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J. ISSAAS Vol. 20, No. 1:104-114 (2014) 104 SOME PHYSIOLOGICAL CHARACTER RESPONSES OF RICE UNDER DROUGHT CONDITIONS IN A PADDY SYSTEM Maisura 1, Muhamad Achmad Chozin 2, Iskandar Lubis 2, Ahmad Junaedi 2 and Hiroshi Ehara 3
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(Received: April 2, 2013; Accepted: May 24, 2014) ABSTRACT The most serious impact of climate change is the El Nino phenomenon that creates increased drought in paddy systems. Drought is the most important limiting factor for the sustainability of rice production.

This research aimed to identify the physiological character of drought tolerant rice varieties in a paddy system under greenhouse conditions. The experimental design used was split plot design with three replications, with drought stress as the main plot and variety as sub-plot. The main plots consisted of a control (normal irrigation) and drought stress (drought imposed three weeks after transplant up to harvest).

The sub-plots consisted of eight rice varieties: IR 64, Ciherang, IPB 3S, Way Apo Buru, Jatiluhur, Menthik Wangi, Silugonggo, and Rokan. Results showed that drought stress led to a decrease in grain yield per hill, chlorophyll a content and chlorophyll a/b ratio, and an increment of proline and total sugar accumulation.

Tolerant varieties (Ciherang, Way Apo Buru and Jatiluhur) accumulated proline over a longer time and increased accumulation of total sugar at the stage of pre anthesis. Jatiluhur varieties showed less reduction in grain yield under drought stress (40.7%) than other varieties. Less reduction in grain yield under drought stress, accumulation of proline in a longer time, and an increased total sugar accumulation at anthesis phase during drought were physiological characters that played an important role in tolerance to drought stress in paddy systems.

Key words : chlorophyll , proline, starch, total sugar INTRODUCTION Drought is the most important limiting factor affecting rice production. This has become a problem for rice developing countries all over the world (Passioura, 2007). Currently, the situation has become more severe by climate change (Kawasaki and Herath, 2011; Prasad et al., 2012).

Rice is a semi-aquatic plant that grows normally in flooded conditions. However, almost 50% of the paddy field system does not have sufficient water for irrigation. Therefore, a serious drought problem can affect rice production and quality. The most serious impact of climate change is the El Nino phenomenon that results in an increased drought in paddy systems, which can occur when soil water content drops below saturation. 105 Rice has a variety of mechanisms by which it reacts to such conditions.

Analysis of data on the impact of drought on paddy systems showed an increasing trend of total area affected by drought reaching 230.000 and 400.000 hectares in 2010 and 2011, respectively (Department of Agriculture, 2011). Planting rice in the Asian region generally follows a bimodal rainfall pattern, thus drought stress during the growing season can be classified into 3 types, namely drought stress: early in the growing season (early stress), in the middle of the growing season (mild - intermittent stress), and in the late growing season (late stress) (Chang et al.,1979).

The response of rice plants to drought depends on the time and periods of drought associated to the growing phase (Fukai and Cooper, 1995). This suggests that drought stress at different growth stages of rice will show a different response. Rice plant growth include three main phases, namely vegetative, reproductive, and maturation (De Datta, 1981). Wopereis et al.

(1996) described the effect of the early phase of drought stress on vegetative tiller growth and delayed flowering. In the reproductive phase, especially during flowering, it is known to be sensitive to drought, followed by gametogenesis (booting) and grain filling. Drought also affects the reproductive phase wherein there is an increase in percent empty grains and a decrease in grain weight causing a decrease in yield.

Rice sensitivity to drought increases when drought happens during the vegetative and flowering period. This leads to a decrease in seed production (O'Toole, 1982). Plant's response to drought stress can be analyzed through identification of characters that play an important role in drought tolerance. Analysis can be done at the morphological, physiological, cellular, biochemical, and molecular levels.

Drought response at the cellular stage depends on the plant stage when drought occurs, duration of drought, and plant species (Prasad et al., 2012). An important physiological response of plants to drought is its ability to maintain turgor pressure by reducing osmotic potential as a tolerant mechanism. The decrease in osmotic potential and the ability to accumulate soluble compounds help maintain turgor pressure.

In the process of osmotic adjustment, the soluble compounds that are usually accumulated are sugar and amino acids, especially proline (Girousse et al., 1996; Szabados and Savoure, 2009). Studies show that drought can accelerate senescence and remobilize assimilation in the stems to the seeds in cereal crops (Yang et al., 2001; Yang et al., 2003).

Tubur et al. (2012) reported that some varieties have a high tolerance to drought. Paddy rice and upland rice varieties are divided into three groups: tolerant varieties (Ciherang, Jatiluhur and Way Apo Buru), moderate (IPB 3S and Silugonggo), and susceptible varieties (IR 64, Menthik Wangi and Rokan) to drought. The same varieties were also used by Supijatno et al.

(2012) who reported that Jatiluhur variety was more efficient in water use than other varieties. Jatiluhur variety had a better yield and used less water per unit of yield. Further research is also needed to examine important physiological characteristics associated with drought stress tolerance.

This research aimed to identify the physiological characters of drought tolerant rice in a paddy system under greenhouse conditions. **MATERIALS AND METHODS** This experiment was done in a plastic house at the Rice Research Bogor Agricultural University in 2012. Plant material used are IR 64, Ciherang, IPB 3S, Way Apo Buru, Jatiluhur, Menthik Wangi, Silugonggo, and Rokan rice varieties. Fertilizers used are 37.5

kg of N ha⁻¹, 36 kg of P₂O₅ ha⁻¹, and 60 kg of K₂O ha⁻¹, that were applied one week after planting. In addition, 37.5 kg of J. ISSAAS Vol. 20, No. 1:104-114 (2014) 106 N ha⁻¹ was applied five and nine weeks after planting. Tools used for this research were a tensiometer (DIK-3151) and thermohygrometer (TL 8010). The experiment was arranged in a split plot design with three replications.

The main plots consisted of control (normal irrigation) and drought stress (drought imposed at three weeks after transplant up to harvest). The sub-plot consisted of eight rice varieties namely: IR 64, Ciherang, IPB 3S, Way Apo Buru, Jatiluhur, Menthik Wangi, Silugonggo, and Rakan. Rice seeds were put in the oven for 72 hours in 43 0 C for uniformity of germination.

After which, seeds were soaked in water for 5 hours and kept in a sealed and cool bag for 2 days. Seeds were planted in seedling trays until 12 days. Eight varieties were planted in every main plot. Thirty plants were planted in each sub-plot in 2 rows with a 20 cm x 20 cm spacing between each plant. Spacing between each variety was 25 cm. For the control, irrigation was given close to harvest, with water level kept 2.5 cm above ground.

The water level was maintained during the vegetative growth, pre-anthesis, grain filling to harvest for the control treatment, by making a canal inlet and outlet. For drought treatment, irrigation was stopped at three weeks after transplant up to harvest. Laboratory analysis involving physiological character of proline was based from Bates et al. (1973).

The anthrone method was used to determine carbohydrate using a spectrophotometer (UV-vis Shimadzu UV 1800) (Hodge and Hofreiter, 1962). Proline accumulation was measured when the plant was at 11 and 13 weeks after transplanting (stage of reproductive), while total sugar accumulation and starch was measured during anthesis and at harvest.

Chlorophyll a, chlorophyll b, and the chlorophyll a/b ratio were measured when the plants were 11 weeks after transplant (reproductive stage). Yoshida et al. (1976) method was used to determine chlorophyll content using a spectrophotometer (UV-vis Shimadzu 1201) . Data were analyzed for significance by analysis of variance in the level of $\alpha = 0.05$ using LSD analysis.

RESULTS AND DISCUSSION Physiological Character of Drought Tolerant Varieties
Chlorophyll content Drought stress has a significant effect on chlorophyll a content of all varieties. Meanwhile, drought stress and variety show significant differences in decreasing chlorophyll a/b ratio (Table 1). Anjum et al. (2003) and Farooq et al. (2009) reported that drought in several plant species can cause a change in the chlorophyll a/b ratio and carotenoid content.

A high chlorophyll a/b ratio was found in Silugonggo, followed by IPB 3S varieties. The

value of the chlorophyll a/b ratio depends on the chlorophyll a and chlorophyll b content of each variety. Table 1. Average chlorophyll-a and a/b content of eight varieties under drought stress Treatment Chlorophyll-a Chlorophyll-a/b Drought stress

----- mg.g

-1 FW ----- Control 3.41 a 3.29 a Drought stress 2.75 b 2.16 b Variety IR 64 3.21 a 2.56 b Ciherang 2.78 a 2.49 b 107 Treatment Chlorophyll-a Chlorophyll-a/b IPB 3S 2.96 a 2.91 ab Way Apo Buru 2.93 a 2.62 b Jatiluhur 2.90 a 2.55 b Menthik Wangi 2.81 a 2.50 b Silugonggo 3.83 a 3.44 a Rokan 3.28 a 2.74 b Note: Values followed by the same letter in the same column are not significantly different according to LSD analysis at P<5% Chlorophyll b content is affected by the interaction of drought stress with variety (Table 2). Drought stress causes an increase in chlorophyll b.

Rokan, followed by Jatiluhur, and then IR 64 varieties contain higher chlorophyll b than other varieties. In more severe stress conditions, namely drought at three weeks after transplant until harvest led to a greater decrease in chlorophyll. This indicates that drought stress caused a strong loss of photosynthetic reaction centers. An increase in chlorophyll-b causes the chlorophyll-a/b ratio to decrease.

Chlorophyll b serves as an antenna that collects light and transfers to the reaction center. Composed of a reaction center chlorophyll a. Light energy is converted into chemical energy in the reaction center which can then be used in the reduction process of photosynthesis (Taiz and Zeiger, 1991). The results showed IR 64 and Silugonggo varieties containing higher chlorophyll b than other varieties.

The response of all varieties in the control was not significantly different. Result from the same research done in two lines of okra showed that drought can cause an increase in chlorophyll-b content (Jaleel et al., 2009). Other research results showed that chlorophyll-a and chlorophyll-b are susceptible to dehydration (Farooq et al., 2009). Table 2.

Effect drought stress and variety on chlorophyll b content Variety Control Drought stress ----- mg/g ----- IR 64 1.14 bc 1.47 a Ciherang 1.12 bc 1.16 bc IPB 3S 1.06 bc 0.99 c Way Apo Buru 1.14 bc 1.10 bc Jatiluhur 0.97 c 1.43 a Menthik Wangi 1.00 c 1.30 ab Silugonggo 1.00 c 1.33 ab Rokan 0.97 c 1.51 a Note: Values followed by the same letter in the same column are not significantly different according to LSD analysis at P<5% Drought stress causes a decrease in chlorophyll content in all varieties, but the response between varieties showed an increase in chlorophyll b.

The increase observed in the Rokan variety, followed by Jatiluhur, and then IR 64,

indicates that an increase in chlorophyll b content is an adaptation to drought stress in order to increase photosynthetic capacity. A decrease in chlorophyll a content has the ability to change the reaction energy of light radiation decreases such that photosynthesis is inhibited.

Chlorophyll a and b play a role in the process of photosynthesis. Chlorophyll b acts as a photosynthetic antenna that collects light. Meanwhile, the decrease in chlorophyll was associated with a reduction in the flux of nitrogen into the J. ISSAAS Vol. 20, No. 1:104-114 (2014) 108 tissue, as well as alteration in activity of enzyme systems, such as nitrate reductase (Taiz and Zeiger, 1991).

Chlorophyll is the main component of the chloroplast which is involved in photosynthesis. A decrease in chlorophyll-a during drought is a sign of oxidative stress caused by photooxidated pigment, which degrades chlorophyll (Verma et al. 2004; Farooq et al. 2009). Decrease in chlorophyll content and changes in chlorophyll level during drought have been reported in several plant species.

This also depends on drought and the level. Chlorophyll plays an important part in the light harvesting process of photosynthesis and in reducing over energy (Anjum et al. 2011). Research conducted by Mannivannan et al. (2007) on sunflower showed a decrease in chlorophyll content upon drought stress.

A reduced chlorophyll content is predicted to occur because the loss of the chloroplast membrane, excessive swelling in lamella vascular, and lipid peroxidation (Kaiser, 1987). Low photosynthesis pigment concentration can directly limit photosynthetic potential. Proline content Interaction between drought and variety has a significant effect on proline accumulation at the ages of 11 and 13 weeks after transplant (Table 3).

Proline accumulation is an early response when a plant is experiencing a water deficit, in which it decreases cell damage (Anjum et al. 2011). Proline accumulation does not only happen in a tolerant variety, but also on susceptible varieties. However, drought-tolerant varieties can accumulate proline for a longer period of time than susceptible varieties (Saruhan et al. 2006).

This was shown in Silugonggo, Rokan, and IR 64, that are categorized as susceptible varieties wherein at 11 weeks after transplant, it accumulated a higher proline concentration compared to other varieties. Even so, there was a decrease in proline concentration in Rokan, followed by Silugonggo, and the IR 64 variety during the 13 weeks after transplant (Table 4).

The characters directly related to drought tolerance are the increased accumulation of proline and total sugar during drought stress. Yue et al. (2006) reported the mechanism of drought tolerance through osmotic adjustment as the increased accumulation of solutes, such as proline and total sugar. The results showed that proline levels at 11 and 13 weeks after transplant accumulate proline over a longer period time for IPB 3S followed by Way Apo Buru, Ciherang, and Jatiluhur varieties (tolerant varieties) (Table 4). Table 3.

Effect drought and variety on proline content at 11 and 13 weeks after transplant (reproductive stage)

Variety	Proline content (11 WAT) $\mu\text{mol.g}^{-1}\text{FW}$	Proline content (13 WAT) $\mu\text{mol.g}^{-1}\text{FW}$
Control	21.92 a	21.04 b
Ciherang	32.16 cde	39.80 ab
IPB 3S	21.56 g	23.63 fg
Way Apo Buru	27.56 d-g	24.50 fg
Jatiluhur	24.13 fg	24.07 fg
Menthik Wangi	34.80 a-d	24.33 fg
Silugonggo	29.03 c-g	35.63 a-c
Rokan	25.93 efg	33.96 bcd

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

Proline plays an important role as an osmoprotectant, an energy sink to regulate redox potential, and as a radical hydroxyl scavenger (Sharma and Dietz, 2006). Furthermore, proline protects macromolecules from denaturation and also reduces acidity in the cell (Kishor et al. 2005) and acts as an antioxidant (Vendruscolo et al. 2007). According to Szabados and Savoure (2009), proline can act as a molecular signal to modulate mitochondrial function, affect cell proliferation or cell death, and initiate certain gene expression that is important role in protecting plants from stress. Table 4.

Variety	Proline content 11 WAT ($\mu\text{mol.g}^{-1}\text{FW}$)	Proline content 13 WAT ($\mu\text{mol.g}^{-1}\text{FW}$)	Relative decrease (%)
IR 64	41.90	23.20	44.63
Ciherang	39.80	25.38	36.23
IPB 3S	23.63	22.08	6.55
Way Apo Buru	24.50	17.35	29.18
Jatiluhur	24.07	15.11	37.22
Menthik Wangi	24.33	12.34	49.28
Silugonggo	35.63	14.24	60.03
Rokan	33.96	14.24	52.41

Besides increasing proline accumulation, drought stress can also increase total sugar and starch content at the pre anthesis stage.

Variety, drought stress, and interaction also have a significant effect. Tolerant varieties (Ciherang, Way Apo Buru, and Jatiluhur) have increased total sugar accumulation, reaching 2-4 times higher during drought than the control (Table 5). Table 5.

Effect drought stress and variety on total sugar at anthesis phase and harvest

Variety	Anthesis	Harvest
Control	mg gBK ⁻¹	mg gBK ⁻¹
Drought stress		
Control		
Drought stress		

64 34.54 gh 42.83 fgh 9.76 d 54.23 b Ciherang 59.66 f 183.48 c 11.30 d 57.84 b IPB 3S
43.75 fgh 139.90 d 14.80 d 85.14 a Way Apo Buru 50.48 fg 131.59 d 12.70 d 46.99 bc
Jatiluhur 128.52 d 138.11 d 17.86 d 17.14 d Menthik Wangi 135.83 d 230.53 a 8.71 d
73.75 a Silugonggo 26.07 h 90.18 e 9.64 d 54.25 b Rokan 58.23 f 289.38 a 7.58 d 34.20 c
Note: Values followed by the same letter in the same column are not significantly
different according to LSD analysis at $P < 5\%$ Tolerant varieties accumulate total sugar in
the stem and then translocate it to other organs for metabolism and growth.

Meanwhile, susceptible varieties accumulate a high amount of total sugar in J. ISSAAS
Vol. 20, No. 1:104-114 (2014) 110 the stem during pre anthesis stage, however when
drought occurs, there is some sugar translocation blockage to other parts of the plant,
especially the seeds. This can be seen in the tolerant variety, Jatiluhur, which
accumulates total sugar at the same concentration as the control and drought
treatments.

During the grain filling period of the control and drought treatment, total sugar re-
translocation in Jatiluhur variety did not experience blockage to seeds. While it is a
susceptible variety (Menthik Wangi), it experiences blockage of sugar re-translocation
under drought conditions and has a high concentration of sugar at harvest. Sugar
accumulation in rice varieties is severely affected by drought. Total sugar accumulation
is one mechanism for a tolerant plant in facing drought.

Total sugar accumulation also plays a role in substrate hydrolysis in a biosynthetic
process, producing energy and acts as a sensor and signal. It also functions as a typical
osmoprotectant to maintain cell stability and maintain turgor pressure (Kishor et al.
2005). During drought stress, total sugar accumulation increases especially in the stem
(Hu et al. 2006).

Total sugar accumulation in the organ of a tolerant variety is more effective because of
the high membrane stability and low water loss than susceptible varieties (Valentovic et
al. 2006). Varieties that are relatively tolerant to drought, especially Jatiluhur, clearly
demonstrate that total sugar accumulation is significantly different in both the control
and drought treatment during anthesis phase and harvesting stage. This due to most
sugar and starch in the stem being actively relocated to seeds.

Mc Dowell and Sevanto (2010) reported that drought can block carbohydrate transport,
usage, and mobilization. If severe drought happens, then the plant will die if it reaches
its critical point. Therefore, a starch content variable that was observed during the stage
of anthesis and harvest in drought treatment can give information on how varieties
experience blocked carbohydrate transport caused by water inefficiency in susceptible

varieties.

Drought stress causes increased accumulation of solutes (proline and total sugar). Tolerant varieties (Jatiluhur, Ciherang, and Way Apo Buru) have a mechanism of tolerance through accumulation of proline over a longer time during drought stress and increasing total sugar reaching 2-3 times higher than the control.

Increase in proline and total sugar accumulation when drought stress occurs is one of the indicators of plant tolerance to drought through osmotic adjustment. Yue et al. (2006) reported that the mechanism of tolerance through osmotic adjustment is an adaptive process which accumulates non-toxic "compatible solutes" in the cell and lowers the osmotic potential during water deficit.

Grain yield Significant differences were observed in grain yield per hill in rice varieties under control and drought conditions. Drought stress reduced grain yield irrespective of rice varieties. The highest reduction in grain yield under drought stress was recorded in Rokan variety (95.22%), followed by Menthik Wangi (82.99%), and Silugonggo (73.94%), while less reduction was noted in Jatiluhur (40.72%) (Table 6).

Rice is a crop that is very sensitive to water shortage in the reproductive phase, with water shortage leading to a higher reduction in grain yield. Decrease in grain yield is due to reduced panicle formation and high sterility. Liu et al. (2008) reported that water stress can abort pollination for up to 67 percent of total grain per panicle.

When pollination occurs, the pollen reaches the ovule longer mikrofil in 1-8 days. Pollen cannot be on the surface of the flower because flowers fail to open due to drought. Grain yield is a multigenic factor, with susceptible varieties producing high grain yield under normal conditions, but it also shows a high percent reduction over control during drought.

The yield stability in tolerant varieties was due to specific adaptive feature that make them able to produce stable grain yield even under stress (Van Heerden and Laurie, 2008; Liu et al. 2008). 111 Drought is a complex mechanism involving various plant adaptive features which produce a stable yield. Tolerant varieties (Jatiluhur) showed stable yield due to a better scavenging system, high accumulation of osmolytes during stress, and fast recovery after relief from stress. Table 6. Effect drought stress and variety on grain yield per hill

Control	Drought stress	Relative decrease (%)
IR 64	4.63 l	15.23 cd
69.60 Ciherang	6.35 gh	15.50 cd
59.03 IPB 3S	5.19 h	23.55 a
77.96 Way Apo Buru	5.06 h	13.95 de
63.73 Jatiluhur	13.48 de	22.74 a
40.72 Menthik Wangi	3.51 jk	20.64 ab
82.99 Silugonggo	3.32 jk	12.74 fg
73.94 Rokan	2.01 k	18.12 b
95.22		

Note: Values

followed by the same letter in the same column are not significantly different according to LSD analysis at $P < 5\%$ CONCLUSION Physiological characters of rice varieties differed in their response to drought stress.

However, drought reduced chlorophyll a, chlorophyll a/b ratio, and grain yield, but increased proline, total sugar, and chlorophyll b content. Tolerant rice varieties experiencing drought had better osmoregulation, in terms of proline and total sugar content. Meanwhile, tolerant varieties (Ciherang, Way Apo Buru, and Jatiluhur) tended to accumulate proline over a longer period time and had a higher total sugar accumulation.

Jatiluhur varieties experiencing drought showed higher yield and stability than other varieties. Lesser reduction in grain yield during drought, accumulation of proline over a longer period of time, and an increase in total sugar accumulation at anthesis phase during drought were physiological characters that played an important role on tolerance against drought in paddy systems.

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