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RESEARCH ARTICLE

Physiology, yield, and water use efficiency of drip-irrigated upland rice cultivars subjected to water stress at and after flowering

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Summary

Water scarcity due to global warming can increase the water demand for upland rice at critical stages of crop development. However, there is little research on cultivar responses to this scenario and technologies that enhance water use efficiency (WUE). To determine the influence of water stress at and after flowering stages of drip-irrigated upland rice cultivars on physiology, yield, and WUE, a shelter experiment was conducted using a randomized block design with a split-plot arrangement of treatments. Three modern and one traditional cultivar were subjected to five irrigation managements: 100% of the field capacity considered the reference management (RM), 70 and 40% of the RM at the flowering stage, and 70 and 40% of the RM at the grain-filling stage. In general, the modern cultivars tended to maintain higher photosynthetic rate, stomatal conductance, transpiration, leaf water potential, and lower crop water stress index compared to the traditional cultivar under water stress. The WUE decreased for all cultivars under severe stress, averaging 0.55 and 0.62 kg m^{-3} when stress occurred at flowering and grain-filling, respectively, whereas moderate stress imposed at grain-filling maintained WUE for all cultivars, averaging 1.21 kg m^{-3}. In addition, grain yield (GY) showed a similar variation trend under drought stress as WUE, and its reduction was mainly associated with low filled grain percentage. Among the five irrigation treatments, both GY and WUE were the highest in the RM; the best cultivar recorded 9.3 Mg ha^{-1} and 1.62 kg m^{-3}, respectively. Findings suggest that attending to the full water demand under precision drip irrigation and appropriate cultivar selection can enhance upland rice production at significant levels.

Keywords: *Oryza sativa*; precision irrigation; photosynthesis

Introduction

Eight percent of the world's rice area is upland rice (*Oryza sativa* L.), and its distribution in Asia, Latin America, and sub-Saharan Africa is approximately 65, 10, and 25 percent of the total rice area, respectively (Saito *et al.*, 2018). Upland rice is cropped primarily under rainfed systems on well-drained soils (Morillas *et al.*, 2019) and to a lesser extent in areas with supplementary irrigation (Stone *et al.*, 1999).

In central Brazil, 65% of the upland rice area corresponds to rainfed systems, which are susceptible to periods of drought (Crusciol *et al.*, 2013); as a result, the cropped area has been reduced by up to 60% in recent decades (Pinheiro *et al.*, 2006). However, upland rice can be integrated into crop rotations with maize and soybean (Nascente and Stone, 2018; Pacheco *et al.*, 2017), allowing the use of irrigation by center-pivot or new areas of subsurface drip irrigation (Sano, 2013). There is evidence that improved upland rice varieties bring out their genetic potential under supplementary irrigation, achieving yields greater than 5 Mg ha^{-1} (Stone *et al.*, 1999), making this system economically viable.

Reduced water availability affects the physiological processes of the plant (Jaleel *et al.*, 2009), producing negative effects on rice yield components (Boonjung and Fukai, 1996). Upland rice physiological responses are poorly understood in comparison to flood rice conditions (Kato and Katsura, 2014). A decrease in stomatal conductance is accompanied by a reduction in the transpiration rate, resulting in low photosynthetic rates and changes in canopy temperature (Ali and Hussain, 2021). Plants have diverse strategies to grow under water stress, such as increasing stomatal resistance (Ohsumi *et al.*, 2007), greater water uptake capacity through high root lengths and density (Miyazaki and Arita, 2020), and increasing water use efficiency (WUE) (Zhao *et al.*, 2004). The response of rice to drought depends on the balance of water relations and damage to cells (Kumar *et al.*, 2017). However, water relations in upland rice when subjected to different drought timing and severity are divergent (Alou *et al.*, 2018; Kato *et al.*, 2006; Luo *et al.*, 2019), making it difficult to develop drought-tolerant cultivars.

Water scarcity is becoming more frequent and may result in an increased incidence of crop drought stress (Kang *et al.*, 2017; Liu *et al.*, 2015). To cope with drought stress in rice, Heinemann *et al.*, 2015) suggest that breeding strategies for drought tolerance have to include spatio-temporal considerations. On the other hand, Bouman *et al.* (2007) emphasize the increase in crop productivity per unit of water required by introducing new water-management technologies. Despite research efforts on water-saving techniques in rice, such as alternate wetting and drying, drip systems, and aerobic rice culture, available information is still limited (Alou *et al.*, 2018; Kato and Katsura, 2014; Sharda *et al.*, 2017). Research that generates information considering the adoption of precision drip irrigation, combined with possible future drought scenarios as projected by Ramirez-Villegas *et al.* (2018) for central Brazil, will be useful for optimizing the upland rice production systems.

It was hypothesized that the negative effect of drought stress on production and physiology is less intensive in modern rice cultivars. To address this hypothesis, the objective of this study was to determine the effects of water stress imposed at and after flowering on the physiology, yield, and WUE of three modern upland rice cultivars and one traditional cultivar under drip irrigation.

Materials and Methods

Plant materials, growth conditions, and drought stress treatments

Four upland rice cultivars commonly cultivated in central Brazil were obtained from the *National Research Center for Rice and Beans* germplasm bank, Embrapa, Brazil (Supplementary Material Table S1). BRS Esmeralda, BRS A501 CL, and BRS Serra Dourada are classified as modern cultivars, while Rio Paraguai is considered a traditional cultivar, no longer used by the Brazilian breeding program.

The experiment was carried out under rain shelter conditions at the Biosystems Engineering Department (LEB), Sao Paulo University (USP/ESALQ), Piracicaba – SP, Brazil (22° 42' 32" S, 47° 37' 45" W and 548 m altitude). The experimental area consisted of a shelter with a ceiling height of 5.2 m, a transparent plastic cover shielded against UV rays, and a black screen on the sides that intercepted 50% of the incident radiation.

The experiment was based on a randomized block design with split plots and four replications per treatment. The main plot was the irrigation management (M), and the subplots were the upland rice cultivars (C). Four upland rice cultivars were evaluated at five irrigation managements: 100% (M1) by keeping the soil moisture close to field capacity (FC) as the reference irrigation

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depth, 70% (M2) and 40% (M3) of the reference irrigation depth at the flowering stage, and 70% (M4) and 40% (M5) of the reference irrigation depth at the grain-filling stage, resulting in an experiment with 80 useful plots plus 20 border plots and 32 canopy temperature reference plots (Total of 132 plots). For M2 and M3, water was withheld from 50% heading, such that the required stress level was reached at the time of flowering and maintained until the end of pollination. After imposing irrigation reductions, plots of M2 and M3 were returned to 100% FC until the last irrigation at the end of the crop cycle. For M4 and M5, water limitation started at the end of pollination and finished at the end of the growing cycle. The periods of water stress differed due to the variation in the phenological development of each cultivar (Supplementary Material Table S2).

The soil type selected was a red-yellow latosol with a sandy-loam texture. The chemical properties of the soil were analyzed before the sowing of the crop and the nutritional management was conducted according to Van Raij *et al.* (1997) recommendations. Nitrogen, phosphate, and potassium mineral fertilizer were applied at the rates of 40 mg N dm^{-3}, 25 mg P_2O_5 dm^{-3}, and 80 mg K_2O dm^{-3}, respectively. All the phosphate was applied in the sowing furrow, while nitrogen and potassium were divided into three soil cover applications (sowing, maximum tillering, and 50% heading).

Seeds of upland rice were manually sown directly on September 1, 2020, in a single row per plot using a seeding rate of 180 seeds per meter. Each plot consisted of a large waterproofed trough with an area of 0.43 m^2 and dimensions of $1.04 \times 0.41 \times 0.76$ m (length, width, and depth). Plants were thinned at 4 and 13 days after emergence, leaving 60 plants per meter row length (60 plants $plot^{-1}$). Weed control was conducted manually throughout the growing cycle, and agro-chemicals were applied to control diseases and pests when necessary.

Irrigation management and micrometeorological measurements

A drip irrigation system was used in this experiment, with self-compensating emitters, anti-siphons, and anti-drainage. A small drip line (1 m long) was installed in each plot with six emitters with a flow rate of 0.6 L h^{-1} spaced at 0.15 m, resulting in a flow rate of 3.6 L h^{-1} per plot. All plots were irrigated individually, controlled through micro-taps installed on a control panel. Irrigation was managed according to soil water matric potential, monitored in four replications of the reference management (RM) (M1) of each cultivar (Supplementary Material Fig. S1). Soil matric potential was measured with a digital portable tensiometer from 16 tensiometer batteries, each battery with three tensiometers installed at 0.10 m, 0.25 m, and 0.35 m depths, providing measurements in the center of three soil layers: 0.0–0.20 m, 0.20–0.30 m, and 0.30–0.40 m. Irrigation for the 100% FC level was computed by adding the water necessary to increase the soil water to field capacity for the two first layers, while the third layer was used for drainage control. Irrigation was carried out whenever the soil water potential fell below −20 kPa at 20 cm depth (Supplementary Material Fig. S1). Volumetric soil water content for each layer before irrigation was estimated from matric potential readings using the van Genuchten soil water retention curve (van Genuchten, 1980). Water depths for M2, M3, M4, and M5 were a fraction of the water applied to the RM plots (M1) of each cultivar.

Measurements of air temperature and relative humidity were recorded with a Vaisala sensor HMP45C-L12 (Campbell Scientific, Logan, Utah, USA) and global solar radiation with a LP02-L12 pyranometer (Campbell Scientific, Logan, Utah, USA). The data were integrated every 10 minutes through an automatic weather station installed inside the greenhouse connected to a CR1000 data-logger (Campbell Scientific, Logan, Utah, USA). For estimating the reference evapotranspiration (ETo), the method of Penman-Monteith was used (Allen *et al.*, 1998).

Canopy temperature and Crop Water Stress Index (CWSI)

Canopy temperature of rice plants was measured using a portable infrared sensor, TIV 6500 (Vonder, Curitiba, Brazil). The measurements were continuously replicated for five readings of each plot at the top of the canopy, which focused on sampling leaves that were fully exposed to the sunlight and with an insertion angle similar in relation to the vertical plane. The measurements were carried out between 11:00 and 13:00 h under clear weather conditions. The time chosen to measure leaf temperature was determined using data from additional plots subjected to an irrigation deficit from 20 days after sowing until the last irrigation (data not shown). Furthermore, these plots made it possible to strengthen the obtaining of the baselines to calculate the CWSI. The CWSI was computed using the formula proposed by Idso (1982):

$$CWSI = \frac{(T_c - T_{air}) - T_{wet}}{T_{dry} - T_{wet}}$$

T_{air} is air temperature (°C), T_c is canopy temperature (°C), T_{wet} is the non-water-stressed baseline (temperature of fully transpiring leaves with open stomata), and T_{dry} is the water-stressed baseline (temperature of non-transpiring leaves with closed stomata). Baselines were calculated following the methodology proposed by Bian *et al.* (2019), where T_{wet} and T_{dry} corresponded to the minimum and maximum difference between T_c and T_{air}, respectively. The CWSI obtained with this methodology is called the 'Observed CWSI' (Costa *et al.*, 2020).

Gas exchange measurements

Leaf net photosynthetic rate (A), transpiration (E), and stomatal conductance (gs) were measured with a portable gas exchange system Li-6400 XT (IRGA/LiCOR-Inc, Lincoln, Nebraska, USA) from 9:00 to 11:00 h on cloudless days. The equipment was set to use concentrations of 400 µmol CO_2 mol^{-1} in the leaf chamber, and the photon flux density photosynthetic active used was 1400 µmol [quanta] m^{-2} s^{-1}. Intrinsic water use efficiency (iWUE) was calculated as the ratio of A to gs based on IRGA measurements. Measurements were taken on three randomly selected flag leaves in each plot at the end of the water stress periods.

Chlorophyll index

The chlorophyll index was determined by averaging five readings per plot using a portable, non-destructive chlorophyll meter, CFL1030 (Falker, Porto Alegre, Brazil), which provides a dimensionless index. Measurements were obtained at the 2/3 position on the youngest fully expanded leaf from the top at the end of the water stress periods of every treatment as indicated by Shrestha *et al.* (2012).

Leaf water potential

Leaf water potential was measured at predawn with a pressure chamber model 3005 (Soil Moisture, Santa Barbara, California, USA). One flag leaf was sampled from each plot at the end of the irrigation treatments. These samples were placed in appropriate containers with ice for transportation to the laboratory to be processed in the chamber as soon as possible.

Yield, yield components, and WUE

At physiological maturity, plants from the center of the row of each plot were harvested (0.22 m^{-2}). For aerial dry matter determination, the plants of each plot were separated into straw and panicles, then dried at 65° C in an oven with forced air circulation for three days and weighed. Each panicle was hand-threshed, and the unfilled spikelets were separated from the filled spikelets with a blower. The leaf weight, number of panicles per plant, spikelets per panicle (SPN), filled grain percentage, 1000-grain weight, and grain yield (GY) were obtained. WUE (kg m^{-3}) was calculated as the ratio of GY to the total volume of water applied.

Table 1. Weather records inside the greenhouse during the experimental period

Month	Temperature (°C)		Solar Radiation ($MJ\ m^{-2}\ day^{-1}$)	Average relative humidity (%)	ETo PM56 ($mm\ day^{-1}$)
	Maximum	Minimum			
September	36.8	16.8	9.4	59.6	3.6
October	35.4	18.5	10.1	65.3	3.7
November	35.0	17.3	12.9	66.5	4.4
December	34.8	20.1	11.2	74.0	3.8
January	35.7	21.0	10.8	74.3	3.8
Mean	35.5	18.7	10.9	67.9	3.9

ETo, reference evapotranspiration.

Statistical analysis

All the statistical analyses were performed with the R software (http://www.r-project.org). Physiological traits were analyzed by three-way ANOVA for linear mixed models with irrigation management and cultivar as fixed effects and phenological stage as a random effect using the R package 'lmerTest' (Bates *et al.*, 2015). Means of physiological parameters were tested by pairwise comparisons through the Tukey test using the R package 'emmeans' (Lenth, 2019). GY, grain yield components, and WUE were analyzed by two-way ANOVA, and the means were compared by the Fisher's least significant difference test at the 5% probability level using the R package 'ExpDes' (Ferreira *et al.*, 2013).

Results and Discussion

Weather conditions and water demand

The mean maximum and minimum air temperatures throughout the growing cycle were 35.5 and 18.7 °C (Table 1), respectively. The average maximum air temperature was just little higher than optimal for rice growth, particularly during booting and flowering (Shah *et al.*, 2011). The average reference evapotranspiration from sowing to maturity (period between 121 days in BRS Serra Dourada to 141 days in Rio Paraguai) was 3.9 mm day^{-1}.

The cumulative reference irrigation ranged from 792 mm in BRS Serra Dourada to 1148 mm in Rio Paraguai (Figure 1), which is consistent with the studies of Kato *et al.* (2009), Heinemann *et al.* (2017), and Alou *et al.* (2018) in Japan, Brazil, and South Africa, respectively. The water depletion for M2, M3, M4, and M5 was on average 58, 121, 51, and 103 mm, respectively, compared to well-irrigated management (M1).

The physiological response to deficit irrigation

The individual effect of irrigation management and cultivar was significant for all physiological traits, whereas the interaction effect of irrigation management × cultivar was significant for net photosynthesis rate (A), transpiration (E), leaf water potential (LWP), and the chlorophyll index (Supplementary Material Table S3).

Deficit irrigation at flowering (M2 and M3) resulted in a significant decrease in A, gs, and E for all cultivars, except for gs in Rio Paraguai which was low even under the full irrigation management (Figure 2A, C, E). At the grain-filling stage, A, gs, and E were statistically the same under moderate water stress (Figure 2B, D, F) compared to the RM (M1) in BRS Esmeralda, BRS Serra Dourada, and Rio Paraguai, whereas these parameters were reduced in BRS A501 CL. Furthermore, at grain-filling, A, gs, and E under severe water stress (M5) decreased for all cultivars and to a greater extent in BRS A501 CL. The reduction in A, E, and gs due to drought stress at

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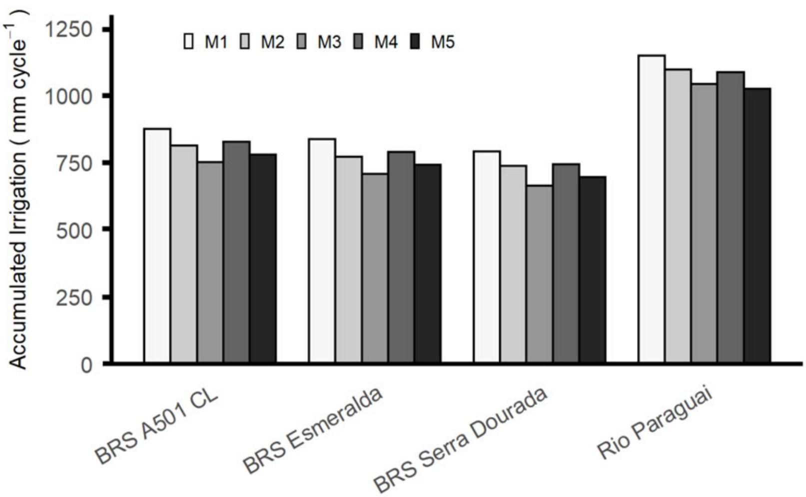

Figure 1. Cumulative irrigation during the growing season for four upland rice cultivars subjected to five irrigation managements. M1, 100% of the field capacity considered the reference management (RM); M2, 70% of the RM at the flowering stage; M3, 40% of the RM at the flowering stage; M4, 70% of the RM at the grain-filling stage; M5, 40% of the RM at the grain-filling stage.

flowering and grain-filling is consistent with previous studies in lowland and upland rice (Dingkuhn *et al.*, 1989; Vijayaraghavareddy *et al.*, 2020). Irrespective of irrigation management and cultivar, values for A, gs, and E were higher during the grain-filling stage (Figure 2B, D, F), which corresponds with the lower GY penalty under water stress imposed at this stage. However, this needs further validation since previous studies showed that plants subjected to drought stress in the late phenological stage can use carbohydrates that were built up during pre-anthesis (Jagadish *et al.,* 2015; Sehgal *et al.*, 2018). Pooled data revealed that BRS A501 CL achieved the highest GY (Table 2), mainly under severe water stress, but this cultivar recorded the lowest values for A, gs, and E among the modern cultivars. This could be because higher leaf gas exchange parameters may not necessarily promote higher productivity of Brazilian upland rice cultivars (Alvarez *et al.*, 2015; Lanna *et al.*, 2020).

The iWUE increased either significantly or non-significantly under moderate withholding irrigation (M2) at flowering or severe withholding irrigation at the grain-filling stage (M5) (Figure 2G, H). However, results differed from the research of Yang *et al.* (2019), which linked drought tolerance to cultivars with higher iWUE. For example, the highest iWUE during flowering was recorded for Rio Paraguai, but this cultivar was the most affected by moderate and severe water stress at this stage, reducing GY by 77 and 94%, respectively. These differences could be produced by morpho-physiological mechanisms and spatio-temporal variations (Blum, 2009; Medrano *et al.*, 2015), which could be another topic of interest.

The LWP of the four cultivars decreased under severe drought stress at flowering and grain-filling stages (M3 and M5) compared with M1 (Figure 3A, B), which is consistent with the study of Kumar *et al.* (2017) on lowland rice. Therefore, severe drought can affect LWP regardless of the genetic constitution of varieties. Under M3, the LWP ranged between −1.0 MPa in BRS Esmeralda and −1.4 MPa in Rio Paraguai, whereas under M5, the LWP ranged between −1.4 MPa in BRS A501 CL and −1.9 MPa in Rio Paraguai. Under moderate stress (M2 and M4), LWP was statistically equal to the RM both at flowering and grain-filling for all cultivars, except for Rio Paraguai at flowering (−1.3 MPa), which decreased similarly to that under severe stress (Figure 3A). This could be because traditional cultivars function with their stomata more closed than modern

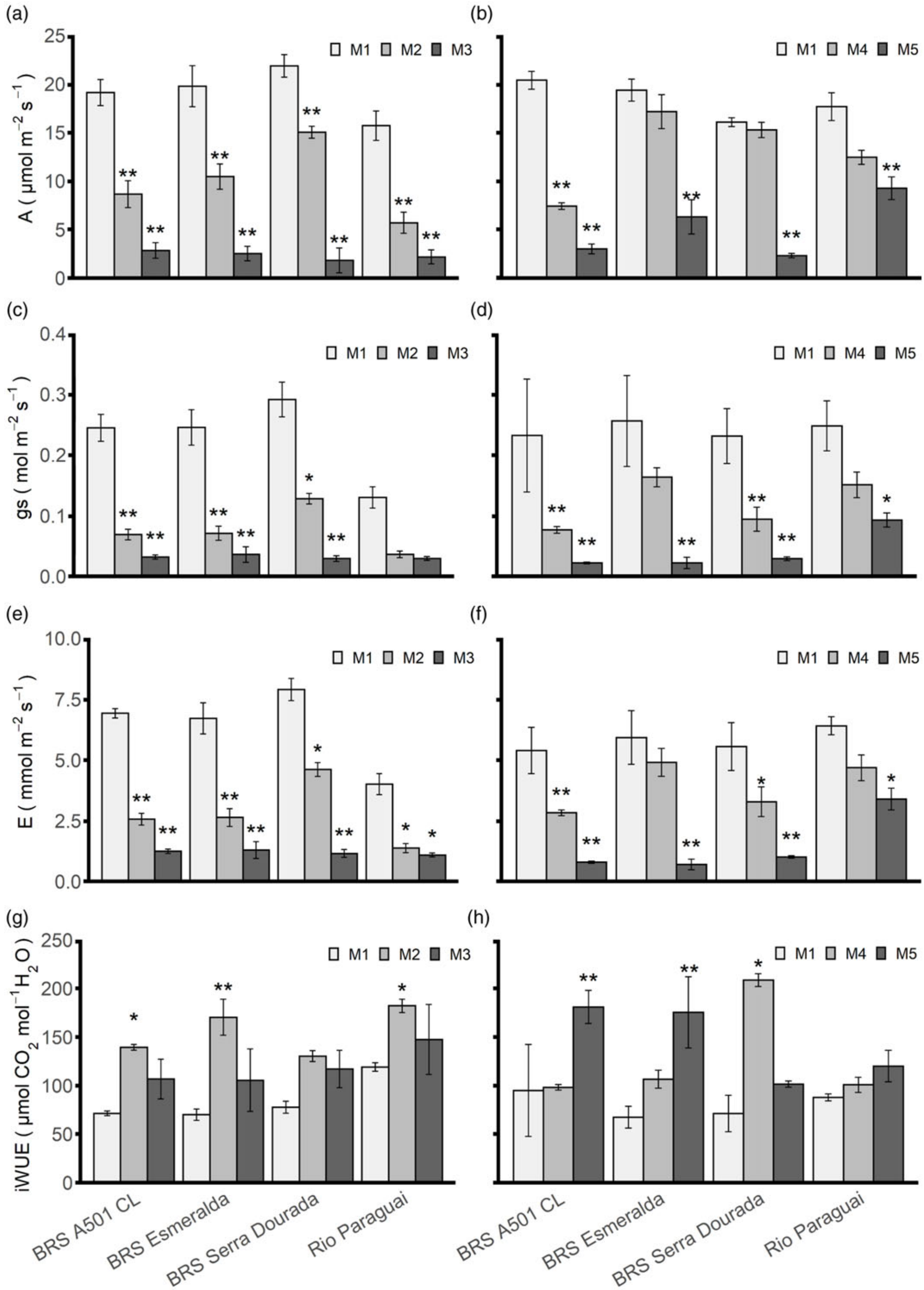

Figure 2. Gas exchange traits of four upland rice cultivars subjected to five irrigation managements. Data indicate mean ± SE ($n = 4$). A, Photosynthetic rate (A) at flowering stage. B, A at grain-filling stage. C, Stomatal conductance (gs) at flowering stage. D, gs at grain-filling stage. E, Transpiration (E) at flowering stage. F, E at grain-filling stage. G, Intrinsic water use efficiency (iWUE) at flowering. H, iWUE at grain-filling stage. * and ** indicate significant differences from M1 at the 0.05 and 0.01 levels within cultivars, respectively. M1, 100% of the field capacity considered the reference management (RM); M2, 70% of the RM at the flowering stage; M3, 40% of the RM at the flowering stage; M4, 70% of the RM at the grain-filling stage; M5, 40% of the RM at the grain-filling stage.

Table 2. Yield and yield components for four upland rice cultivars subjected to five irrigation managements

Yield component	Irrigation management	Upland rice cultivars				
		BRS A501 CL	BRS Esmeralda	BRS Serra Dourada	Rio Paraguai	Mean
Grain yield (Mg ha^{-1})	**M1**	9.3aA	8.3aA	9.2aA	5.2aB	8.0a
	M2	6.7bcAB	5.5bB	7.7aA	1.2bC	5.3c
	M3	5.0cdA	3.2cB	2.1bBC	0.3bC	2.7d
	M4	7.5abA	6.7abA	7.9aA	3.8aB	6.5b
	M5	4.7dA	2.8cB	3.4bAB	1.8bB	3.2d
	Mean	6.7a	5.3b	6.1ab	2.5c	
Spikelets per panicle	**M1**	122.7aB	165.0aA	158.2aA	106.9aB	138.2a
	M2	105.5aB	139.2aAB	142.0aA	63.2bcC	112.5b
	M3	110.0aA	140.6aA	109.4bA	62.9cB	105.7b
	M4	126.3aAB	156.5aA	157.1aA	94.0abB	133.5a
	M5	124.3aAB	157.8aA	135.4abA	101.1aB	129.7a
	Mean	117.7B	151.8A	140.4A	85.6C	
Filled grain percentage (%)	**M1**	78.8aA	69.8aA	78.4aA	59.5aA	71.6a
	M2	64.5abcAB	52.2bcB	73.6aA	30.4bC	55.2b
	M3	54.0cA	36.1cdB	26.9bB	6.0cC	30.7c
	M4	71.4abA	63.3abAB	74.5aA	50.2aB	64.8a
	M5	56.0bcA	32.5dB	37.1bB	30.1bB	38.9c
	Mean	64.9A	50.8B	58.1AB	35.2C	
1000-grain weight (g)	**M1**	23.1aB	22.2aB	19.5aC	30.0aA	23.7a
	M2	22.5aB	22.7aB	19.0aC	25.9bA	22.5ab
	M3	21.5aB	21.0aB	18.2aC	28.1abA	22.2ab
	M4	20.9aBC	22.0aB	18.9aC	28.7aA	22.6ab
	M5	17.7bB	20.0aB	17.9aB	23.4cA	19.7b
	Mean	21.2B	21.6B	18.7C	27.1A	

Means followed by distinct lowercase letters within a column and distinct capital letters within a row are different by the LSD test at 0.05 significance. M1, 100% of the field capacity considered the reference management (RM); M2, 70% of the RM at the flowering stage; M3, 40% of the RM at the flowering stage; M4, 70 % of the RM at the grain-filling stage; M5, 40 % of the RM at the grain-filling stage.

cultivars under moderate decreases in soil moisture (Heinemann *et al.*, 2011). Accordingly, modern Brazilian cultivars are demonstrating an effective tolerance to moderate drought stress.

The CWSI is used to quantify water stress in plants and ranges from 0 (no water stress) to 1 (extreme water stress). The CWSI under moderate stress at flowering and grain-filling (M2 and M4) did not differ statistically compared to M1 (Figure 3C, D). However, severe stress at flowering (M3) increased CWSI in BRS A501 CL and BRS Serra Dourada, and at grain-filling (M5) in BRS Esmeralda and BRS Serra Dourada compared to M1. The maximum CWSI values under severe stress were obtained in Rio Paraguai and BRS Serra Dourada at flowering (CWSI = 0.76) and in BRS Serra Dourada at grain-filling (CWSI = 0.83). In general, these cultivars recorded greater yield penalties under severe stress, which is consistent with the study of Olalekan *et al.* (2022) who reported that upland rice cultivars with warmer canopies under drought stress conditions exhibit low GY. This could be because high CWSI values affect canopy photosynthesis and hence GY (Biju *et al.*, 2018).

Severe drought stress reduced the chlorophyll index values for BRS A501 CL and BRS Esmeralda, but this reduction was significant only at the grain-filling stage (Figure 3E, F). In contrast, BRS Serra Dourada and Rio Paraguai, subjected to severe stress, maintained the chlorophyll index values compared to the RM. The chlorophyll index is frequently used to evaluate drought tolerance since plants under environmental stress lose their green chlorophyll tissues (Vijayaraghavareddy *et al.*, 2020). However, rice genotypes that have a substantial reduction in stomatal conductance (traditional cultivars) tend to maintain chlorophyll index values under water stress (Singh *et al.*, 2017).

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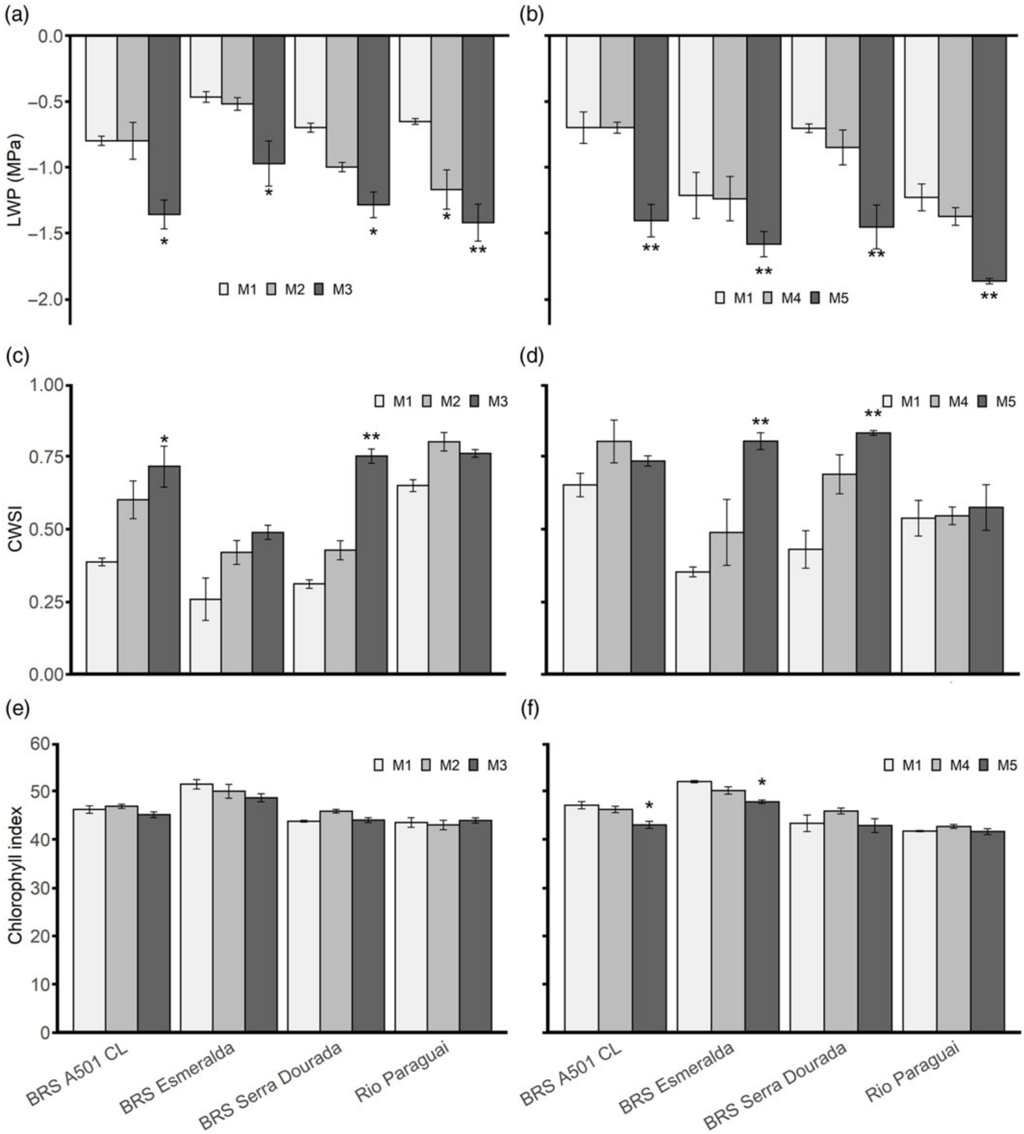

Figure 3. Leaf water potential (LWP), crop water stress index (CWSI), and the chlorophyll index of four upland rice cultivars subjected to five irrigation managements. Data indicate mean ± SE ($n = 4$). A, LWP at flowering stage. B, LWP at grain-filling stage. C, CWSI at flowering stage. D, Chlorophyll index at grain-filling stage. E, Chlorophyll index at flowering stage. F, LWP at grain-filling stage. * and ** indicate significant differences from M1 at the 0.05 and 0.01 levels within cultivars, respectively. M1, 100% of the field capacity considered the reference management (RM); M2, 70% of the RM at the flowering stage; M3, 40% of the RM at the flowering stage; M4, 70% of the RM at the grain-filling stage; M5, 40% of the RM at the grain-filling stage.

Effects of deficit irrigation on GY, grain yield components, and WUE

The individual effect of irrigation management and cultivar was significant for GY, grain yield components, and WUE whereas the interaction effect of irrigation management × cultivar was significant for filled grain percentage (FG), 1000-grain weight (TGW), GY, and WUE (Supplementary Material Table S4).

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Moderate water stress at flowering (M2) caused a significant reduction in the GY of each cultivar except for BRS Serra Dourada, whereas severe stress at flowering (M3) reduced GY for all cultivars (Table 2). Drought stress at flowering reduced GY to a greater extent in the traditional cultivar (Rio Paraguai). For example, when moderate stress occurred, GY of Rio Paraguai was reduced by 76.9%, compared with 28.0, 33.7, and 16.3% in BRS A501 CL, BRS Esmeralda, and BRS Serra Dourada. These differences were expected since traditional cultivars limit GY by early stomatal closure (Heinemann *et al.*, 2011). Moderate water stress at grain-filling (M4) caused a significant reduction in the GY of Rio Paraguai, whereas severe stress at grain-filling (M5) reduced GY for all cultivars (Table 2). Drought stress at grain-filling reduced GY to a lesser extent in BRS A501 CL. For example, when severe stress occurred, the GY of BRS A501 CL was reduced by 49.5%, compared with 66.3, 63.0, and 65.4% in BRS Esmeralda, BRS Serra Dourada, and Rio Paraguai. The differences of GY between modern cultivars in response to water stress could be explained by the different genetic constitutions of their parents (Lanna *et al.*, 2020). In the current experiment, the highest yield was obtained under the RM (M1), with an average of 8.0 Mg ha^{-1} (Table 2). Similar results were obtained for upland rice under aerobic conditions irrigated by sprinkler systems in Japan (Kato and Katsura, 2014).

Moderate and severe stress introduced at flowering reduced the number of SPN by 19 and 24%, respectively, whereas when stress occurred at grain-filling, SPN was the same as for the RM (Table 2). There were no significant changes in the filled grain percentage (FG) under moderate stress compared with the RM, except for BRS Esmeralda and Rio Paraguai at flowering, which reduced FG by 52 and 30%, respectively (Table 2). However, severe water stress resulted in a great reduction of FG in all cultivars, to a greater extent in Rio Paraguai, which reduced FG at flowering to 5% and at the grain-filling stage to 30%. When water stress was imposed at flowering, 1000-grain weight was similar between the reference (M1) and stress treatments (M2 and M3), whereas when severe stress occurred at grain-filling, TGW was reduced by 23% in BRS A501 CL and Rio Paraguai (Table 2).

Decreases in GY when stress was applied at flowering (M2 and M3) were mainly associated with low spikelet fertility (low filled grain percentage) and low spikelet number (Table 2). This could be because water stress during flowering in rice can decrease yield due to incomplete panicle exertion and poor anther dehiscence, which reduces spikelet fertility and produces grain abortion in the early stages following fertilization (Barnabás *et al.*, 2008). In addition, low filled grain percentage may be caused by temperature (Table 1), as reported by Shah *et al.* (2011) and Sharma *et al.* (2018), who indicated that temperatures for rice during flowering above 33 °C are critical. Reduction of GY when stress was applied at grain-filling (M4 and M5) was mainly associated with low filled grain percentage (severe stress) and low 1000-grain weight (Table 2). According to Boonjung and Fukai (1996) and Vijayaraghavareddy *et al.* (2020), stress at the grain-filling stage causes a reduction in photosynthetic rate as a consequence of leaf rolling and leaf death, as well as negative source-sink interactions, harming spikelet fertility, and lowering the level of assimilates needed to fill grains.

The WUE across treatments ranged from 0.16 to 1.75 kg m^{-3} (Figure 4). Moderate stress at flowering (M2) reduced WUE in BRS A501 CL, BRS Esmeralda, and Rio Paraguai by 22, 29, and 76%, respectively. However, WUE under moderate stress at grain-filling (M4) was similar to the WUE of the RM (M1) in all cultivars, averaging 1.2 and 1.4 kg m^{-3}, respectively. Severe stress (M3 and M5) decreased WUE for all cultivars, with the greatest reduction in Rio Paraguai by 94% when stress occurred at flowering and the lowest reduction in BRS A501 CL by 43% when stress occurred at grain-filling. WUE reductions under drought stress in this trial suggest that the crop was less efficient as water inputs were reduced, which is consistent with the studies of Zhao *et al.* (2004) and Alou *et al.* (2018), who demonstrated that even light stress at critical stages (reproductive or terminal drought) cannot improve WUE in upland rice. Under the RM (M1), all cultivars presented the highest WUE, but Rio Paraguai differed from the modern cultivars. The WUE under well-watered conditions in Rio Paraguai was 0.72 kg m^{-3}, compared with 1.50, 1.62,

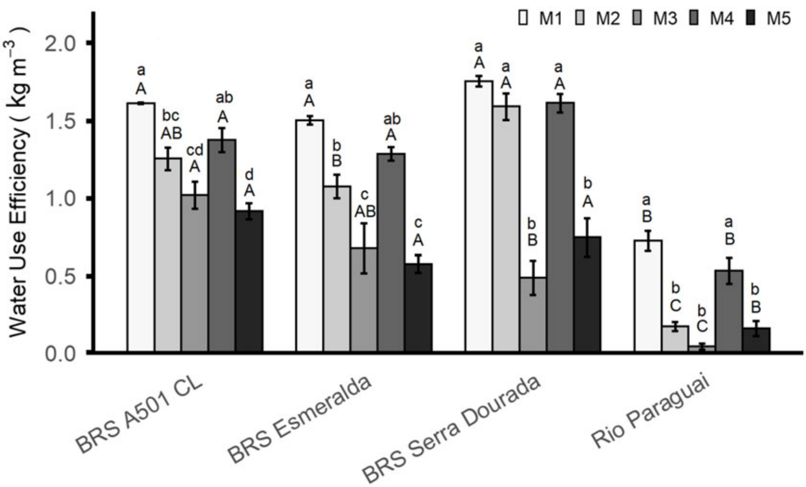

Figure 4. Water use efficiency of four upland rice cultivars subjected to five irrigation managements. Data indicate mean ± SE ($n = 4$). Distinct lowercase letters within a variety and distinct capital letters within an irrigation management are different by the LSD test at 0.05 significance. M1, 100% of the field capacity considered the reference management (RM); M2, 70% of the RM at the flowering stage; M3, 40% of the RM at the flowering stage; M4, 70% of the RM at the grain-filling stage; M5, 40% of the RM at the grain-filling stage.

and 1.75 kg m^{-3} in BRS Esmeralda, BRS A501 CL, and BRS Serra Dourada, respectively. Thus, the WUE of modern cultivars under full irrigation was higher than WUE values reported for upland rice systems (Alou *et al.*, 2018; Kumar *et al.*, 2017), where water replacement is commonly performed when the soil moisture tension in the root zone reaches −50 kPa (O'Toole and Moya, 1981). Yet, the WUE values found in this trial were similar to those of the aerobic rice systems (Bouman *et al.*, 2007; Tao *et al.*, 2015), where water in the root zone is managed in the range of −10 to −30 kPa (Belder *et al.*, 2005). Similar conditions were adopted in this trial where upland rice was subjected to high-frequency irrigation, and the seasonal mean soil moisture tensions ranged from −13 to −15 kPa at 10 cm depth.

Conclusions

Modern Brazilian upland rice cultivars maintained higher yields (GY) and WUE under temporal water stress compared to a traditional reference cultivar, since these new cultivars were less affected by the negative effects of deficit irrigation on net photosynthetic rate, stomatal conductance, transpiration, leaf water potential, and CWSI, indicating that breeding programs have also improved drought resistance.

The study indicated the importance of attending to the full water demand with precision drip irrigation to meet the highest GY and WUE of upland rice, more so in modern cultivars; the best rice cultivar recorded a GY of 9.3 Mg ha^{-1} and a WUE of 1.62 kg m^{-3}. When moderate stress was applied at grain-filling, GY and WUE were minimally affected, whereas severe stress reduced GY and WUE for all cultivars.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0014479722000205

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Conflict of Interest. None.

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