Australian Journal of Crop Science
AJCS
AJCS 7(9):1277-1283 (2013) ISSN:1835-2707

Chlorophyll fluorescence parameters of cultivated (*Oryza sativa* L. ssp. indica) and weedy rice (*Oryza sativa* L. var. nivara) genotypes under water stress

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Abstract

Plant responses to water stress vary according to species, severity of the stress and the plants growth stage when the water limitation occurs. The study was focused to evaluate the effect of pre-anthesis water stress on the leaf water potential, chlorophyll fluorescence and its consequence on spikelet fertility in cultivated and weedy rice to understand whether weedy rice can be controlled by imposing water stress at reproductive stage or not. Two widely cultivated rice varieties of MR 219 and MR 232 (*Oryza sativa* L**.** ssp. indica), and two weedy rice strains of Bertam and Ketara **(***Oryza sativa* var. nivara**)** were grown in polybags in rice culture system-like situations. Water stress condition (no water supplied) was then applied for 9 days prior to anthesis phase. Results showed that both cultivated rice varieties and weedy rice strains were sensitive to pre-anthesis water stress for 9 days. Almost similar responses to leaf water potential and photosynthetic traits were observed under water stress. The minimum fluorescence (Fo) of the studied genotypes significantly increased under water stress, whereas the maximum quantum yield (Fv/Fm) and maximum primary yield (Fv/Fo) of PS II were significantly declined, which resulted in drastic increased spikelet sterility (> 80%) in all studies varieties/ecotype. However, weedy rice strains were comparatively more tolerant under water stress condition than cultivated rice varieties, in respect to maintaining chlorophyll fluorescence traits and spikelet sterility, indicating these two weedy rices, Bertam and Ketara, cannot be controlled by imposing water stress at reproductive growth stages.

Keywords: Chlorophyll fluorescence, leaf water potential, water stress, weedy rice.
Abbreviations: Fo_minimum fluorescence; Fv/Fm_maximum quantum yield of photosystem II; Fv/Fo_maximum primary yield of photosystem II; LWP_leaf water potential; PSII_photo system II; SWC_soil water content; WR_weedy rice

Introduction

Water availability is one of the most important limiting factors in crop production. Plant responses to water deficit stress vary according to species, severity of the stress and the plants growth stage whenever the water limitation occurs (Gonzalez et al., 2008). Decreasing water supply affects physiological, morphological and biochemical processes in plants and if that water stress occurs in critical phase of plant growth, it can decrease yield and even cause crop failure (Liu et al., 2006). It has been reported that the reproductive developments in grain crops was more sensitive to water stress than the vegetative development. In other words, the presence of water stress during plant reproduction stage is detrimental to the reproductive organ (Saini, 1997). The flowering phase is one of the most sensitive period to water stress in rice (Liu et al., 2006) and environmental stresses can cause a decline in spikelet fertility (Khan and Abdullah, 2003; Jagadish et al., 2010). The spikelet sterility increases under water stress condition which might be due to the reduction of many key metabolic functions and physiological processes in plants (Tezara et al., 2002). Reduction in chlorophyll fluorescence parameters of rice at water stress have been reported by many authors (Chaerle et al., 2007; Cha-um et al., 2010; Sikuku et al., 2010). Based on our knowledge, no research work has been conducted with weedy rice in tropical region so far. Therefore, studies on chlorophyll fluorescence parameters in cultivated and weedy rice plants exposed to water stress are necessary to gain knowledge on the effect of increase water stress on spikelet sterility for weed managements.

Leaf water status and photosynthesis, which related to productivity and crop yield, may be affected by water stress (Chaves et al., 2009). Currently, measurement of chlorophyll fluorescence is widely used to measure photosynthetic performance in leaves and the function of the photosynthetic apparatus (Baker and Rosenqvist, 2004). This method is commonly used to investigate the response of plants to environmental stress (Resco et al., 2008; Wu et al., 2011). Furthermore, chlorophyll fluorescence parameters suggested that water stress sensitivity in plant is associated with decreased photosynthetic efficiency of PS2 and enhanced non-photochemical quenching (Lichteuthaler and Miehe, 1997). The modulated chlorophyll fluorescence techniques have successfully been used along with measurements of net CO_2 exchange and leaf water potential for rapid screening of cereal crops for drought tolerance (Sayed, 2003).

Rice is an important food crop in many countries. It has been documented that lowland rice is susceptible to water deficit and the flowering period is one of the critical phase to water limited condition (Liu et al., 2006). Weedy rice (*Oryza*

Table 1. Effect of water stress on chlorophyll fluorescence parameters in rice genotypes at 8 days of stress treatment imposed.

Treatment		Fo	Fv/Fm	Fv/Fo	Spikelet sterility (%)
Water regime					
Well water		588.2 b	0.78 a	3.51 a	10.5 b
Water stress		770.2 a	0.60 b	1.85 b	81.0 a
Rice group					
Cultivated rice		716.0 a	0.66 ^{ns}	2.68 ^{ns}	46.8 ^{ns}
Weedy rice		542.4 b	0.72 ^{ns}	2.78 ^{ns}	44.8 ^{ns}
Genotype					
Cultivated rice	MR219	708.9 a	0.70 ^{ns}	2.77 ^{ns}	49.0 ^{ns}
	MR232	723.2 a	0.63 ^{ns}	2.60 ^{ns}	44.5 ^{ns}
Weedy rice	Bertam	625.4 b	0.72 ^{ns}	2.81 ^{ns}	45.5 ^{ns}
	Ketara	659.5 b	0.71 ^{ns}	2.75 ^{ns}	44.0 ^{ns}
CV (%)		6.77	5.86	11.2	9.72

In a column, either within water treatment or rice group or genotype, the figures bearing the same letter (s) do not differ significantly at $P \leq 0.05$ by DMRT; ns, not significant, Fo, minimal fluorescence; Fv/Fm, maximum quantam yield of PS II; Fv/Fo, maximum primary yield of PS II.

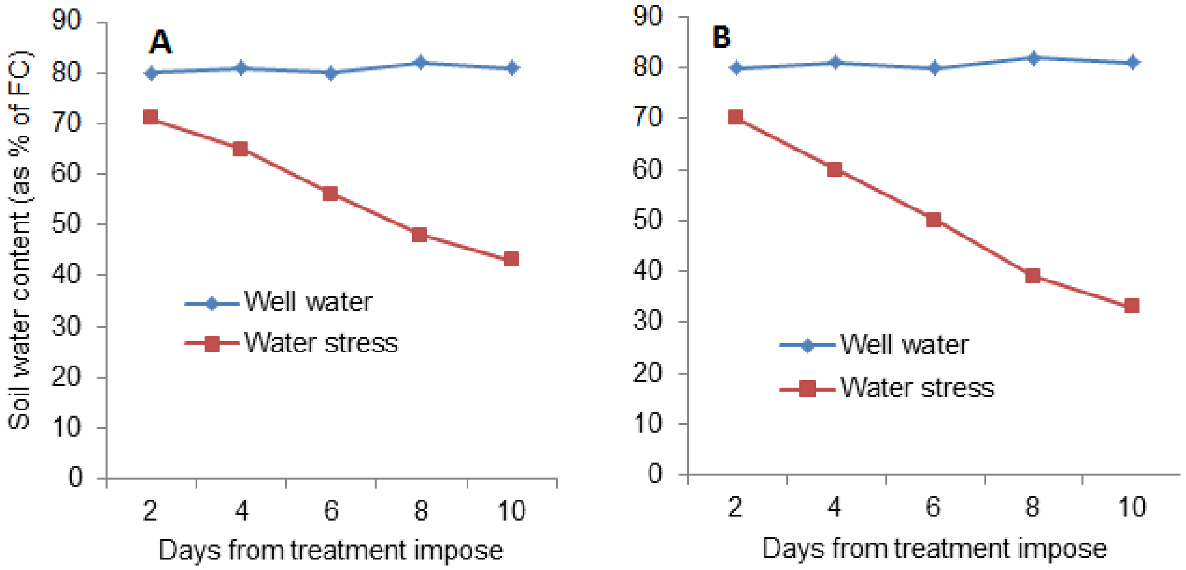

Fig 1. Changes in soil water content over time due to water stress in one cultivated rice variety, MR219 (A) and one weedy rice ecotype, Bertam (B).

sativa var. nivara) is an annual grass. It is morphologically similar to cultivated rice and usually grows in the same field conditions (Mansor et al., 2012). The early and easy seed shattering is its main characteristics of them (Akasaka et al., 2011). Currently it appears as one of the noxious weeds in rice cultivation due to its similar morphology and traits to cultivated rice varieties (Londo and Schaal, 2007). Its invasion is throughout the rice growing areas of the world (Chung and Nam-Chon, 2003; Olsen et al., 2007; Hamid et al., 2007; Prathepha, 2009). There have been numerous studies on effect of water stress on the leaf water potential and photosynthetic capacity in cultivated rice varieties (Jongdee et al., 2002; Lafitte, 2002; Sibounheuang et al., 2006; Cha-um et al., 2010). However, despite its (weedy rice) similarity to cultivated rice variety, information on physiological characters of weedy rice like its responses to water stress is very limited. This experiment studies the weedy rice behavior under water stress during reproductive stage and compares it with the cultivated rice in order to provide an understanding in photosynthetic apparatus of rice, particularly weedy rice. So, the suitable genotypes and management practices can be developed to maximize rice production. This study aimed to compare the leaf water potential, chlorophyll fluorescence and its consequence of percentage of spikelet sterility responses of both cultivated and weedy rice to water stress during the pre-anthesis phase.

Results

Soil water content and leaf water potential

The changes in soil water content (SWC) and leaf water potential (LWP) in rice genotypes during the water stress period are shown in Figs. 1 and 2. After exposing to water stress, the SWC and LWP of the stressed plants, in both cultivated varieties and weedy rice ecotypes, reduced consistently with the increase time of withholding water. Meanwhile, the SWC and LWP of untreated plants were relatively constant. A positive correlation between SWC and LWP was observed in all genotypes (Figs. 1 and 2). Data showed that LWP decreased upon the reduction of SWC. The LWP in water stress plants remained constant in both cultivated varieties and weedy rice ecotypes at first five days after withholding water and then decline sharply to -16.2 MPa in MR 219, -18.1 MPa in MR 232, -20.3 MPa in WR Bertam and to -27.2 MPa in WR Ketara on day 9 (Fig. 2). The LWP of well watered plants remained constant (Fig. 2). From the results obtained, it is obvious that the stress period for 9 days was severe enough and caused extreme reduction in the leaf water potential in both cultivated varieties and weedy rice ecotypes.

Table 2. Interaction effect of genotype and water regime on chlorophyll fluorescence parameters and spikelet sterility in rice (at day eight of water stress period).

Interaction		Fo	Fv/Fm	Fv/Fo	Spikelet sterility (%)
Genotype	Water regime				
MR219	WW	627.2 ^{ns}	0.78 a	3.53 a	10.0 d
	WS	790.5	o.62 b	2.00 b	88.0 a
MR232	WW	583.7	0.78 a	3.58 a	9.10 d
	WS	862.7	0.47 c	1.62 c	80.0 bc
Bertam	WW	579.4	0.77 a	3.44 a	10.9 d
	WS	671.4	0.67 b	2.18 b	80.1 bc
Ketara	WW	562.5	0.78 b	3.49 a	10.3 d
	WS	756.4	0.64 b	2.03 b	75.7 c
CV (%)		6.77	5.86	11.2	9.72

Figures bearing the same letter (s) within a column do not differ significantly at $P \leq 0.05$ by DMRT; ns, not significant; WW = Well watered, WS = Water stress; Fo, minimal fluorescence; Fv/Fm, maximum quantam yield of PS II; Fv/Fo, maximum primary yield of PS II.

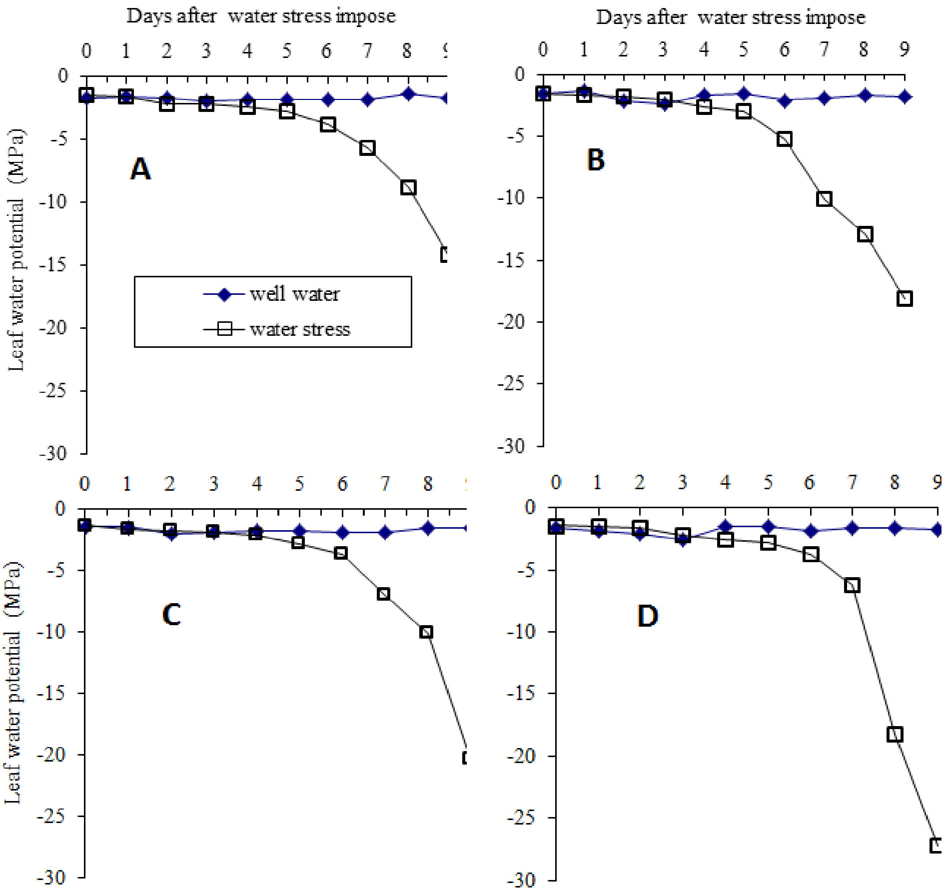

Fig 2. Changes in leaf water potential over time due to water stress of two cultivated rice Varieties, MR219 (A) and MR232 (B), and two weedy rice ecotypes, Bertam (C) and Ketara (D).

Chlorophyll fluorescence parameters

Minimal fluorescence (F_o) value

Under stressed condition, the F_o value of both weedy rice ecotypes and cultivated varieties increased as the duration of stress treatment lengthened as compared to their control (Fig. 3). Under well watered condition, the F_o values were stable and hardly affected throughout the stress treatment period. After eight days of water deficit period, the weedy rice Bertam showed the lowest increment of F_o value among the genotypes. The value of F_o increased by 21% in variety MR-219, by 32% in variety MR 232, and by 26 % in weedy rice Ketara and only 14 % in weedy rice Bertam as compared to well watered control plants (Table 2). On average, cultivated rice varieties showed higher value of F_o than weedy rice ecotypes after eight days of water stress period (Table 1).

The Maximum quantum yield of photosystem II (Fv/F_m ratio)

The chlorophyll fluorescence parameter F_v/F_m ratio declined after four days of water stress imposed and the Fv/Fm ratio reduction continued as the water limitation progressed in both cultivated varieties and weedy rice ecotypes. In well water plants, the F_v/F_m ratio unchanged during the stress period (from day 0 to day 8) (Fig. 4). Results showed that Fv/Fm value was lower in water stress plants than in well water plants (Table 1). Within the groups, cultivated rice varieties showed lower Fv/Fm value than weedy rice ecotypes.

The interaction between water status and genotypes on F_v/F_m was significant at day eight of water stress period (Table 2). Under well water condition, there were no significant differences of F_v/F_m values in all rice genotypes. Meanwhile, under water stress condition cultivar MR 232 had lower F_v/F_m value than MR 219, WR Bertam, and WR Ketara (Table 2).

The maximum primary yield of photochemistry of photosystem II (F_v/F_o)

Under water stress condition, the F_v/F_o ratio of cultivated rice and weedy rice decreased gradually as the water deficit progressed compared to their control (Fig. 5). The interaction between water status and genotypes was significantly differed on F_v/F_o ratio at day eight after imposition of water stress treatment (Table 2). At day eight, the F_v/F_o ratios in four rice genotypes were not significantly different under well water condition; however, it decreased under water stress in general. However, there was no significant difference in Fv/Fo between cultivated and weedy rice genotypes. Under water stress treatment, cultivated rice MR 232 exhibited the lowest F_v/F_o value compared to other genotypes, whereas there was no difference among the other three genotypes. Variety MR 219 and MR 232 reduced F_v/F_o about 43.3 % and 65.9%, respectively, while weedy rice Bertam and Ketara declined by 36.6 % and 42.6 %, respectively as compared to well water control (Table 2).

Spikelet sterility percentage

There were significant effects of water regime treatment and interaction between water regime and genotypes on spikelet sterility percentage (Table 2). Sterility percentage was about eight times higher in water stress plants than control plants (well watered plants). Sterility percentage of cultivated varieties and weedy rice strains was not significantly different. The highest sterility percentage under water stress condition was recorded in MR219 (88.0%) and the lowest was recorded in WR Ketara (75.7%).

Discussion

Water stress is one of the most common plant stresses that is the bottleneck for agriculture development in many regions of the world. Anthesis stage in rice plant is one of the critical periods in water stress that can cause yield reduction if water deficit occurs during it. During a severe enough water stress, reduction in physiological activities such as retarded vegetative growth and reduced carbon gain can be observed (Sayed, 2003). Reduced physiological activity during reproductive growth leads to abortion of reproductive effort (Nilsen and Orcutt, 1999).

Among the physiological attributes, leaf water potential (LWP) can be used as indicators of water stress (Lafitte, 2002). The high LWP might diminish the negative effect of water deficit occurring at reproductive phase (Jongdee et al., 2002). The differences in plant water status during the stress period reflects the differences in drought tolerance among

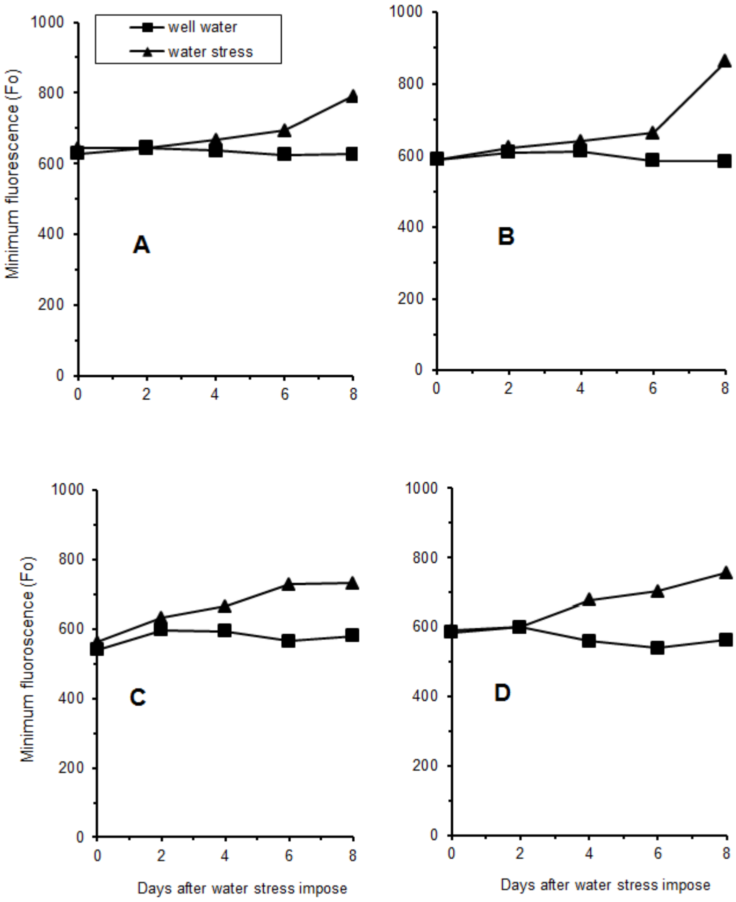

Fig. 3. Changes in minimum fluorescence (F_0) over time of two cultivated rice varieties, MR219 (A) and MR232 (B), and two weedy rice ecotypes, Bertam (C) and Ketara (D) under well water and water stress conditions.

rice genotypes (Lafitte, 2002). However, no significant difference in leaf water potential observed among the four cultivated varieties and weedy rice ecotypes. After subjected to water stress for 9 days, very low leaf water potential values were achieved by all rice genotypes tested, as compared to well water condition (Fig. 2). This indicated that both cultivated rice and weedy rice experienced a severe water deficit. The reduction of LWP in response to water stress is a well-known phenomenon and it has been reported earlier in rice (Sibounheuang et al., 2006).

The impact of water stress on photosynthesis is caused by stomatal closure due to leaf-sourced abscisic acid (Davies and Zhang, 1991). Water stress-induced stomatal closure depletes inter-cellular CO_2 leading to photoinhibition (Kokubun et al., 2001). In the present study, water stress affected the photosynthetic apparatus as seen from the changes of chlorophyll fluorescence values in both cultivated and weedy rice (Tables 1 and 2). The values of chlorophyll fluorescence parameters under well-water conditions did not differ significantly between cultivated and weedy rice (Table 2).

Water stress caused an increased in minimum fluorescence (F_o) value of cultivated and weedy rice compared to well-water condition. The increment was obvious after eight days of withholding water. The F_o is the primary chlorophyll fluorescence yields, which measures the stability of the light-

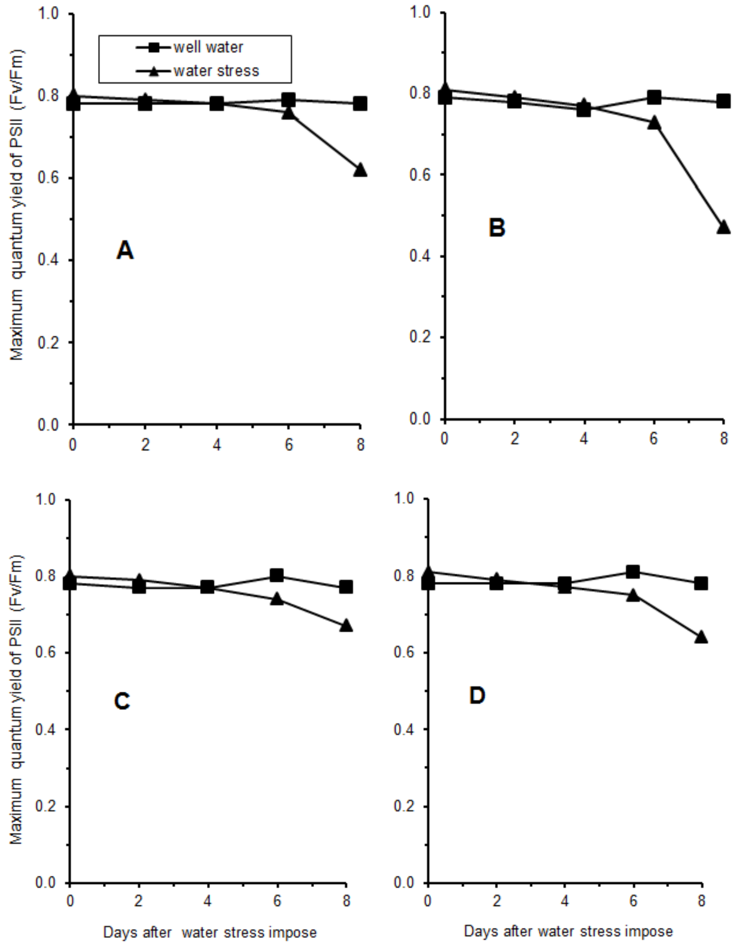

Fig 4. Changes in chlorophyll fluorescence, Fv/Fm ratio over time of two cultivated rice varieties, MR219 (A) and MR232 (B), and two weedy rice ecotypes, Bertam (C) and Ketara (D) under well water and water stress conditions.

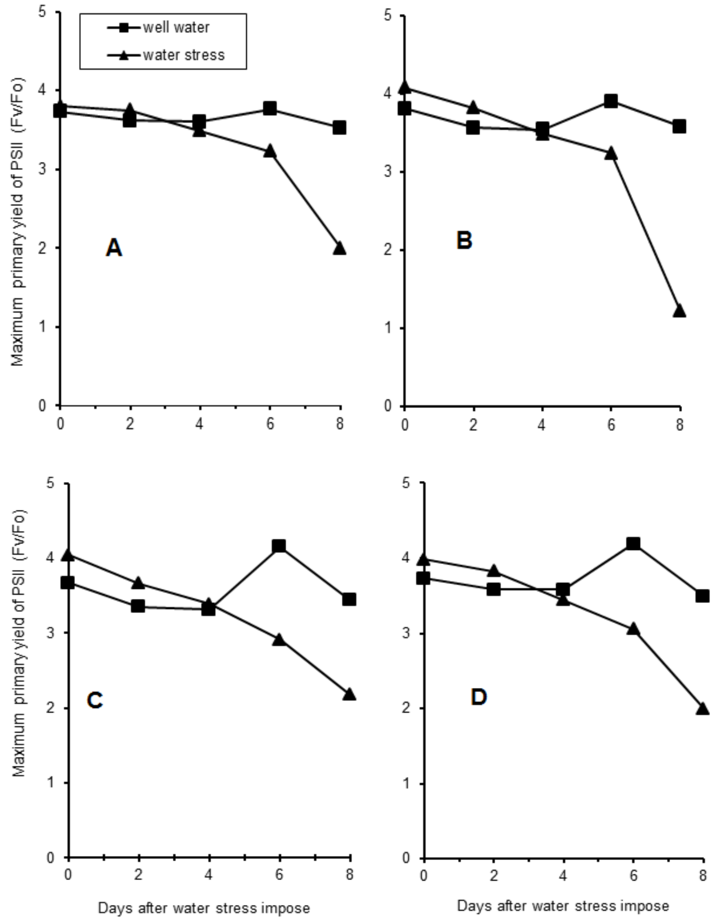

Fig 5. Changes in chlorophyll fluorescence, Fv/Fo ratio over time of two cultivated rice varieties, MR219 (A) and MR232 (B), and two weedy rice ecotypes, Bertam (C) and Ketara (D) under well water and water stress conditions.

harvesting complex (Percival et al., 2003). The increase of F_o value indicates the disruption of photosynthetic apparatus. The increase of F_o due to abiotic stresses has been reported, such as those due to temperature stress (Yamada et al., 1996). Weedy rice Bertam exhibited the lowest F_o under water stress condition than the other three genotypes (Table 2), indicating WR Bertam is more tolerant to water stress than the other three rice varieties/ecotypes.

Compared to the well-watered treatments, the decline of F_v/F_m and F_v/F_o value due to water stress observed in four genotypes studied. The F_v/F_m ratio represents the maximum quantum yield of PS II, which is correlated with the quantum yield of net photosynthesis. It is frequently used as an indicator of the photo-inhibitor or other injury caused to the PS II complexes (Rohacek, 2002). The F_v/F_m ratio values are about 0.80 and almost constant for different plant species measured under the non-stressed condition (Oyetunji et al., 2007). In the present study, water stress was found to reduce the F_v/F_m ratio in cultivated and weedy rice tested. This suggests that the total amount of light energy transformed in PS II reaction centre were decreased. Thus, the changes observed in photochemical activity of PS II can contribute to the limitations of photosynthesis activity under water deficit (Tezara et al., 2002). Weedy rice Bertam showed a little reduction in F_v/F_m compared to other cultivated varieties under stress condition, indicating WR Bertam is tolerant to water stress. The reduction was more pronounced in variety MR 232, indicating the variety MR232 is susceptible to water stress at reproductive stage.

F_v/F_o ratio is the maximum ratio of quantum yields of photochemical and concurrent non-photochemical processes in PS II. It estimates the maximum primary yield of photochemistry of PS II and provides an estimation of leaf photosynthetic capacity. The F_v/F_o value is in the range of 4 – 6 (Rohacek, 2002; Percival et al., 2003). According to Borkowska (2002) a reduction in F_v/F_o under severe stress could be associated with a disruption of photosynthesis in donor part of the PS II. In this study, in response to photosynthetic process, both cultivated and weedy rice ecotypes were found sensitive to water stress with being the more sensitive in variety MR232 as can be seen through the chlorophyll fluorescence measurements. From the drop of F_v/F_m, F_v/F_o values and the increase of F_o value, the water stress treatment possibly damaged the photosynthetic apparatus and led to disturbance in photosynthetic process.

Water stress caused an increase in spikelet sterility of both cultivated and weedy rice. Puteh et al. (2009) reported that weedy rice has relatively lower spikelet sterility than the cultivated ones. However, in this study, after experiencing water deficit, the spikelet sterility in both cultivated and weedy rice increased more than 75%. Increased spikelet sterility of rice under water deficit condition at flowering stage has been reported earlier (Liu et al., 2006; Jongdee et al., 2002). The data presented in this investigation demonstrates that photosynthesis decreased upon the reduction of the plant water status as represented with leaf water potential. This reduction of photosynthetic activity might have reduced assimilates translocation to reproductive organs and this leading to increased sterility rate under water stress condition.

Materials and methods

Plant materials and crop establishment

This study was conducted at the Faculty of Agriculture, Universiti Putra Malaysia in 2011. Two cultivated rice varieties (MR 219 and MR 232) and two wild genotypes of *Oryza sativa* L. × *Oryza nivara* (WR Bertam and WR Ketara) were used.

Rice culture system was developed where plants were grown in black perforated plastic polybags (diameter 40 cm, height 45 cm) containing approximately 20 kg of soil obtained from a rice growing area. The experimental soil was Jawa series. Prior to sowing, the seeds were allowed to germinate in petridishes layered with wet filter papers for 48 hours. Fifteen pre-germinated seeds were sown in each polybag. Plants were thinned to three plants per polybag about two weeks after sowing. The polybags were submerged in water in polyethylene tanks (diameter 100 cm, height 56 cm). Eight polybags were housed in each polyethylene tank. The polyethylene tanks containing seedlings in polyethylene bags were placed in an enclosed cage and were arranged in a completely randomized design factorial, combination of four rice genotypes and two water status treatments in three replications. Standard procedures of rice growing culture were followed throughout the studies. Seedlings growth and development were monitored daily.

Water stress treatments

At late booting stage the plants in all the eight polybags were taken out from the polyethylene tanks to impose water stress treatment for 9 days. The stress treated plants were put back into the polyethylene tank on day 10 as re-watering. The control plants were left submerged in water in the polyethylene tanks throughout the study. Within this imposition of water-stress period, the polybags were placed under the shade to avoid additional water from rain.

Measurement of soil moisture

A portable soil moisture meter (Irrometer, Model SR, Riverside, CA, USA) was inserted into each polybag of both well watered and the stressed plants at a depth of 15 cm to monitor soil moisture during the water stress period (Fig. 1).

Measurement of leaf water potential

Leaf water potential was determined daily from freshly cut flag leaves from five plants per treatment during the water stress period for nine days. A potentiometer (Model WP4-T, Decagon Devices, Inc, Pullman, Washington) was used for leaf water potential measurement (Fig. 2).

Measurement of chlorophyll fluorescence parameters

Chlorophyll fluorescence emission from the adaxial surface on the leaf were measured on randomly selected flag leaves of the rice genotypes using a portable fluorescence spectrometer Handy PEA (Hansatech Instrument, Norfolk, UK) following the manufacturer's instruction. For each treatment, five measurements were made during the water stress period in alternating days. Dark adaptation period for all the measurements was about 20 minutes using leaf clips. Fluorescence values recorded, including the initial/minimal fluorescence (F_o), the ratio of variable to maximum fluorescence (F_v/F_m) which represents the maximum quantum yield of Photosystem II (PS II) and the ratio of variable to minimum fluorescence (F_v/F_o) which estimates the maximum primary yield of photochemistry of PS II. The F_m is the maximal fluorescence value, and F_v is variable fluorescence calculated as F_m - F_o (Rong-hua et al., 2006).

Determination of spikelet sterility percentage

At harvest, when the seed reached full maturity, 15 undisturbed panicles from each experimental unit were randomly selected for the determination of seed yield components. The panicles were cut and then the spikelets were threshed from the panicles by hands. From each panicle, filled and unfilled spikelets were separated. Spikelet sterility was estimated as the ratio of unfilled grains or sterile florets to total number of reproductive florets or spikelets and expressed as percentage.

Data analysis

Analysis of variance (ANOVA) was carried out using SAS software to determine the significance of variation for all the traits measured for this study and the means were tested using the Duncan's multiple range test (DMRT) at 5% level of probability (Steel and Torrie, 1960).

Conclusions

Cultivated and weedy rice showed almost similar responses to water stress on leaf water potential and spikelet sterility. Chlorophyll fluorescence parameters decreased under water stress in both cultivated and weedy rice but the decline in chlorophyll fluorescence parameters due to water stress was less in weedy rice as compared to cultivated rice. Imposing of water stress at pre-anthesis phase for 9 days is severe enough to cause a drastic reduction of leaf water potential and disruption of photosynthetic apparatus as measured by chlorophyll fluorescence and result in high grain sterility percentage. However, weedy rice strains are comparatively more tolerant to water stress than the cultivated rice varieties, in respect to maintaining chlorophyll fluorescence traits with the highest tolerance in WR Bertam. Further study with more numbers of weedy rice variants is needed to confirm the response of weedy rice to water stress applied at pre-anthesis phase for 9 days. Since water stress treatment increased the spikelet sterility of weedy rice, combination of water stress treatment and other technique might be used as an alternative to control weedy rice in order to reduce the amount of weedy rice seed bank in soil.

Acknowledgements

The authors are grateful to the Ministry of Higher Education, Malaysia for providing financial support from Long-Term Research Grant Scheme (LRGS) to carry out the experiments and also for providing publication cost.

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