38±4.21^{d-k}	10.170±2.50^{f-p}
		14	25.13±3.39^{k-o}	17.46±2.36^{g-k}	7.540±1.03^{l-p}
		17	26.73±2.53^{h-o}	17.63±1.68^{f-k}	8.970±0.85^{i-p}
		22	40.44±4.10^{a-g}	25.27±071^{a-e}	14.980±3.36^{a-j}
Ciherang	WW	0	27.53±2.37^{g-o}	17.870±1.59^{f-k}	9.53±0.77^{g-p}
		7	28.88±4.30^{f-o}	18.53±2.19^{d-k}	10.210±2.09^{f-p}
		14	37.42±5.10^{a-k}	23.59±2.42^{a-g}	13.660±2.63^{b-n}
		17	28.32±4.46^{g-o}	18.56±2.62^{c-k}	9.628±1.856^{g-p}
		22	31.31±9.53^{d-o}	18.52±4.49^{d-k}	12.660±5.11^{e-p}
	WD	0	27.53±2.37^{g-o}	17.87±1.59^{f-k}	9.530±0.77^{g-p}
		7	20.46±2.75^{o}	13.80±1.92^{k}	6.560±0.82^{op}
		14	32.23±5.93^{c-o}	21.30±3.34^{a-j}	10.770±2.59^{f-p}
		17	38.13±2.87^{a-k}	23.99±1.04^{a-g}	13.970±1.82^{b-l}
		22	32.44±5.90^{c-o}	21.78±3.00^{a-j}	10.490±2.96^{f-p}

Means with the different letter within a column indicate significant difference at p<0.01 using least significance difference (LSD), ns: Non-significant

Table 2: Pearson correlation coefficient of physiological characters in six North-Sulawesi- cultivated-rice cultivars at 22 days after withholding water at the vegetative stage

Parameters	Soil water content	Leaf water content	Leaf relative water content	Total chlorophyll	Chlorophyll a	Chlorophyll b
Cumulative transpiration	-0.148^{ns}	-0.5120*	-0.4302^{ns}	0.21200^{ns}	0.5520*	0.0600^{ns}
Soil water content		0.781*	0.7980*	-0.78300*	-0.7690*	-0.7550*
Leaf water content			0.992*	-0.70400*	-0.8420*	-0.6160*
Leaf relative water content				-0.659*	-0.7830*	-0.5780*
[Total chlorophyll]					0.926*	0.9870*
[Chlorophyll a]						0.855*

*p<0.01 significant, ns: Non-significant

Table 3: The level of drought tolerance in six North Sulawesi-cultivated-rice cultivars based on the physiological characters at 22 days after withholding water at the vegetative phase

	Cultivars					
Physiological characters (x±SD)	Cigeulis	Superwin	Serayu	IR64	Sultan	Ciherang
Leaf water content (mL g^{-1} dry mass) 1.426±0.402	1.316 (ST)	1.544 (ST)	1.278 (ST)	1.066 (NT)	1.175 (ST)	2.179 (T)
Leaf relative water content (%) 45.549±15.117	43.925 (ST)	51.452 (ST)	37.496 (ST)	30.582 (ST)	37.024 (ST)	72.816 (T)
[Total chlorophyll] (mg L^{-1}) 41.200±5.474	42.900 (ST)	46.045 (ST)	38.125 (ST)	47.252 (T)	40.444 (ST)	32.437 (NT)
[Chlorophyll a] (mg L^{-1}) 24.932±1.653	26.076 (ST)	25.917 (ST)	24.524 (ST)	26.018 (ST)	25.273 (ST)	21.783 (NT)
[Chlorophyll b] (mg L^{-1}) 16.084±3.982	16.631 (ST)	19.936 (ST)	13.419 (ST)	21.041 (T)	14.984 (ST)	10.493 (NT)
Total level	0 (T)	0 (T)	0 (T)	2 (T)	0 (T)	2 (T)
	5 (ST)	5 (ST)	5 (ST)	2 (ST)	5 (ST)	0 (ST)
	0 (NT)	0 (NT)	0 (NT)	1 (NT)	0 (NT)	3 (NT)
Category of level	Semi tolerant	Semi tolerant	Semi tolerant	Semi tolerant	Semi tolerant	Non tolerant

xi: Mean in certain cultivar, x: Mean in all cultivars, SD: Standard deviation, Tolerant (T): xi>x+SD, Semi tolerant (ST): x-SD≤xi≤x+SD, Non tolerant (NT): xi<x-SD

water content at 14 days after treatment. The drought-tolerant plants were able to osmotically adjust by maintaining turgor, higher leaf water content and other metabolic activities. The high ability of osmotic adjustment in plants enabled the plants to avoid leaf cell dehydration and then the plants could continue the other physiological processes, such as cell enlargement, photosynthesis and water extraction[8]. This study indicated that leaf water content was a potential indicator of drought stress in rice plants.

The chlorophyll concentration was a potential indicator for evaluating metabolism imbalances between photosynthesis and crop production under water deficit. The chlorophyll concentration decreased intolerant and sensitive sweet sorghum (*Sorghum bicolor* L. Moench) genotypes. The reduction of chlorophyll concentration in the sensitive genotype was larger than the tolerant genotype under drought. The chlorophyll concentration in drought-tolerant-sorghum-genotype was higher than in the sensitive genotype[9]. The concentration of chlorophylls (total, a and b) in North Sulawesi local rice (*Oryza sativa* L.) cultivars declined as a response to PEG (polyethylene glycol) 8000-induced water deficit. The concentration of leaf chlorophyll total and chlorophyll a were potential physiological indicators for water deficit induced by PEG 8000 in rice[14]. The chlorophyll concentration decreased in plants under water deficit. The chlorophyll concentration declined under drought as the chlorophyll synthesis was slow and this pigment was fast breakdown, protein complexes were unstable and the chlorophyll was destructed by elevated activity of chlorophyll degrading enzymes as well as chlorophyllase[15,16]. The decrease of chlorophyll concentration has also resulted from the degradation of chloroplast and other apparatus of photosynthesis. In addition, chlorophyll concentration was positively correlated with photosynthesis capacity and plant production[9].

This study indicated that the concentration of leaf chlorophylls (total, a and b) in rice cultivars under WD was larger than under WW. The concentration of leaf chlorophylls (total, a and b) after 4 weeks of water deficit was lower than in well-watered potato (*Solanum tuberosum* L.) plants; however, these variables did not clearly describe the drought sensitivity or drought tolerance[17]. The concentration of chlorophylls (total, a and b) in *Vigna sinensis* L. was not significantly different between irrigation treatments of 50 and 150% field capacity[18]. The concentration of leaf chlorophylls (total, a and b) in rice cultivar Serayu and IR 64 under WD induced by polyethylene glycol was higher under WW condition[19]. The increase of chlorophyll b concentration under drought was also reported in rice cv. Rokan, Jatiluhur and IR 64[20]. These rice cultivars adapted to drought stress by increasing chlorophyll b concentration in order to elevate photosynthetic capacity. Chlorophyll b is a photosynthetic antenna pigment that absorbs light and then transfers it to other pigments as the center reaction in the photosystem of photosynthesis. The light energy as the photon is converted to chemical energy as ATP and NADPH that are used in the reaction of CO_2 reduction[21]. These results could be related to the decreased leaf water content in WD to be 50% lower than in WW and this condition affected the measurement of leaf chlorophyll concentration based on the fresh weight of leaf tissue. The potency of chlorophyll concentration as a drought stress indicator, therefore, was not shown in these six rice cultivars used in this study. The measurement of chlorophyll concentration based on the leaf fresh weight in this study should be modified to be based on the leaf dry weight or leaf unit area in the future study.

Leaf water content was the potential physiological indicator in rice as a response to water deficit in this study. The leaf water content in six rice cultivars (Cigeulis, Superwin, Serayu, IR 64, Sultan and Ciherang) under WD were lower than in WW at 22 days after treatment. Cigeulis, Superwin, Serayu, IR 64 and Sultan were semi tolerant rice cultivars and could be recommended to be cultivated in the dry season when the water availability was limited. Evaluation of drought tolerance in this local rice based on the morphological[7] and physiological characteristics (this study) at the vegetative stage, however, should be enriched with anatomical, biochemical, molecular and other characteristics as a response to drought stress.

CONCLUSION

The study showed that cv. Cigeulis, Superwin, Serayu, IR 64 and Sultan were semi tolerant rice cultivars, whereas cv. Ciherang was a nontolerant rice cultivar based on the total level of drought tolerance using five physiological characteristics observed in each rice cultivar. Leaf water content was a potential physiological indicator of drought tolerance in rice.

SIGNIFICANCE STATEMENT

The results of this study indicated that cv. Superwin as North Sulawesi local rice as well as Cigeulis, Serayu and IR 64, were potential to be used as germplasm source for staple food, especially in the dry season. This study also provided information on leaf water content as a physiological indicator that could be used in a simple selection method for obtaining drought- tolerant-rice cultivars that were required to minimize the loss of rice production during dry season.

ACKNOWLEDGMENTS

This work was financially supported by the Directorate of Research and Community Service, Ministry of Education and Culture, Republic of Indonesia, through for Fundamental Research fiscal year 2012 (grant number: 0748/023-04.2.01/27/2012). Special thanks to Balai Pengawasan Sertifikasi Benih Tanaman Pangan dan Hortikultura, North Sulawesi Province, Indonesia, for the seeds. Sincere thanks to Dr. Agus Darwanto, Novartis Institute for Biomedical Research, Cambridge USA for useful suggestions.

REFERENCES

1. Liu, H.Y., S, Hussain, M.M. Zheng, S.B. Peng, J.L. Huang, K.H. Cui and L.X. Nie, 2015. Dry direct-seeded rice as an alternative to transplanted-flooded rice in Central China. Agron. Sustainable Dev., 35: 285-294.
2. Anjum, S.A., X.Y. Xie, L.C. Wang, M.F. Saleem, C. Man and W. Lei, 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res., 6: 2026-2032.
3. Perekonomian R.I.M.B., 2011. Master plan for the acceleration and expansion of Indonesia's economic development 2011-2025-Sulawesi corridor. Paper presented at Seminar Nasional Orientasi Program Pembangunan Sulawesi Utara, Manado, 16 April, 2011.
4. Kumar, A., A.K. Nayak, B.S. Das, N. Panigrahi and P. Dasgupta *et al*., 2019. Effects of water deficit stress on agronomic and physiological responses of rice and greenhouse gas emission from rice soil under elevated atmospheric CO_2. Sci. Total Environ., 650: 2032-2050.
5. BPS, 2013. Temporary production of corn, rice and soybeans. http://banten.bps.go.id/pdf/1314-Asem 2012- 010313.pdf.
6. Nio, S.A., 2015. Plant physiology under drought stress. http://repo.unsrat.ac.id/2386/1/Fisiologi_Tanaman_Pada_Saat_Kekeringan.pdf.
7. Nio, S.A. and D.P.M. Ludong, 2014. Comparing the drought tolerance of local rice cultivar Superwin with other cultivars cultivated in North Sulawesi Province based on dry matter partitioning. Proc. Int. Conf. Global Resour. Conserv., 4: 17-22.
8. Nio, S.A., G.R. Cawthray, L.J. Wade and T.D. Colmer, 2011. Pattern of solutes accumulated during leaf osmotic adjustment as related to duration of water deficit for wheat at the reproductive stage. Plant Physiol. Biochem., 49: 1126-1137.
9. Kristanto, B.A., D. Indradewa, A. Ma'as, and R.D. Sutrisno, 2014. Leaf senescence, leaf chlorophyll content and grain yield in sweet sorghum (*Sorghum bicolor* L. Moench) under water-stressed condition. AgroUPY, 6: 38-49.

10. Sigala, C., N.G. Songke, K.P. Tumoka, R.R. Butarbutar and S.A. Nio, 2019. The concentration of leaf chlorophyll in croton plant (*Codiaeum variegatum* L.) under shading treatment. Jurnal Ilmiah Sains, 19: 70-73.
11. Susilawati, R.A. Suwignyo, Munandar and M. Hasmeda, 2012. Agronomic and physiological characteristics and tolerance of red chili varieties under waterlogging stress. J. Agron. Ind., 3: 196-203.
12. Nio, S.A., R. Siahaan and D.P.M. Ludong, 2019. Partial submergence tolerance in rice (*Oryza sativa* L.) cultivated in North Sulawesi at the vegetative phase. Pak. J. Biol. Sci., 22: 95-102.
13. Koda, M., S.A. Nio, and P. Siahaan, 2017. Leaf water content of North Sulawesi local rice at the vegetative phase under waterlogging and water deficit condition. J. Bios Logos, 7: 22-26.
14. Nio, S.A., M. Pirade, D. Peter and M. Ludong, 2020. Leaf chlorophyll content in North Sulawesi (Indonesia) local rice cultivars subjected to polyethylene glycol (PEG) 8000-induced water deficit at the vegetative phase. Biodiversitas, 20: 2462-2467.
15. Akram, H.M., A. Ali, A. Sattar, H.S.U. Rahman and A. Bibi, 2013. Impact of water deficit stress on various physiological and agronomic traits of three basmati rice (*Oryza sativa* L.) cultivars. J. Anim. Plant Sci., 23: 1415-1423.
16. Amini, R., M.A. Milani and A.D. Mohammadinasab, 2013. Physiological response of lentil (*Lens culinaris* Medick.) to water limitation affected by wheat straw mulch application. J. Agron. Plant Prod., 4: 2548-2553.
17. Van der Mescht, A., J.A. de Ronde and F.T. Rossouw, 1999. Chlorophyll fluorescence and chlorophyll content as a measure of drought tolerance in potato. South Afr. J. Sci., 95: 407-412.
18. Hendriyani, I.S. and N. Setiari, 2009. Chlorophyll content and growth of long beans (*Vigna sinensis* L.) plants at different water supply rates. J. Sains Matematika, 17: 145-150.
19. Banyo, Y.E., S.A. Nio, P. Siahaan and A. Tangapo, 2013. The chlorophyll concentration in rice leaves under polyethylene-glycol-induced water deficit. J. Ilmiah Sains, 13: 1-8.
20. Maisura, M.A.C., I. Lubis, A. Junaedi and H. Ehara, 2014. Some physiological character responses of rice under drought conditions in a paddy system. ISSAAS J., 20: 104-114.
21. Sujinah and A. Jamil, 2016. Response mechanism of rice under drought stress and tolerant varieties. Iptek Tanaman Pangan, 11: 1-7.