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Growth, Physiological and Biochemical Responses of Malaysia Rice Cultivars to Water Stress

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ABSTRACT

The response of water deficit on rice plants varies substantially according to cultivars. Drought tolerant cultivars possess better morphological, physiological and biochemical adaptation to reduce water availability. An experiment involving water stress on rice varieties was carried out under rain shelter to examine the morphological changes (leaf rolling, root depth), stomatal responses and biochemical processes (proline and peroxidase accumulation) of five different local rice varieties. These varieties were selected based on their drought tolerant potential from an earlier varietal screening trial. The varieties were taken from both traditional (Muda, Jawi Lanjut and newly breed commercial varieties, MR 84, MR219 and MR 220) obtained from Genebank, MARDI Research Station, Seberang Prai, Kepala Batas, Pulau Pinang. These varieties were exposed to two different water regimes; water stress by withholding water and well watered condition (control). The experiment was carried out in a Complete Randomized Design (CRD) with 4 replicates. Water stress plants exhibited lower growth rate with obvious variation among rice varieties on the sensitivity to water stress. Meanwhile, the overall sensitivity of the varieties to water stress was ranked in the order; MR220>Muda>MR84>MR219>Jawi Lanjut. Water deficit decreased stomatal conductance, relative water content and root depth while peroxidase activities and proline accumulation were increased in rice grown under water stress treatment.

Keywords: Leaf rolling, relative water content, root growth, stomatal conductance, water stress and rice cultivars

INTRODUCTION

Water scarcity is a severe environmental limitation to plant productivity. Drought-induced loss in crop yield may exceeds loses from all other causes, since both the severity and duration of the stress are critical (Farooq *et al*., 2008). A slow pace in revealing drought tolerance mechanisms has hampered both traditional breeding efforts and the use of modern genetics approaches in the improvement of drought tolerance of crop plants. Fukai and Cooper (1995) listed the traits of rice under four categories; drought escape, drought avoidance, drought tolerance and drought recovery. The physiological avoidance can be achieved by cultivars which were able to reduce water loss, such as by leaf rolling and decreased stomatal conductance. In retrospect, these features may

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also affect the net photosynthesis that will in turn cause a detrimental effect to yield. The optimization of these processes will lead to a significant achievement in sustaining growth under limited water conditions.

Plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to scavenge the generation of reactive oxygen species (ROS), as a result of environmental stresses. Peroxidase is thought to increase during abiotic stress as it is an important antioxidant enzyme in scavenging or utilizing hydrogen peroxide (Okuda *et al*., 1991).

Rice is the most important cereal crop in the world and it is the primary source of food and calories for about half of mankind (Khush, 2005). Rice provides as much as 80% of the dietary calories in some Asian countries. The predominantly rice-growing areas in Asia (130 million hectares, more than 85% of the total world rice production) are often threatened by severe abiotic stresses, and the most common being the drought. Climate change has rendered several areas unsuitable for rice cultivation, especially the 'rice bowl' area in Kedah and this has caused losses in millions of Ringgit. The Malaysian Agricultural Research and Development Institute (MARDI is trying to develop new varieties of rice which are resistant to flood, drought, and high temperatures. The development of rice tolerance to limited water conditions particularly for granary areas, has not been successful in general, although there are cultivars reputedly tolerant to water deficit. The present study was undertaken to determine the physiological and biochemical changes in rice cultivars, when exposed to limited water conditions.

MATERIALS AND METHODS

Origin of Plant Material

Seeds of five rice genotypes (*Oryza sativa* cv. MR84, MR219, MR220, MUDA and Jawi Lanjut) were obtained from Genebank, MARDI Research Station, Seberang Prai, Kepala Batas, Pulau Pinang.

Growth Condition and Plant Culture

Seeds were sown on a plastic tray for raising seedlings and placed in the dark until seedlings emerged. At the 1.5-leaf stage, seedlings of uniform size were transplanted into plastic pots with clay loam soil from BERTAM. The experiment was conducted at a rain shelter house at Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia.

Water Stress Treatment

Water stress was imposed by withholding irrigation until leaf rolling symptoms were observed as a stress indicator while equal amount of water (1 liter per pot per day) was given in well watered treatment. The experiment was carried out in a Complete Randomized Design (CRD) with 4 replicates.

Measurements

Leaf rolling

Leaf rolling was assessed visually in each pot, in all the treatments. Several plants were assessed and the pots were given a mean leaf rolling score, ranging from 1 to 5, with 1 being flat and 5 a tightly rolled leaf (O'Toole and Moya, 1978). These ratings were made during midday, i.e. about twice per week during the period of water deficit of all the treatments.

Stomatal conductance

Stomatal conductance of leaves was determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK). The measurements were taken on the abaxial surface of the leaf once a week between 11.00 h and 14.00 h. The readings were accomplished during one-hour to avoid the diurnal pattern of variation of the leaves. The terminal part of the main leaf lobe was placed into the cup, i.e. on the head unit which was positioned normal to the sun. The measurements were conducted during cloudless periods on the exposed leaves between 10.00 h and 14.00 h.

Relative water content

The leaves were cut, and the relative water content (*RWC*) was determined according to the procedures by Ghannoum *et al*. (2002). The relative water content of leaf was determined as follows:

$$RWC = (fresh\ weight - dried\ weight) / (fully\ turgid\ weight - dried\ weight) \times 100$$

To determine the fully turgid weight, the leaves were kept in distilled water in the darkness at 4˚C to minimize respiration losses until they reached a constant weight (full turgor, typically after 12 h). The leaf dry weight was obtained after 48 h at 70˚C in an oven. Five to six replicates were obtained per treatment.

Proline measurement: Fresh flag leaf tissue (0·5 g) was ground in liquid nitrogen and then extracted in 20 mL of hot water for 30 min with a moderate shaking. The homogenate was centrifuged at 5000 g for 10 min. The proline concentration was quantified using the ninhydrin acid reagent method described by Bates et al. (1973) using the L-proline as a standard.

Protein expression: For protein expression, leaves were collected using liquid nitrogen and protein was extracted in a buffer 62.5mM Tris-HCL pH 6.8; 10% glycerol 2% SDS and 1.4M 2-mecapthanol (2ul/mg of tissue) and incubated the mixture at 70˚C for 10 minutes and centrifuged at 15000rpm for 10minutes at 40˚C. The supernatants (protein) were taken for the treatment with the cracking buffer before electrophoresis was conducted in the SDS-PAGE.

Peroxidase activity**:** Peroxidase activity was assayed as an increase in optical density due to the oxidation of guaiacol to tetra-guaiacol. The 3 ml reaction mixture contained 16 mM guaiacol, 2 mM H_2O_2, 50 mM phosphate buffer (pH 6.1) and 0.1 ml enzyme extract. Enzyme extract was prepared as in case of SOD. The reaction mixture consisted of 50 mM, pH 6.1, phosphate buffer, 16 mM guaiacol, 2 mM H_2O_2, 0.1 ml enzyme, 0.4 ml water, to make final volume of 3.0 ml. The absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and the enzyme activity was calculated as per extinction co-efficient of its oxidation product, tetra-guaiacol 2 ¼ 26.6 mM71 cm71. The enzyme activity was expressed as mmol tetra-guaiacol formed per min per g fr. wt. or per mg protein.

Root shoot ratio: The shoot samples were harvested by cutting about 2 cm above the soil surface at maturity stage and were separated into grain and straw. The shoots were washed and oven dried for 70 to 72 hours and weighed.

Statistical analysis

All data were analyzed using SAs software (SAS Institute Inc. 1997). Each treatment was analyzed in four replications. When ANOVA showed significant treatment effects, the Duncans multiple range test was applied to compare the means at P‹ 0.05.

RESULTS

Growth Parameters

Root depth

The root depth of rice was reduced when the soil subjected to drought condition (water stress) for all the rice varieties, as shown in *Fig. 1*. Under well watered treatment, root depth was higher than under water stress condition for all varieties. Under the well watered treatment, the root depth of MR220 and MR84 were comparable with a minor difference and were higher than the other varieties. The Jawi Lanjut variety indicated lower root depth under well watered treatment than the other varieties. However, under the water stress treatment, the MR220 indicated lower root depth than the other varieties. From the observation, the Jawi Lanjut variety indicated a higher root depth than the other varieties under the water stress treatment.

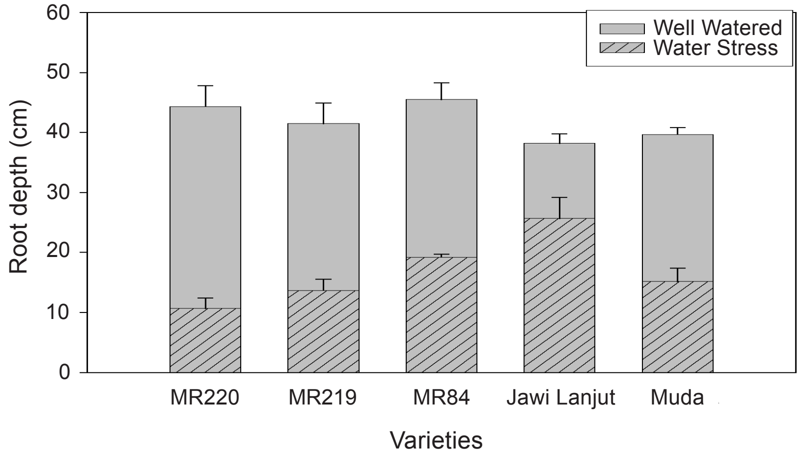

Fig. 1: Root depth of five cultivars exposed to well-watered and drought conditions. Vertical bars indicate ± standard errors, n = 4

Leaf rolling

In the present study, leaf rolling was observed first in MR220, which also showed the higher leaf rolling score at subsequent measurements, indicating that this cultivar was the most sensitive to water deficit (*Fig. 2*). Leaf rolling occurred later in Jawi Lanjut and it was lower than the other cultivars, showing a maximum score of 4. Similarly, the extent of leaf rolling was found to be gradual in MR84 and MUDA. Five days after the stress treatment, the leaves of all cultivars were partially rolled at midday, starting with MR220, and this was followed by MR219, MUDA, MR84 and JAWI LANJUT which showed a higher mean score in leaf rolling.

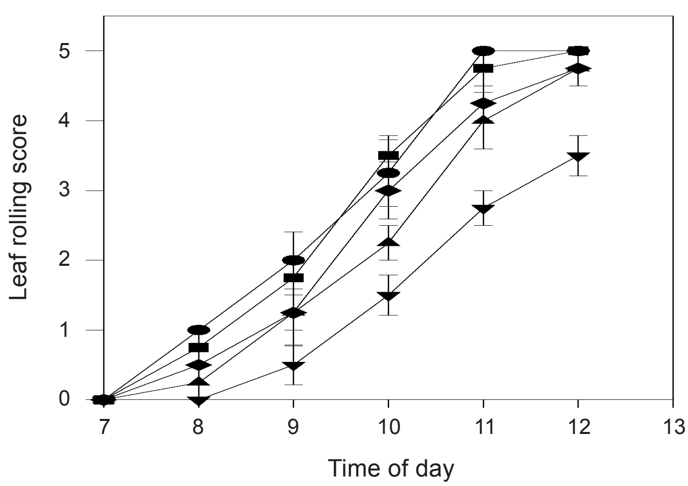

Fig. 2: Diurnal changes in leaf-rolling score of five cultivars (● MR220, ■ MR219, ▲ MR84, ▼ Jawi Lanjut and ◆ MUDA). Vertical bars indicate ± standard errors, n = 4

Physiological Parameters

Relative water contents (RWC)

The relative water contents of all the varieties were similar under the well-watered condition on all the measurement occasions. However, it declined progressively in stressed plots with the development of severe water deficit (*Fig. 3*). The decline in the RWC was more rapid in MR220 than in the other varieties. The Jawi Lanjut had relatively higher water content than the other varieties, even after 10 days of exposure to soil drying. However, all the varieties had similar and lowest values of the RWC at the end of the soil drying cycle.

Stomatal conductance

Stomatal conductance decreased in all the varieties of rice as the intensity of water deficit increased with the time of soil drying (*Fig. 4*). The decline in stomatal conductance was faster after 6 days of stress development than under well watered condition. Stomatal conductance of MR220 and MUDA declined more rapidly than in other varieties; however, after 10 days of soil drying, all varieties (except for Jawi Lanjut) showed a considerable decrease in stomata conductance. Jawi Lanjut exhibited a higher stomatal conductance under stress than the other varieties under stress although it also had consistently lower values after 6 days of stress treatment.

Root-shoot ratio

The root shoot ratio was reduced when the soil was subjected to drought condition (water stress) for all the rice varieties, as shown in *Fig. 5*. Under the well watered treatment, the root shoot ratio was higher than under water stress treatment for all the varieties. Under the well watered treatment, MR220 and MR219 did not show much difference in the root shoot ratio, and were higher than the other varieties. However, under water stress treatment, MR220 showed a lower root shoot ratio than the other varieties. Based on the observation, the Jawi Lanjut varieties showed high root shoot ratio than the other varieties under the water stress treatment.

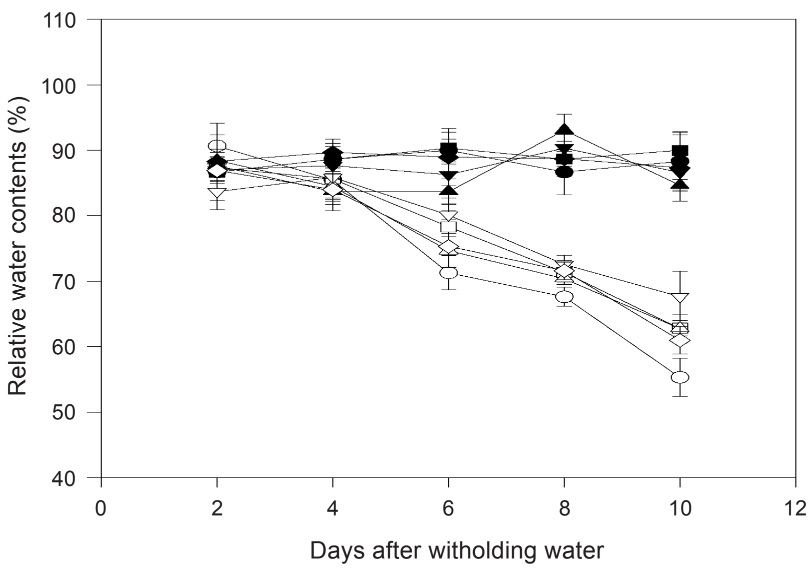

Fig. 3: Relative leaf water content of five cultivars exposed to well-watered (closed symbol) and drought (open symbol) conditions (○ MR220, MR219, △MR84, ▽ Jawi Lanjut and ◊ MUDA). Vertical bars indicate ± standard errors, n=4

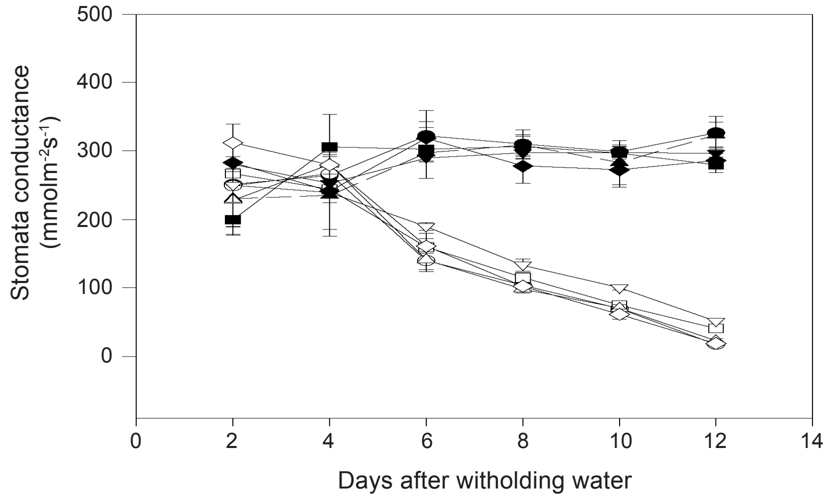

Fig. 4: Stomatal conductance of five cultivars exposed to well-watered (closed symbol) and drought (open symbol) conditions (○ MR220, MR219, △MR84, ▽ Jawi Lanjut and ◊ MUDA). Vertical bars indicate ± standard errors, n=4

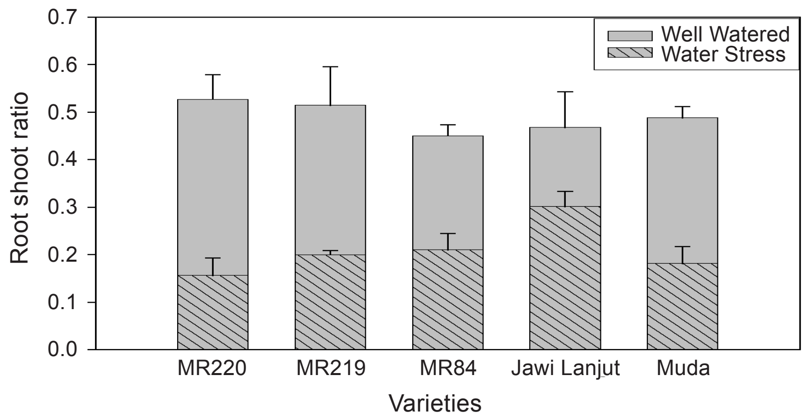

Fig. 5: Root-shoot ratio of five cultivars exposed to well-watered and drought conditions. Vertical bars indicate ± standard errors, n=4

Biochemical Parameters

Proline accumulation

The accumulation of proline was affected by water regimes and rice varieties, as depicted in *Fig. 6*. The accumulation of proline was higher under water stress treatment than the well watered treatment for all the rice varieties. The level of increase in the proline concentration in response to water stress varied between the rice varieties. In particular, Jawi Lanjut was observed to have a high proline accumulation under water stress treatment than the other varieties, while MR220 had a lower proline accumulation than the other rice varieties.

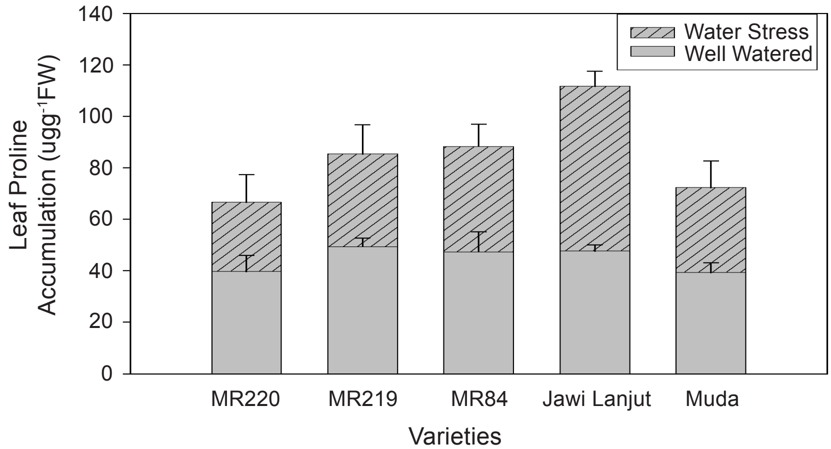

Fig.6: Leaf proline accumulation ratio of five cultivars exposed to well-watered and drought. Vertical bars indicate ± standard errors, n=4

Peroxidase activity

The peroxidase activity was also affected by the water regimes and rice varieties, as shown in *Fig. 7*. The peroxidase activity was high under the water stress treatment than under the well watered treatment for all the rice varieties except for MUDA. Jawi Lanjut was observed to have a high peroxidase activity under the water stress but lower peroxidase activity under well watered treatment than the other varieties. However, MR219 showed a high peroxidase activity for both the treatments (water stress and well watered), and MR220 showed a lower peroxidase activity than the other varieties under water stress treatment.

Protein expression

The protein expression of the rice varieties, under different water regime, is shown in *Fig* 8. The MR219 and Jawi Lanjut varieties showed more protein when exposed to the soil drying

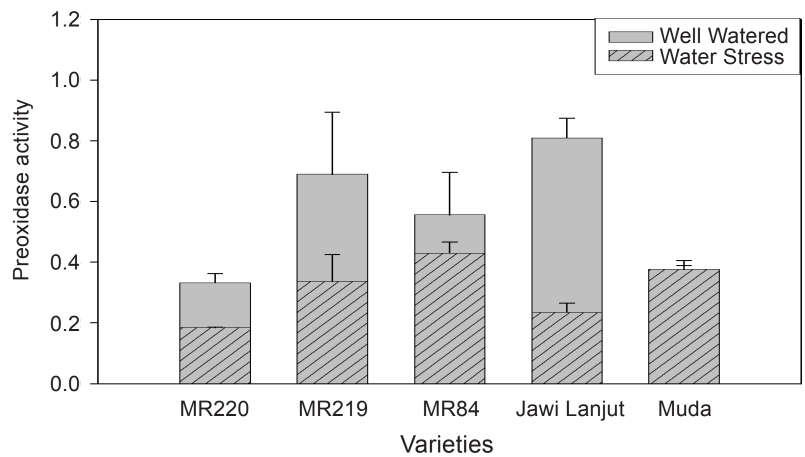

Fig. 7: Peroxidase contents of five cultivars exposed to well-watered and drought conditions. Vertical bars indicate ± standard errors, n=4

(water stress treatment). However, it is different between the MR220, MR84 and MUDA varieties because they did not show much difference in terms of protein expression. However, under the well watered treatment, all the varieties were shown to be comparable and did not differ much in protein expression.

DISCUSSION

As observed in the first experiment, a rice variety was varied by its sensitivity to soil drying. In general, leaf rolling score indicated that the sensitivity of the varieties follows the order MR220>Muda>MR219>MR84>Jawi Lanjut (*Fig. 2*). Therefore, leaf rolling is commonly used as an important criterion during screening of genotypes for drought tolerance (Cutler *et al*., 1980; Sloane *et al*., 1990; Rosario *et al*., 1992; Lilley and Fukai, 1994). Accordingly, several varieties such as MR220, Muda and MR84 were found to be more sensitive than MR219 and MR84, while other variety like Jawi Lanjut was relatively tolerant to the water stress treatment imposed. Leaf rolling and leaflet closure during periods of soil moisture depletion have also been observed in rice (Lilley and Fukai, 1994). These leaf movements, such as the adjustment of leaf angle or modification of leaf orientation to reduce the interception of solar radiation and, thus, decrease leaf temperature and water loss by transpiration, are regarded as one of the drought avoidance mechanisms evolved in plants (Pugnaire *et al*., 1999; Carr, 2001). On the other hand, lower rate of stress development (leaf rolling) as a result of maintenance of turgor has been used as an important criterion during screening genotypes, such as mungbean (*Vigna radiate*) accessions (Rosario *et al*., 1992), soybean plant introductions (Sloane *et al*., 1990; Carter and Rufty, 1992) and rice cultivars (Cutler *et al*., 1980; Price *et al*., 1992; Lilley and Fukai, 1994) for tolerance towards drought. It has been suggested that greater leaf rolling may be an important attribute linked to drought tolerance and may have a positive impact on crop yield under water stress conditions (Joshi, 1999; Lima *et al*., 2002).

Relative water content (RWC) substantially declined with prolonged period of soil drying, but the rate of decline was faster at the later stages of the stress (*Fig. 3*). Relative water content of all the varieties were similar under well-watered condition on all the measurement occasions. However, it declined progressively in stressed plots with the development of severe water deficit. The decline in the RWC was more rapid in MR220 than in the other varieties. In particular, Jawi Lanjut had relatively higher relative water content than the other varieties, even after 10 days of exposure to soil drying. All the varieties had similar and lowest values of RWC at the end of the soil drying cycle. On the other hand, the leaf RWC of MR220 was found to be similar to that of the control plants, but it decreased sharply in water stressed plants of Jawi Lanjut, two days after withholding water. The differences among the rice varieties in terms of the rate of decline in the leaf RWC could also be associated with the variations in other physiological responses to water stress, such as reduction in stomatal conductance (g_s).

Stomatal conductance (g_s) considerably decreased as the intensity of water stress increased with the time of soil drying (Figure 4). The stomatal conductance decreased in all the rice varieties, as the intensity of water deficit stress increased with the time of soil drying. The decline in the stomatal conductance was faster after 6 days of stress development. Stomatal conductance of MR220 and MUDA declined more rapidly than the other varieties. However, after 10 days of soil drying, all the varieties (except for Jawi Lanjut) showed a considerable decrease in the stomata conductance. Instead, Jawi Lanjut exhibited a higher stomatal conductance under stress than the other varieties, under stress although it also had consistently lower values, after 6 days of the stress treatment. Such a reduction in g_s appeared to be the primary response and a common phenomenon during water stress, which is believed to be one of the most important desiccation-avoidance mechanisms evolved in plants (Pugnaire *et al*., 1999; Carr, 2001).

The accumulation of proline was high under water stress treatment than the well-watered treatment for all the rice varieties (*Fig. 6*). Similarly, that the leaves of the unstressed plants have been reported to be free from proline contents, which are very small and its accumulation increases 100-folds when the plants are subjected to drought stress (Widyasari and Sugiyarta, 1997). Thus, proline is known to play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity.

Water regimes treatment and interaction between the varieties have been shown to have effects on the peroxidase activity (*Fig. 7*). During soil drying, the different mechanisms of protection appear to act at the different stages of water loss. The survival strategy during early dehydration is to avoid protein unfolding and restrict membrane disturbance by preferential hydration. Upon further removal of water from the hydration shell, sugar molecules have to replace water at hydrogen bonding sites to preserve the native protein structure and spacing between phospholipids (Folkert *et al*., 2001). These results suggested that plants were capable of surviving surface soil drying. This capability could be related to increases in antioxidant activities, particularly the SOD and catalase (CAT). However, full drying suppressed antioxidant activities and induced lipid peroxidation (Fu and Huang, 2001). The protein expression of rice varieties exposed to different water regime are shown in *Fig. 8*. The MR219 and Jawi Lanjut varieties showed that more protein was expressed when they were exposed to soil drying (water stress treatment). Based on the observation, the well-watered treatment for all the varieties showed a low level of protein express, as compared to water stress treatment, except for in Jawi Lanjut and MR219. The findings therefore positively agree with Gulent and Eris (2004), who studied on the effect of heat stress on the total protein content in strawberry plants.

CONCLUSIONS

In general, the differences between rice varieties were observed in their sensitivity to water stress. Based on the mean values of visual stress score, leaf rolling during the drought period, overall sensitivity of the varieties was ranked in the order of MR220>Muda>MR84>MR219>Jawi Lanjut. Water deficit decreased stomata conductance, relative leaf water content and root depth. However, peroxidase activity and proline accumulation were increased in rice grown under stress treatment.

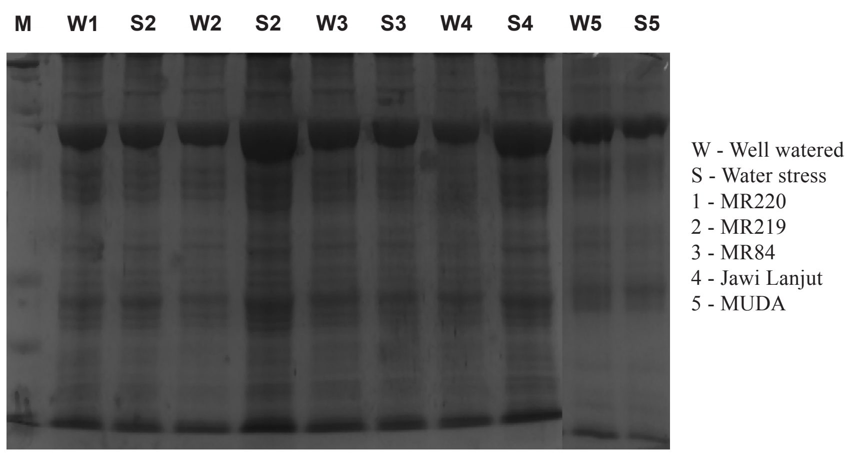

Fig. 8: Protein expression in rice leaves exposed to well-watered and drought conditions

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