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WATER STRESS AFFECTS GROWTH, BIOMASS PARTITIONING, GRAIN FILLING AND PRODUCTIVITY OF MR219, A HIGH YIELDING RICE VARIETY OF MALAYSIA

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ABSTRACT

Oryza sativa, rice is the major staple food for half the human population especially Asians. However, the crop which belongs to the grass family is highly susceptible to drought which causes huge yield losses in the areas of cultivation. This condition affects physiological and biochemical processes in rice plant which in turn impacts its productivity. Grain filling which is the most important determinant of yield is affected if water stress were to occur during the booting stage. The effect of different water regimes (well watered and water stressed) on the high yielding rice variety, MR219 was investigated both under rain shelter and field condition. The objectives of this study were to evaluate the growth, physiological processes, yield and biomass partitioning of rice plants subjected to water stress. Biomass partitioning, harvest index and photosynthesis were reduced by 50% under water stress due inefficient translocation of assimilates. Results from the field study pointed to yield losses that ranged from 60 to 90% under drought condition as compared to control

Keywords: Drought, grain filling, rice, water stress, yield

1. INTRODUCTION

Nigeria accounts for 19% of world output and 34% of Africa's output (Ezike, Nwibo, & Odoh Tropical countries are often thought to experience a wet and humid weather. As such, Malaysia which is located on the equatorial belt is assumed to experience abundant rainfall, where water is plentiful and available throughout the year. However, this is a misconception, as in reality, the country experiences periods of excess water as well as periods of drought, both being limitation factors for rice production. Thus, rice cultivation is exposed to different water conditions depending on months and locations due to uncertainties in weather that influences the plant's microclimate.

MR219 is a local indica rice variety that is grown in 53% of major granary areas in Malaysia (Ministry of Agriculture and Agro-Based Industry, Malaysia, 2014). MR219 may be high yielding, but it is also susceptible to drought stress if it were to occur at the reproductive stage

(Talei et al., 2013). It requires plenty of water for cultivation, depending on both rainfall and irrigation system with water requirement reaching approximately an average of 1240 mm or 86.4 m^3 ha^{-1} day^{-1} (8.64 mm day^{-1}) to produce optimum yield (MARDI, 2002).

Although drought stress has a marked inhibitory effect on plants at every growth and development phases, its imposition at early growth stages causes considerable reduction in both cell expansion and its elongation (Ashraf et al., 2013). Drought stress mainly hampers the rate of carbon assimilation which results in decreased growth and yield of most crops (Mafakheri et al., 2010). The optimum proportion of dry mass should be partitioned if the final yield is to be maximized (Mondal et al., 2014). Drought stress during grain filling period decreased the net photosynthesis rate and grain filling of rice directly (Thameur et al., 2012). It is generally believed that grain filling in rice is closely related with sink strength (Yang et al., 2003). The sink strength can be described as the product of sink size and sink activity (Fu et al., 2011). Sink size is a physical restraint that includes cell number and cell size, and sink activity is the physiological constraint upon a sink organs assimilate import (Ho, 1988). A proper water management system could increase growth rate during grain production and/or enhance the remobilization of assimilates from vegetative tissues to grains during the grain-filling period which usually leads to a higher harvest index (HI) (Fletcher and Jamieson, 2009).In many situations, HI (0.17-0.56) is closely associated with water use efficiency (WUE) and grain yield in rice (Yang and Zhang, 2010).

Little information were available on the pattern of assimilates partitioning that contributes to grain filling and yield of MR219 rice variety in relation to water availability. Thus, the present study was designed to evaluate growth, physiological processes, biomass partitioning and yield of MR219 rice plants subjected to different water regimes.

2. METHOD

2.1. Experimental site and soil condition

Two experiments were conducted to evaluate MR219 rice plant responses to water deficit. The experiments were conducted under both rain shelter and in the field. For the latter, only yield was determined due to practical issue. The former was conducted between the months of August to November 2012 under the rain shelter facilities at Rice Research Centre, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia (3.3°N latitude and 101.5°E longitude with an elevation of 21 m from sea level). During the experimental period, monthly average maximum and minimum temperatures and relative humidity ranged from 25 to 29°C and 70 to 85%, respectively. The “Tok Yong” soil series having a clay loam texture (20.23% sand, 46.72% silt and 33% clay) with pH 5.3 and 2.2% organic carbon was used as cultivation medium. Soil nutrient status was 0.21% total N, 36 mg kg^{-1} available P and 67 mg kg^{-1}available K. Field trial was carried out during off-season 2013 (February-May) under field conditions in Kemubu Agriculture Development Authority (KADA), Kelantan, Malaysia (5° 58’ N 102° 18’ E, 34 m elevation). Soil properties and type followed that of rain shelter experiment.

2.2. Planting materials and plant establishment

For rain shelter experiment, the seeds of MR219 were soaked overnight in water containing seed priming product from ZAPPA-PLUS (PeladangTech, Bangi, Selangor, Malaysia) and spread on wet tissue in a flat tray overnight. The seeds were sowed approximately after two days of soaking by direct seedling technique with 4 seeds per pot with size 390 width × 390 diameter × 350 mm height containing approximately 17 kg of soil.

For the field trial, ten days old seedlings were transplanted at 20 x 15 cm spacing. Eight plots for each experiment with 16 m^2 (4 x 4 m) areas were prepared and separated by 0.5 m barrier. Both experiments were fertilized with NPK compound fertilizer at 360 kg/ha, 175 kg/ha and 175 kg/ha at 15, 50 and 70 days after transplanting (DAT), respectively and urea was supplemented (top-dressed) at 100 kg/ha at 35 DAT (MADA 2015).

2.3. Experimental design and treatments

The rain shelter experiment consisted of 2 treatments [well watered (water level at 5 cm according to regular farmer practice) and water stress (cyclic water stress at 10 days intervals)]. The imposition of cyclic water stress was followed according to methods described by Puteh et al. (2013). The treatments were arranged in a complete randomized design with four replications. The well watered plants were left in flooded pot throughout the study. The water stress treatment was started on the 30th day after sowing (DAS) for eight cycles. A portable soil moisture meter (HH2 Moisture Meter, Delta-T, UK) was inserted into each pot at a depth of 5 cm to monitor soil moisture during growing period.

The field trial comprised of four water conditions (T1: Plants supplied with water for four weeks, T2; Plants supplied with water for two weeks, T3: Plants supplied with water for one week and T4: well-irrigated (Plants supplied with water for nine weeks), were arranged in Randomized Complete Block Design (RCBD) with four replications. Water was maintained at 5 cm by irrigation for four weeks (T1), two weeks (T2) and one week after transplanting throughout the rice cultivation periods. In control plot, water level was maintained at 5 cm by continuously pumping water from underground water until maturity due to water scarcity. In between treatments, 0.5 m boundary was prepared to avoid assortment.

2.4. Plant height and tiller number

Plant height was measured according to methods described by Yoshida (1981) where measurement was made from the plant base to the tip of the highest leaf blade. Tiller number per hill was counted by fully expanded tiller. Four hills per pot were taken randomly and measured at 45, 65 and 95 DAS.

2.5. Total leaf area meter per plant

All leaves collected at 45, 65 and 95 DAS were directly measured for their areas by a bench top leaf area meter (Li-Cor 3100C, Li-cor Inc., Lincoln, NE, USA). Due to the nature of rice leaves which would roll inward (rapid water loss from the bulliform cells) upon removal from the stems, they were first soaked in the water to get it rehydrated before being flattened and measured (Yoshida, 1981).

2.6. Photosynthesis rate and stomatal conductance

Photosynthesis rate was measured on fully expanded young leaves (third leaf from the top) at 0900- 1100 using an open-top portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, Nebraska – USA). The measurements were taken on the abaxial surface at a CO_2 reference rate of 400µmol $m^{-2}s^{-1}$ at 45, 65 and 95 DAS. The photosynthetic photonflux density (PPFD) was 900 mmol$m^{-2}s^{-1}$. Stomatal conductance was derived from the same photosynthesis measurements earlier.

2.7. Biomass partitioning

Three plants were harvested from each treatment at 45, 65 and 95 DAS. They were partitioned into roots, culms and leaves to determine dry weights of each part (QC35EDE-S Sartorius, Germany) after drying in an oven at 72°C for three days until the weight became constant. The total biomass was calculated based on total dry weights of leaf, culm, root and total yield. The root to shoot ratio was calculated using the following formula (Hunt, 1978).

Root: Shoot Ratio = Total Root Dry Weight / Total Shoot Dry Weight

2.8. Yield components

In the rainshelter trial, all the plants were harvested at 115 DAS. The grain weight and yield components were determined after drying (72 hours, 60°C). The grain yield was based on the weight of filled grains per hill and expressed in grain per hill (g $hill^{-1}$). The grain yield was determined using digital balance (QC 35EDE-S Sartorius, Germany). Panicle per hill, grain number per panicle and percentage of filled grain per panicle were counted and calculated manually. The 1000 grains weight (g) was also obtained using the same balance. Ten panicles bearing tillers from each treatment were sampled. Prior to weighing the grains, fully filled grains were separated from the unfilled grains manually. The percentage of filled grains per panicle was derived from the ratio of the number of fully ripened grains (filled grains) to the total number of grains per panicle per average hill. Thus, the total percentage of filled grains was calculated using the following formula (1):

$$\text{Percentage of filled grain per panicle} = \frac{\text{Number of filled grains}}{(\text{Filled} + \text{Unfilled grains})} \times 100\,\% - -(1)$$

The partitioning of dry matter between grain yield and vegetative part is indicated by harvest index. Harvest index was calculated as the ratios of grain dry weight to the total weight (2);

$$\text{Harvest Index} = \frac{\text{Grain dry weight (economic yield)}}{\text{Total dry weight (biological yield)}} - -(2)$$

Both percentage of filled grain and harvest index were determined according to Yoshida (1981). As for field trial, the plants were harvested at 80% maturity from one meter squared plot and the yield was converted to ton per hectare (Yoshida, 1981).

2.9. Statistical analyses

Rain shelter data were analysed using SAS software (Windows version 9.1, SAS Institute, Cary, NC, USA). The differences between treatments were determined using T-test at P≤0.05 was used to test significant differences among treatments for rain shelter experiment. As for field trial, all data were analyzed using the ANOVA procedure in the SAS software. The differences among treatments were determined using the least significant difference (LSD) test at P≤0.05.

3. RESULTS

3.1. Rain shelter experiment

3.1.1. Soil water content

Results showed that soil water content (SWC) decreased from day-2 of water stress imposition compared to well watered treatment (Figure 1). The SWC dropped to 30% at day 10, a reduction of 48% since water stress was imposed on day one. Meanwhile the SWC in well watered plants remained between 75 to 85%.

3.1.2. Plant height

The result showed that imposition of water stress significantly reduced plant height by 8 and 12% at 45 and 65 DAS, respectively as compared to well watered treatment (Figure 2). The results further revealed that water stressed treatment imposed at 95 DAS had the highest reduction in plant height.

3.1.3. Tiller number per hill

Tiller number was significantly different in well watered treatment as compared to water stressed treatment at 65 (24 tillers) and 95 (19 tillers) DAS (Figure 3). The results further revealed that reduction of tiller number per hill by 21 and 8% at 65 and 95 DAS in water stressed over well watered treatment. Tiller number was the most affected in water stressed treatment at 65 DAS.

3.1.4. Leaf area

At the early stages of 45 and 65 DAS, leaf area was highly significant in well watered as compared (828 and 1829 cm^2 $hill^{-1}$) to water stressed treatments (702 and 656 cm^2 $hill^{-1}$) (Figure 4). In contrast, leaf area was significantly different in water stressed treatment at 95 DAS with

1663 cm^2 $hill^{-1}$. The results further revealed that, the lowest leaf area was observed at 65 DAS with a 64% reduction as compared to well watered treatment.

3.1.5. Photosynthesis rate

The photosynthesis rate (Pn) at different DAS presented in Figure 5 showed a significantly higher Pn rates in well watered treatments as compared to water stressed treatment. At 45, 65 and 95 DAS, water stressed treatment had led to a reduction in Pn. There were reductions of 23, 17 and 39% in Pn at 45, 65 and 95 DAS in water stressed plants as compared to control treatments.

3.1.6. Stomatal conductance

Similar to Pn, stomatal conductance was significantly higher in well watered as compared to water stressed treatments at 45, 65 and 95 DAS (Figure 6). At 45, 65 and 95 DAS, the lower stomatal conductance were observed with water stress treatment with values of 0.17, 0.62 and 0.67 $\mu molm^{-2}s^{-1}$, respectively. The result indicated a reduction of 17, 16 and 19% at 45, 65 and 95 DAS, respectively in water stressed treatment.

3.1.7. Biomass partitioning

Table 1 and Figure 7 illustrates pattern of biomass partitioning in MR219 rice plants subjected to well watered and water stressed treatments imposition. At 45 DAS, no significant difference was observed on biomass partitioning to leaves, culm and roots in both treatments. But there were significant differences on biomass partitioning to leaves and culm at 65 DAS. In contrast, no significant difference was observed for biomass partitioning to roots at this particular phase. It was observed that in water stressed treatment, more biomass were partitioned to culm and roots. Higher dry matter partitioning to roots (57%), leaves (42%) and grains (40%) in well watered treatment were observed at 45, 65 and 95 DAS. In contrast, biomass were partitioned to roots (54%), leaves (36%) and grains (26%) at 45, 65 and 95 DAS in water stressed treatment. Leaves were the most affected by the partitioning changes under water stressed treatment as compared to other parts of plant especially at 65 DAS. Days after sowing were significant at 65 and 95 DAS in well watered treatment with 1.78 and 0.65 for root per shoot ratio. According to observation on biomass partitioning, reduction of 8% leaves at 65 DAS directly lowered grain weight by 14% and grain filling percentage by 4% in water stressed treatment.

3.1.8. Yield

As shown in Table 2, yield component was significantly affected with water stress as compared to control treatments. The reductions of 60, 15, 4, 9 and 31% of grain weight, thousand grain weight, percentage filled grain, number of grains per panicle and panicles per hill, respectively, were observed in water stressed plants compared to control treatments. The reduction in the yield was attributed by lower filled grain in water stress treatments. The harvest index of control was greater compared to water stress treatments with values of 0.66 and 0.34, respectively.

3.2. Field experiment

3.2.1. Rainfall, relative humidity and temperature

The trial was conducted at Kemubu Agricultural Development Authority (KADA), Kelantan that is located in east coast of Malaysia. This granary area often experiences water deficit during off-planting season normally in the months of January to July. During period of the present study, the average rainfall was recorded less than 200 mm per month. As illustrated in Table 3, the monthly average maximum and minimum temperature and relative humidity ranged from 25.6 to 28.9°C and 75.5 to 77.7%, respectively, while total rainfall ranged from 6.2 to 131.4 mm/month. The mean temperature, relative humidity and total rainfall in KADA were obtained from Meteorological Department of Malaysia as shown in Table 3.

3.2.2. Yield

The study showed that yield reduction of 96, 92 and 63% when plants received water for 1 (T1), 2 (T2) and 4 (T3) weeks, respectively compared to well-irrigated plants (T4, weeks) (Figure 8,9,10,11). There was a significant reduction in yield when water was only available for the first 4 weeks of planting that coincided was with low rainfall in the region.

The rice yield in response to water supply (Figure 9) showed a linear relationship with the following function:

Yield = 0.0052x with R^2= 0.98* (n = 16).

4. DISCUSSION

The results from the rain shelter experiment showed growth and yield decreased tremendously under water stress imposition compared to well watered treatment. In the present study, change in growth was evaluated in terms of plant height, tiller number and leaf area which decreased markedly under water stress conditions. Water stress reduced plant height, tiller number and total leaf area through obstruction of water absorption by roots and when transpiration rate becomes excessive. Total leaf area was the most affected, as it was reduced by 64% when water stress was imposed at 65 DAS. To reduce water loss through transpiration, many leaves were found dead, thus the reduction in leaf area. At 95 DAS, the higher leaf area observed in water stressed plant compared to well watered could have been due to an increase in assimilation to the leaves rather than to the grains for recovery processes as part of plant adaptation strategy. Such reduction in the parameters above under water deficit conditions was attributed to reduced cell division, expansion and elongation (Farooq et al., 2009; Ashraf et al., 2011). These results were in agreement with previous studies on rice (Hubbart et al., 2013), mungbean (Uddin et al., 2013) and chickpea (Shamsi et al., 2010).

The limitation of carbon uptake by the leaves through reduction in stomatal conductance indicated that stomatal conductance inhibition reduced photosynthesis rate (Schreiber, 2004; Mafakheri et al., 2010). The photosynthesis rate was reduced severely (39%) at 95 DAS in water stressed plants through reduction in stomatal conductance. The reduction in stomatal conductance indicated that stomata was closed to lessen the water loss through transpiration. These results with respect to stomatal conductance were in agreement with many previous studies on rice crops (Yokota et al., 2002; Farooq et al., 2009).

Water stress altered the assimilate partitioning pattern. As consequences of water stressed treatment, dry matter production and partitioning of the rice plants diminished particularly in leaves and culm (Farooq et al., 2009). Similar pattern was observed in root dry matter distribution whereby it was reduced by 26 (45 DAS), 48 (65 DAS) and 37% (95 DAS) in water stressed treatments. In both treatments, dry matter was partitioned away from shoot organs and redistributed into the roots. There was 47% reduction of assimilate partitioning in water stressed whole plant dry matter (net assimilate accumulation) at 65 DAS which was in accordance to reports by Kobata and Takami (1983). The ratio of dry matter accumulation in root to shoot of water stressed plants were significantly lower than well watered plants at 65 and 95 DAS (15 and 28%), which clearly indicated the altered partitioning of resources within the rice plants under different water conditions. The reduction of dry matter partitioning to leaves and grains was affected by 6 and 14%, respectively at 65 DAS compared to well watered treatment. This resulted decrease in harvest index (49%) in water stressed plants which was substantially lower than that reported for rice species as reported by Prasad et al. (2006), Kim et al. (2011) and Puteh and Mondal (2014). The decrease in grain dry matter comes as a direct result of resource partitioning to the culms and leaves rather than to the grains in water stressed treatment. In addition, poor translocation and partitioning of assimilates into the grains reduces yield. Similar results were reported by Swarbick et al. (2006) and Farooq et al. (2009). In terms of sink strength, the poor capacity of yield organs to import carbon, which is subject to regulation within the sink region in the water stressed treatment might be the reason of lower yield production.

Water stressed markedly reduced the grain weight and grain filling up to 60 and 4% respectively. The decreases in all yield components under water stressed treatment reduced grain weight. This contributed to a lower harvest index in water stressed treatment, indicating that lesser assimilates from photosynthesis, carbon partitioning and dry matter production related to yield production (Yang and Zhang, 2010). Based on percentage of biomass partitioning, more assimilate partitioned to culm and leaves due to a higher photosynthate translocation in water stressed treatment rather than to the grains. It might be due to the inability to convert starch into sucrose after photosynthesis, leading to increased starch accumulation in leaves and culm. Similarly, Mafakheri et al. (2010), Ji et al. (2012), Fu et al. (2011) and Afzal et al. (2014) reported that water stressed treatment influenced the translocation of source-path sink system.

Among the three water stressed conditions, treatments that received water at one and two weeks were severely affected, with yield losses of 96 and 92% compared to well-irrigated. Prediction of rice yield in the field with four week reduced by 63% due to hot (maximum 28°C) and dry (maximum 75.7% humidity) weather conditions (DOA, 2013; Akinbile et al.,2011). Likewise, Alderfasi et al. (2010) reported that yield drastically reduced due to low water availability. The severe loss of yield depended on many factors such as timing, length and severity of the water availability (Alderfasi et al., 2010). Rising temperatures during cultivation periods will therefore increase crop water demand, deplete soil moisture faster and increase irrigation demand (Peng et al., 2004; Singh et al., 1996). Based on the linear relationship, yield depended on water availability during rice cultivation period. At least 800 mm day^{-1} of water is needed to achieve an average yield of 4 ton ha^{-1} at KADA as 52% of the total paddy areas in Malaysia are rainfed (Akinbile et al., 2011).

5. CONCLUSION

The reduced thousand grain weight, percentage filled grain, number of grains per panicle and panicles per hill resulted from drastic reduction in leaf area were responsible for the yield losses of rice during reproductive stage. Water stressed treatment influence the translocation of source-path sink pattern especially to leaves and roots rather than to the grains during ripening stage.

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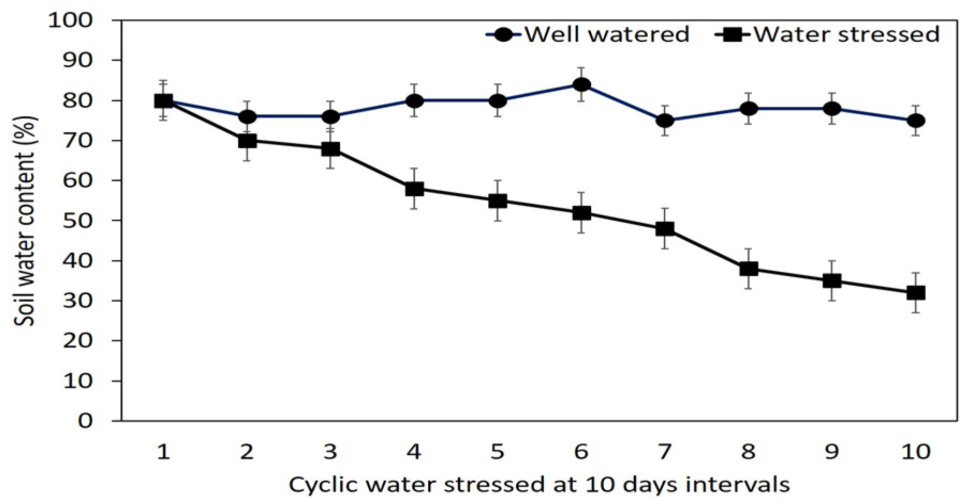

Figure 1.Soil water content at reproductive stage as affected by well watered and water stress conditions in rice plants.

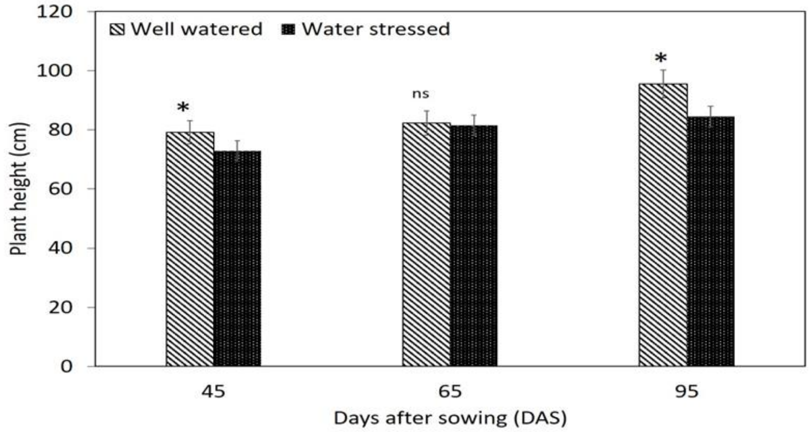

Figure 2.Plant height at different days after sowing as affected by water stress and well watered treatments in rice plants.*: Significant at P≤0.05 and ns: not significant.

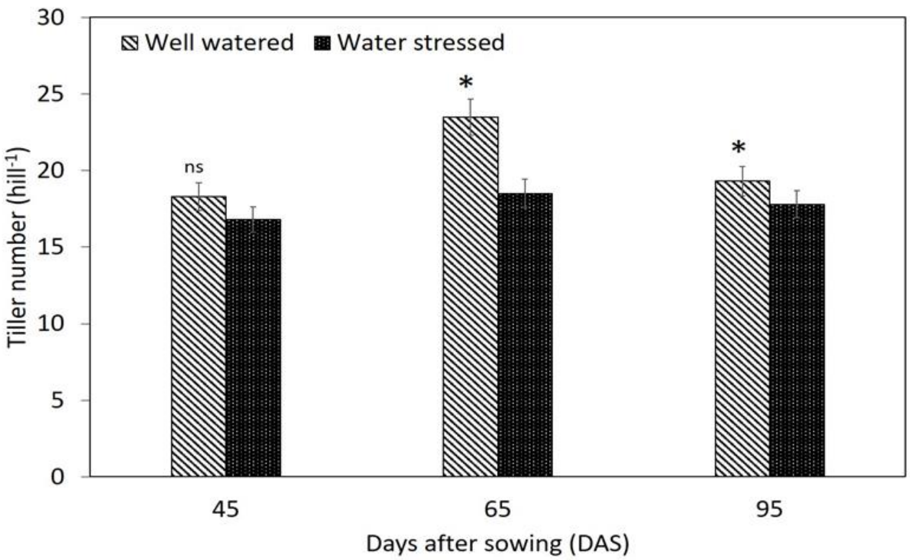

Figure 3.Tiller number per hill at different days after sowing as affected by water stress and well watered treatments in rice plants. *: Significant at P≤0.05 and ns: not significant.

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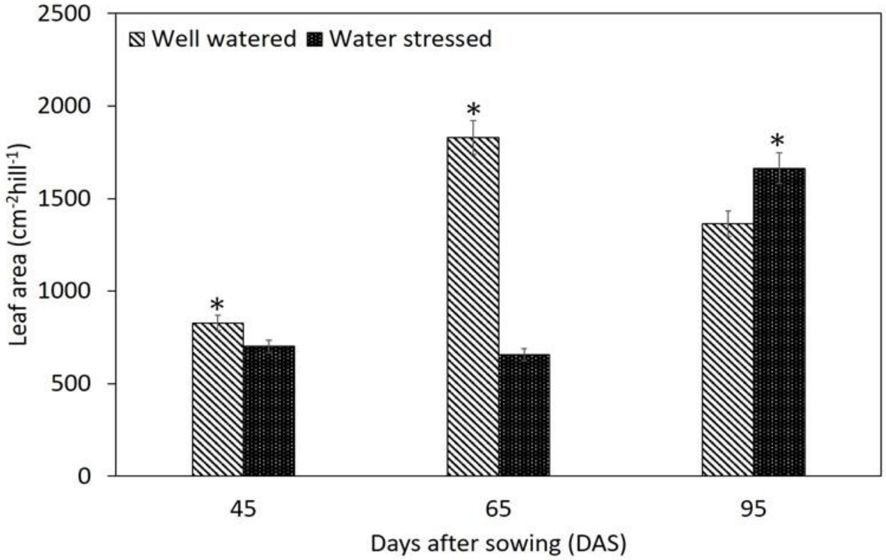

Figure 4.Leaf area in rice at different days after sowing as affected by water stress and well watered treatments in rice plants. *: Significant at P≤0.05

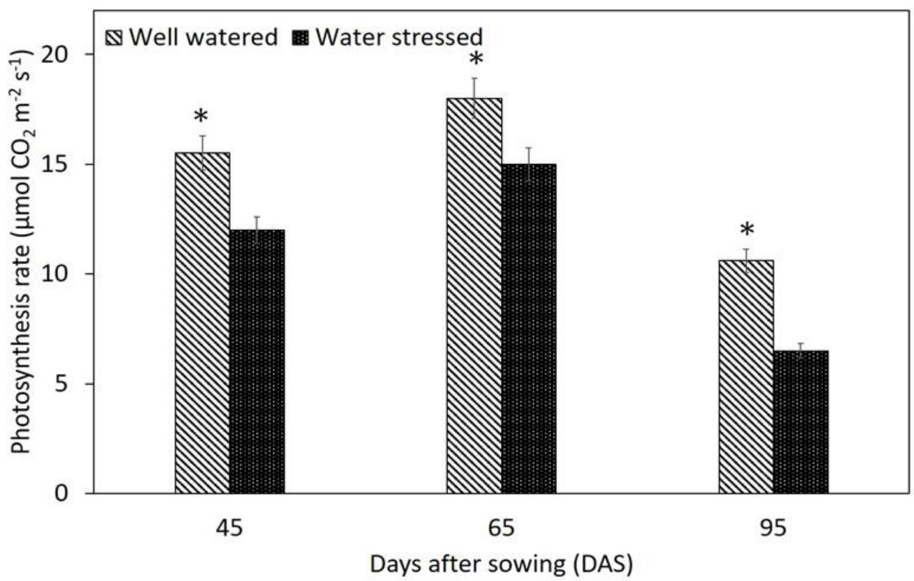

Figure 5.Photosynthesis rate at different days after sowing as affected by water stress and well watered treatments in rice plants. *: Significant at P≤0.05

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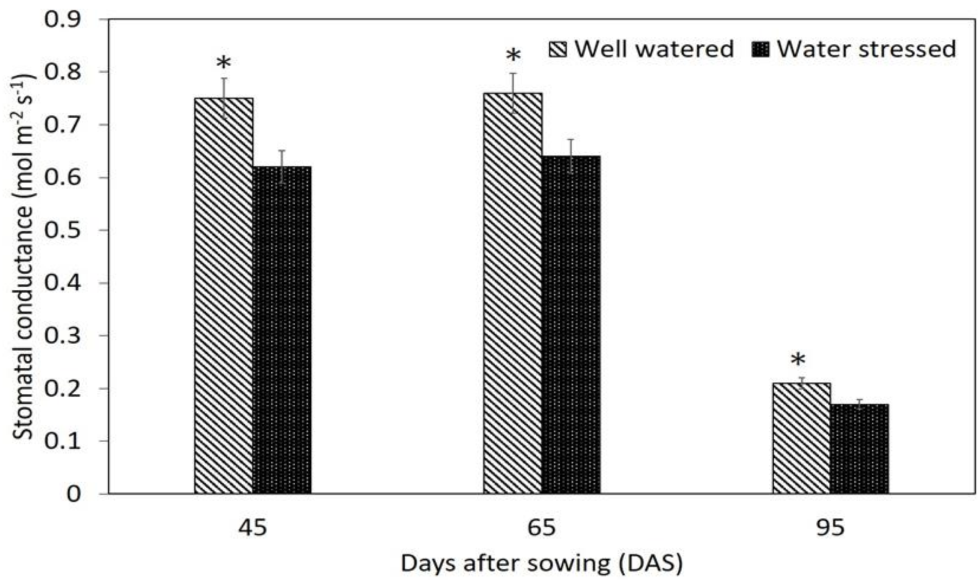

Figure 6.Stomatal conductance at different days after sowing as affected by water stress and well watered treatments in rice plants. *: Significant at P≤0.05

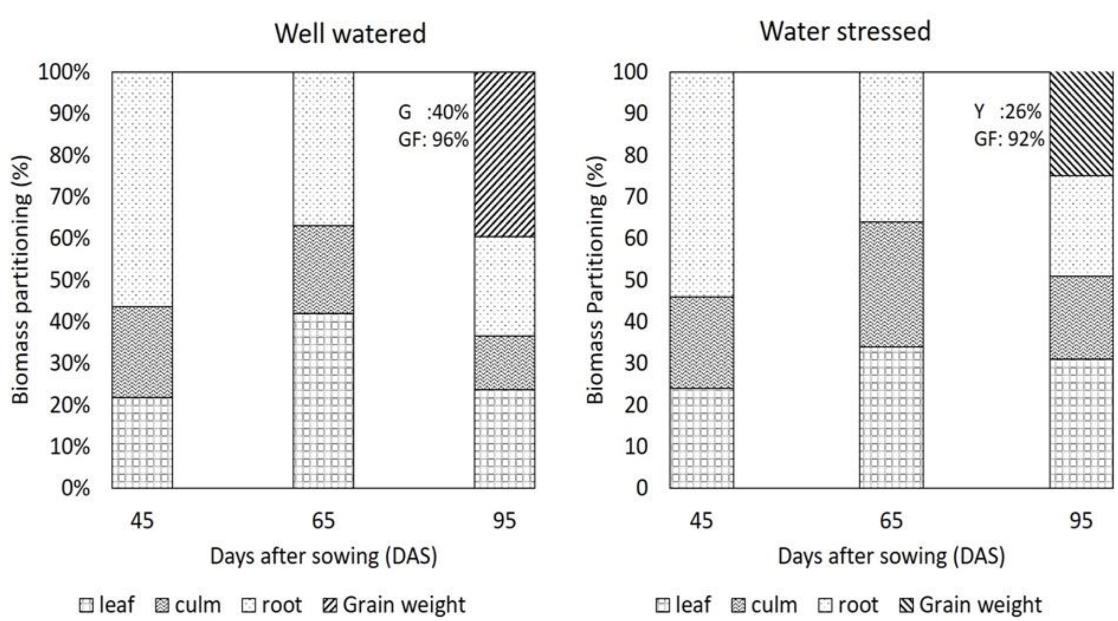

Figure 7. Whole plant percentage dry matter partitioning at different days after sowing as affected by water stress and well watered treatments in rice plants. Y: yield production and GF: grain filling percentage.

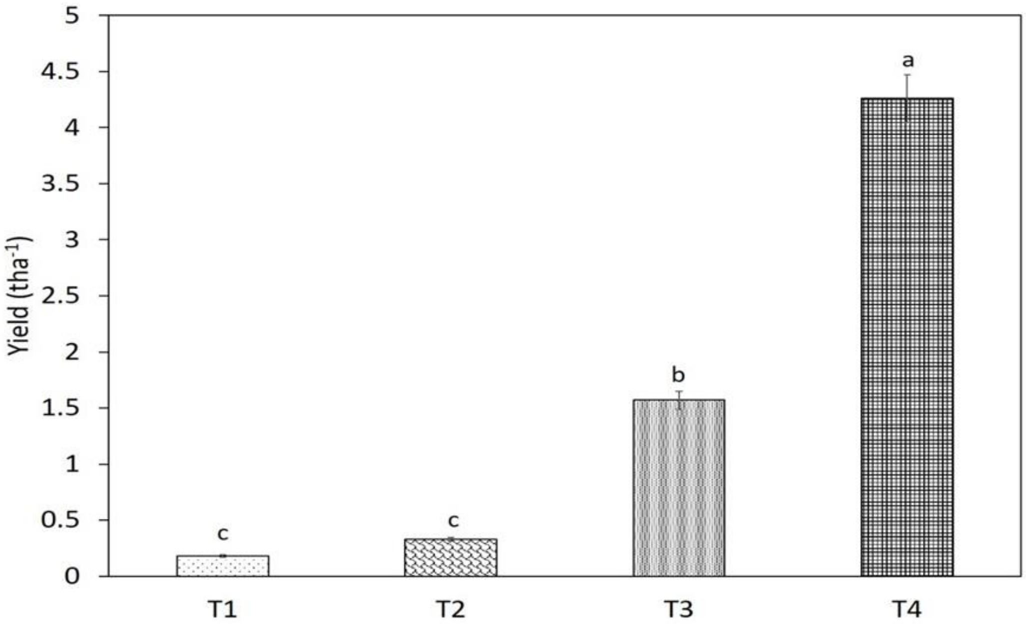

Figure 8. Yield of rice during off season at KADA, Kelantan. Plants received water for 1 (T1), 2 (T2), 4 (T3) and 9 (T4) weeks, respectively. Vertical bars indicate standard errors of means.

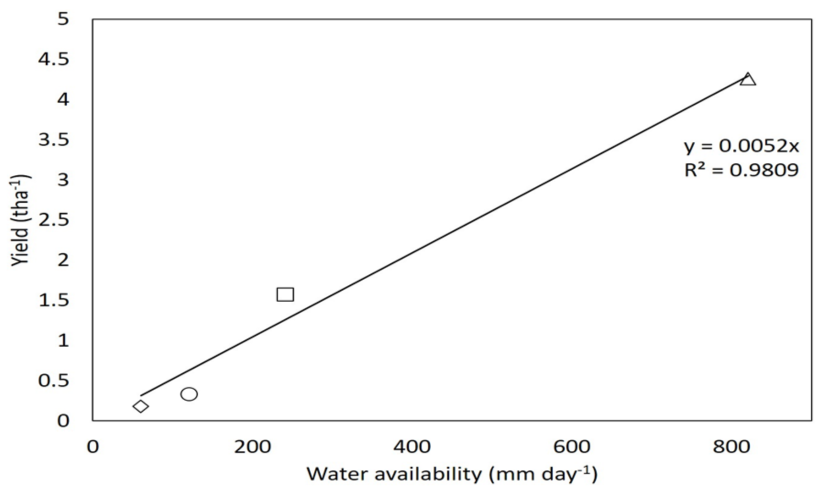

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Figure 9. The relationship between yield and water availability in the field trial. Symbols represent: T1: ◊, T2: ○, T3: □, and T4: ∆.Referring to the water requirement approximately an average of 8.64 mm day^{-1} to produce optimum yield (MARDI, 2002).

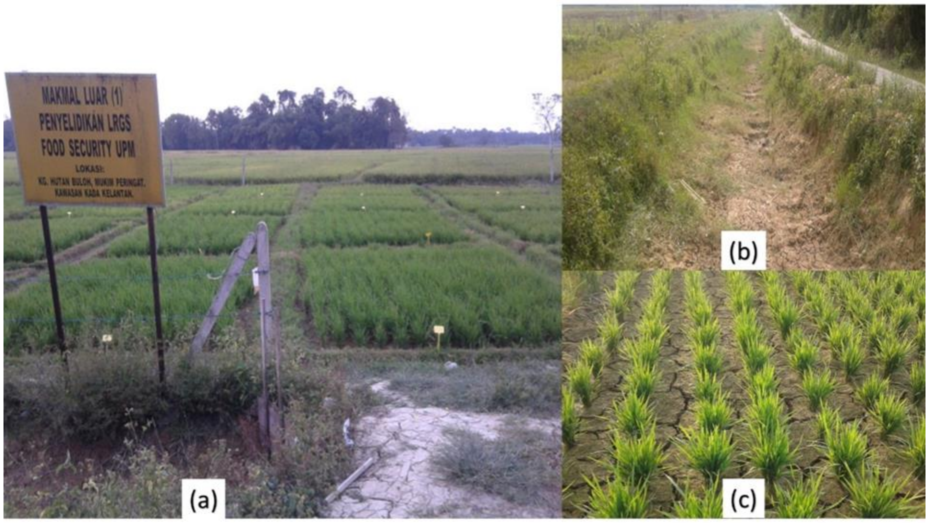

Figure 10. (a) Field experiment plot at KADA, Kelantan, (b) Dried canal nearby plot and (c) soil condition and growth of rice plants at the experimental plot during vegetative stage.

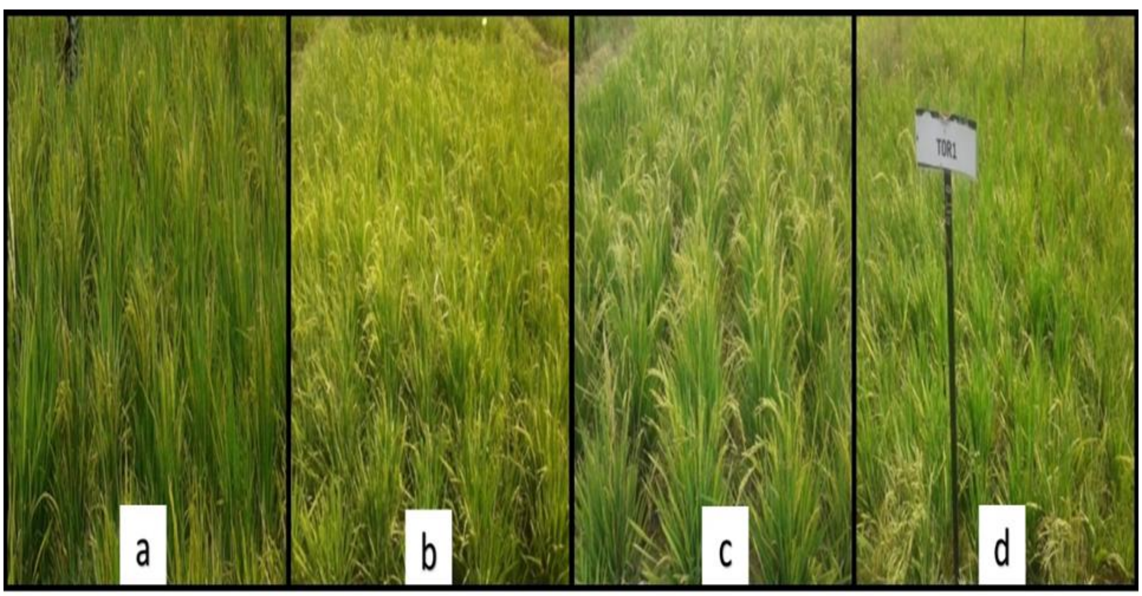

Figure 11. Well-irrigated plants (a), plants received water for 4 (b), 2 (c) and 1 (d) weeks at KADA, Kelantan at ripening stage.

Table 1. Dry matter production (g hill-1) and partitioning (%)at different days after sowing as affected by water stress and well watered treatments in rice plants.

Biomass Partitioning	Leaf (g hill^{-1})			Culm (g hill^{-1})			Root (g hill^{-1})			Total (g hill^{-1})			Root: shoot ratio		
DAS	45	65	95	45	65	95	45	65	95	45	65	95	45	65	95
Well watered	8.9ns	29.7*	22.1*	9.0*	14.6*	12.3*	23.4*	25.9*	22.4*	41.2*	70.2*	56.8*	1.31 ns	1.78*	0.65*
Water stressed	7.7ns	12.6	18.4	7.0	11.4	11.6	17.3	13.5	14.2	32.0	37.5	44.2	1.19 ns	1.51	0.47

*: Significant at P≤0.05 and ns: not significant, CV: Coefficient of variation, DAS: days after sowing.

Table 2: Yield components at different days after sowing as affected by water stress and well watered treatments in rice plants at maturity.

Treatments	Panicles per hill (no.)	Number of grains per panicle	Filled grain (%)	1,000 grain weight (g)	Grain weight (g hill^{-1})	Harvest index
Well watered	13*	118*	95.8*	24.2*	37.7*	0.66*
Water stressed	9	107	91.6	20.6	15.2	0.34

*: Significant at P≤0.05

Table 3. Mean temperature, relative humidity and total rainfall for experiment conducted in KADA, Kelantan.

Month	Mean Temperature (° C)	Mean Relative Humidity (%)	Total Rainfall (mm)
February 2014	25.6	77.4	6.2
March 2014	26.8	76.1	43.6
April 2014	28.8	74.8	28.0
May 2014	29.0	76.5	131.4