University of São Paulo
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Water use efficiency of upland rice and common bean under precision drip irrigation

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Dissertation presented to obtain the degree of Master in Science. Area: Agricultural Systems Engineering

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Advisor:
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In memory of my grandfather, Eliodoro.
I dedicated

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To God for the wisdom and strength he gave me to face this study period.

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¨There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle. ¨

Albert Einstein

SUMMARY

RESUMO

Eficiência do uso da água de arroz de terras altas e feijão comum sobre irrigação de precisão por gotejamento

O presente trabalho teve como objetivo quantificar a eficiência do uso da água do arroz de terras altas (*Oryza sativa* L.) e do feijão comum (*Phaseolus vulgaris* L.) sob manejo de irrigação de precisão, bem como as respostas morfológicas e fisiológicas destas culturas a situações de seca. Os experimentos foram realizados em um ambiente protegido localizado no Departamento de Engenharia de Biossistemas (LEB), Universidade de São Paulo (ESALQ), Piracicaba - SP, Brasil. No experimento de arroz de terras altas, foram testadas quatro cultivares e os níveis de reposição de água foram de 70 e 40% CC, os quais foram impostos em duas fases fenológicas: floração e enchimento de grãos. No experimento do feijão comum, os níveis de reposição de água foram 75 e 50% CC e as estratégias de irrigação incluíram períodos de longo prazo (20 DAS até o fim do ciclo de cultivo) e períodos de curto prazo (fases vegetativas e de floração). O manejo da irrigação foi baseado em medições de tensiometria em um tratamento testemunha (100% da capacidade do campo). As reduções dos demais tratamentos foram frações do tratamento testemunha. Em condições de irrigação plena, a eficiência do uso da água (WUE) para o arroz de terras altas e feijão comum foi em média de 1,4 e 1,6 kg m^{-3}, respectivamente. A WUE para o arroz de terras altas foi superior aos valores tradicionais reportados na literatura, enquanto que para o feijão comum foi semelhante aos valores reportados para esta cultura sob irrigação por gotejamento. A WUE não foi melhorada em nenhum experimento sob estratégias de irrigação deficitária. O estresse hídrico provocou diminuições significativas no rendimento e componentes de rendimento do arroz de terras altas e do feijão comum. Além disso, os caracteres fisiológicos foram afetados pela retenção de irrigação em maior medida com os níveis mínimos de reposição. A WUE derivada das medições de trocas gasosas não esteve correlacionada com a WUE medida no final do ciclo de cultivo. Em conclusão, a eficiência do uso de água pode ser aumentada com o uso de irrigação de precisão, enquanto que os déficits de irrigação podem afetar a WUE e a produtividade dos grãos tanto no arroz de terras altas como no feijão comum.

Palavras-chave: *Phaseolus vulgaris*, *Oryza sativa*, Déficit hídrico, Fisiologia, Rendimento de grão

ABSTRACT

Water use efficiency of upland rice and common bean under precision drip irrigation

The objective of this study was to quantify the water use efficiency of upland rice (*Oryza sativa* L.) and common bean (*Phaseolus vulgaris* L.) under precision irrigation management as well as the morphological and physiological responses of these crops to drought situations. The experiments were carried out under rain shelter conditions at the Biosystems Engineering Department (LEB), Sao Paulo University (ESALQ), Piracicaba – SP, Brazil. In the upland rice experiment, four cultivars were tested and water depletion levels were 70 and 40%, which were imposed at two phenological stages: flowering and grain filling. In the common bean experiment, water depletion levels were 75 and 50% and irrigation strategies included long-term periods (20 DAS until the end of the crop cycle) and short-term periods (vegetative and flowering stages). Irrigation management was based on tensiometry measurements in control plots (100% of the field capacity). Irrigation depth reductions were a fraction of this control management. Under well-watered conditions, water use efficiency (WUE) for upland rice and common bean was on average of 1.4 and 1.6 kg m^{-3}, respectively. WUE for upland rice was higher than traditional values reported in the literature, whereas for common bean it was similar to reported values for this crop under drip irrigation. WUE was not improved in any experiment under deficit irrigation strategies. Water stress occasioned significant decreases in grain yield and grain yield components of upland rice and common bean. Also, physiological traits were affected by irrigation withholding to a greater extent with the least replenishment levels. WUE derived from gas exchange measurements was not correlated with WUE measured at the end of the crop cycle. In conclusion, water use efficiency can be increased with the use of precision irrigation management, whereas irrigation deficits can affect WUE and grain productivity in both upland rice and common bean.

Keywords: *Oryza sativa*, *Phaseolus vulgaris*, Water deficit, Physiology, Grain yield

1. INTRODUCTION

Irrigated agriculture is the largest user of all water resources and consumes over 70% of available fresh water (Ritchie and Roser, 2017). However, water resources for agriculture range between more than 80% in many developing countries and less than 60% in developed countries (Singh, 2014). 23% of the total area cultivated is irrigated and it provides more than 40% of agricultural production (Comas et al., 2019). Irrigation and nutrient input intensification will be vital for food security, increasing global crop production volumes by up to 150% (Folberth et al., 2020), though this growth projection may be affected by water scarcity in many regions.

Among the factors causing water shortages are the loss of soil water resources due to deforestation, run-off patterns associated with the use of water, and the impact of climate change on rainfall distribution (Rockstrom et al., 2009). Strategies to deal with water scarcity include desalination of saline water, re-use of wastewater, virtual water and food trade, increasing agricultural yields, and improving water use efficiency in agriculture (Falkenmark, 2013). Among these strategies, improving water use efficiency may be a key to meeting current and future demands for food, fibers, and bioenergy (Bouman et al., 2007; Coelho, 2021). Water use efficiency is an indicator of physiological, agronomic, and management practices (Kang et al., 2021). Initially, this term was introduced for deficit irrigation strategies and is defined as the ratio of the mass of marketable yield to the volume of water consumed by the crop (Geerts and Raes, 2009).

Numerous studies have reviewed the approaches to improving water use efficiency in agriculture (Stanhill, 1986; Calvache et al., 1997; Lafitte et al., 2006; Blum, 2009; Geerts and Raes, 2009; Kumar et al., 2017). The gap between actual and potential water use efficiency includes low-efficient irrigation technology, lack of precise control of field water use, and an imbalance between cropping areas and water resources (Kang et al., 2017). It is necessary to consider these factors as a whole to promote water-saving strategies in agriculture (Liu et al., 2021). Therefore, cooperation between crop physiologists, irrigation scientists, and irrigation engineers has led to a series of new irrigation strategies and technologies (Kang et al., 2021).

In plant breeding, water use efficiency has been proposed to identify water-use efficient genotypes in situations of heat, water deficit, and interactions among them (Condon et al., 2004; Hatfield and Dold, 2019). Water use efficiency involves different spatial scales, from molecular to field levels, and temporal scales, from seconds to growth season (Condon, 2020). Water use efficiency is commonly measured at the leaf level by facilities using portable leaf gas exchange equipment (Condon et al., 2004). Nevertheless, when these measurements are compared with whole-plant estimates of water use efficiency, there is no correlation (Medrano et al., 2015; Nadal and Flexas, 2019). In addition, improvements in leaf level water use efficiency may not translate into higher crop yields (Condon et al., 2004) and inconsistences are more accentuated under water stress conditions (Flexas et al., 2010; Kang et al., 2017). For these reasons, it has been difficult to carry out selection programs for water use efficiency (Medrano et al., 2015).

In this work, two of the main crops produced in the world (rice and common beans) were considered. For these crops, across South America, a decrease in climatic suitability has been projected, principally due to heat and drought stress (Heinemann et al., 2017b; Ramirez-Villegas et al., 2018). Thus, there are important strategies to cope with drought in the regions of these crops. In this way, Hatfield and Dold (2019) concluded that under climate change, increasing water use efficiency will result on two fronts. First, identification of techniques that can be used for phenotypic screening relative to water use efficiency, and second, adoption of management practices that will reduce soil water evaporation and shift the water use by the crop to more crop transpiration. These strategies are defined as

effective use of water and they are the major engine for agronomic and genetic improvement of crop production under limited water conditions (Blum, 2009).

Irrigation technologies can not be integrated into the genetic material because they depend on human interaction (Coelho, 2021). These technologies are intensive on information and require efforts of rural extension to field implementation. It has been demonstrated that irrigation technologies have a positive effect on water use efficiency, for example, irrigation methods such as drip and micro-sprinkler irrigation (Hatfield and Dold, 2019), adjusting planting density (Heinemann et al., 2017a), application of plastic and straw mulch (Abd El-Wahed et al., 2017), and deficitary irrigation (Mathobo et al., 2017).

High-efficient water use also includes the knowledge of exact crop life water requirements (Kang et al., 2021). Water requirement is defined as the sum of transpiration and soil evaporation (Allen et al., 1998). Plant transpiration is affected by water content in the root zone, leaf stomatal closure, and atmospheric vapor pressure deficit (Serraj et al., 2009; Gupta et al., 2020). Soil evaporation is controlled by meteorological factors, leaf canopy coverage and soil moisture (Boonjung and Fukai, 1996; Lozano-Parra et al., 2018). Methods for determining crop water requirements are based on climatological, plant and soil measurements (Kang et al., 2021). Irrigation scheduling based on the variation of in-situ soil water content is widespread due to advanced technologies in soil moisture sensing devices. Among soil moisture sensors, tensiometers are a reliable and cheap alternative to scheduling irrigation because they work correctly in the wet range (-1 to -50 KPa) (Coelho, 2021).

In this work, different strategies to improve water use efficiency in common bean and upland rice cultivars were considered. Thus, the objective of this research was to quantify the water use efficiency of crops under precision drip irrigation management. In addition, some physiological traits were included to evaluate drought responses.

The two chapters of this dissertation are:

Chapter I:

Water use efficiency of contrasting upland rice cultivars.

Chapter II:

Water use efficiency of common bean subjected to water stress during different growth stages under drip irrigation.

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2. WATER USE EFFICIENCY OF CONTRASTING UPLAND RICE CULTIVARS

ABSTRACT

Water scarcity associated with global warming requires the adoption of new water-saving strategies as well as upland rice cultivars adapted to this scenario. Although multiple efforts have been made, water consumption in rice cultivation remains controversial. The objective of this study was to quantify the water use efficiency of contrasting upland rice cultivars under precision irrigation with full and deficit irrigation strategies, as well as morphological and physiological responses to drought stress. A rain shelter experiment was conducted using a randomized block design with a split-plot arrangement of treatments with four replications. Three modern and one traditional cultivar were subjected to five irrigation managements: 100% of the field capacity (FC), 70 and 40% FC at the flowering stage, and 70 and 40% FC at the grain-filling stage. Yield and yield components were affected by water deficit irrigation to a greater extent in the traditional cultivar. Drought stress resulted in a reduction in photosynthetic rate, stomatal conductance and transpiration, and an increase in intrinsic water use efficiency. Leaf water potential, SPAD and crop water stress index were also affected by withholding irrigation. Under well-watered conditions, water use efficiency ranged between 0.7 and 1.75 kg m^{-3}, higher than traditional values in the literature. However, the water use efficiency decreased for all cultivars under severe stress. The principal component analyses showed that intrinsic water use efficiency had no relation with water use efficiency, whereas the crop water stress index, photosynthetic rate, stomatal conductance, and transpiration were closely related to grain yield. In conclusion, increasing water use efficiency in upland rice cultivars is possible nowadays under precision irrigation, although the effects of severe water stress at flowering and grain-filling stages cause great reductions in water use efficiency and productivity.

Keywords: *Oryza sativa*, Precision irrigation, Brazilian cultivars, Deficit irrigation, Grain yield, Photosynthesis, Principal component analysis

2.1. Introduction

Upland rice (Oryza sativa L.) is cropped in a rainfed system on unsaturated and well-drained soils (Kato and Katsura, 2014). Nearly eight percent of the world's rice area is upland rice and the distribution in Asia, Latin America and sub-Saharian Africa is approximately 65, 10 and 25% of the total area, respectively (Saito et al., 2018). Hardly ever, this crop grows under supplementary irrigation, confirming the importance of rainfall (Crusciol et al., 2021). It is not different in central Brazil, the largest area of upland rice cultivation in this country, where productivity is unstable because of the vulnerability of the region to water scarcity. Consequently, in the last decades, the upland rice cropped area has been reduced by up to 60% (Pinheiro et al., 2006). However, upland rice can be integrated into crop rotations with maize and soybean (Pacheco et al., 2017; Nascente and Stone, 2018), allowing the use of irrigation by center-pivot, or to a lesser extent, of subsurface drip irrigation (Sano, 2013).

Coupled with climate change, water scarcity may result in an increased incidence of crop drought stress (Liu et al., 2015; Kang et al., 2017). Heinemann et al. (2015) suggest that upland rice breeding strategies have to include spatio-temporal considerations and specific characteristics of drought. Furthermore, Bouman et al. (2007) and Coelho (2021) emphasize the increase of crop productivity per unit of water required by introducing new water-management technologies. New water-management technologies are associated with the effective use of water as the major engine for agronomic and genetic improvement (Blum, 2009). Despite research efforts on water-saving in rice, such as

alternate wetting and drying and aerobic systems, available information is still limited (Kato and Katsura, 2014; Alou et al., 2018). Therefore, it may be interesting to include technology that increases water use efficiency considering a possible future scenario of drought for central Brazil, as proposed by Ramirez-Villegas et al. (2018), in response to reproductive drought stress only, as well as reproductive and terminal drought stress.

Drought stress in upland rice reduces the yield potential by up to 35% (Heinemann et al., 2015). According to Kumar et al. (2014), the sensitivity of rice to water stress depends on the duration, severity, genotype, and growth stage. The reduction of water availability and diminished leaf water potential characterize water stress potential, affecting rice yield components (Boonjung and Fukai, 1996); drought also affects the normal plant physiological process (Jaleel et al., 2009); and upland rice physiological responses are poorly understood in comparison to lowland rice responses (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, like 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) (Kato et al., 2006). 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 (Kato et al., 2006; Alou et al., 2018; Luo et al., 2019), making it difficult to develop drought-tolerant cultivars.

It was hypothesized that upland rice cultivars grown under precision irrigation increase reference literature values of water use efficiency. Thus, the goal of this study was to quantify the water use efficiency of four upland cultivars under a high-precision irrigation management subjected to full and deficit irrigation. Three modern upland rice cultivars, including one recently bred herbicide-resistant cultivar, and one traditional cultivar were used. In addition, to provide information to assess the feasibility of adaptation of upland rice cultivars under different drought conditions, morphoagronomic and physiological traits were evaluated.

2.2. Materials and methods

2.2.1. Plant materials, growth conditions and drought stress managements

Four upland rice genotypes were obtained from the *National Research Center for Rice and Beans* germplasm bank, Embrapa, Brazil. The cultivars evaluated were Rio Paraguai, BRS Esmeralda, BRS A501 CL and BRS Serra Dourada. BRS Esmeralda, BRS A501 CL and BRS Serra Dourada are classified as modern cultivars, whereas Rio Paraguai is known as a traditional cultivar, which means that this cultivar is no longer used by the Brazilian breeding program (Additional file 1).

The experiment was carried out in a greenhouse under rain shelter conditions at the Biosystems Engineering Department (LEB), Sao Paulo University (ESALQ), Piracicaba – SP, Brazil (22° 42' 32" S, 47° 37' 45" W and 548 m altitude). The greenhouse consisted of three contiguous spans and a ceiling height of 5.2 m. The upland rice trial was installed in the middle span while the rest of the area was occupied by irrigated soybean experiments.

In a useful area of 130 m^2, 132 plots were used (Fig. 1), each with a volume of 0.33 m^3 and dimensions of 1.04 x 0.41 x 0.76 m (length, width and depth) filled with soil classified as red-yellow latosol with a sandy-loam texture. Plots were sown on the first of September of 2020, using 180 seeds per meter, and emergence occurred on the 8th of September. Thinning was done two times 4 and 13 days after emergence (DAE) to maintain 60 plants per plot. The

soil was analyzed before the sowing of the crop and the nutritional management was conducted according to Van Raij (1997) recommendations. Nitrogen, phosphate and potassium fertilizer were applied at the rates of 80 kg N ha^{-1}, 50 kg P$_2$O$_5$ ha^{-1} and 160 kg K$_2$O ha^{-1}, respectively. Weed control was conducted manually throughout the experiment cycle and agro-chemicals were applied to diseases and pests when necessary.
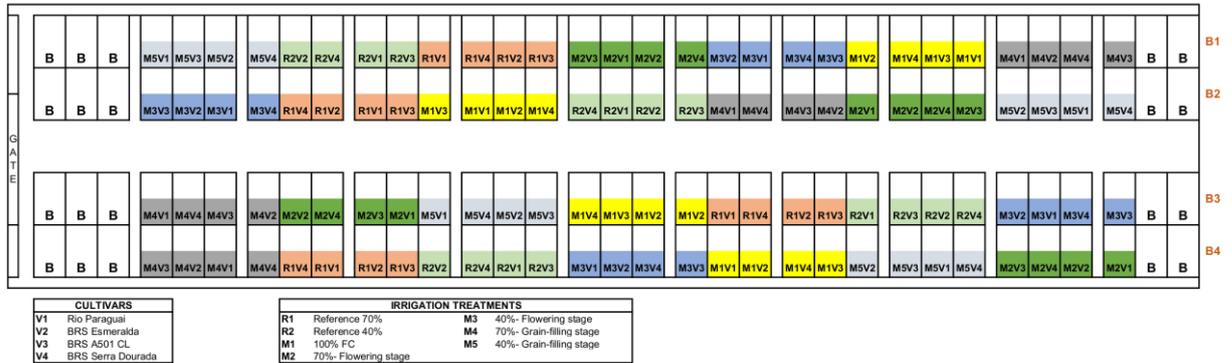

Fig. 1. Treatments distribution in the experimental upland rice area

The experiment was based on a randomized block design with split-plots and four replications per treatment (Fig. 1). The main plot was the irrigation management and the subplots were the upland rice cultivars. Four rice genotypes were evaluated at five irrigation managements: well-irrigated at field capacity soil moisture (100% FC) (M1), 70% and 40% FC at the flowering stage (M2 and M3), and 70% and 40% FC at the grain-filling stage (M4 and M5). 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 depth 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 flowering and finished at the end of the crop cycle. The periods of water stress differed because of the variation in the phenological development of each cultivar (Table 1).

Table 1. Water stress managements for four upland rice cultivars

Irrigation management	Cultivar	Imposition of water stress (Days after emergence)	Stress period (d)
M2-M3	BRS A501 CL	77-95	18
	BRS Esmeralda	76-93	17
	BRS Serra Dourada	71-88	17
	Rio Paraguai	91-110	19
M4-M5	BRS A501 CL	96-110	14
	BRS Esmeralda	93-107	14
	BRS Serra Dourada	89-103	14
	Rio Paraguai	111-126	15

A drip irrigation system was used in this experiment, with self-compensating emitters, anti-siphons, and anti-drainage. A small drip line (1 m) 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 well-irrigated management (M1) of each cultivar (Additional file 2). Soil matric potential was measured with a digital portable tensimeter 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 done whenever the soil water potential fell below 20 KPa at a 20 cm depth. The amount of soil water in each layer before irrigation was estimated from the matric potential using the van Genutchen soil water retention curve equation (van Genuchten, 1980) and the depth of applied water to M2, M3, M4 and M5 was a fraction of the depth of water applied to the reference management (M1) of each cultivar.

Measurements of air temperature and relative air humidity were recorded with a Vaissala 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).

2.2.2. 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 sun light 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 with data from additional plots submitted for the irrigation deficit from 20 DAS 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 of Idso (1982), as in Equation (1):

$$CWSI = \frac{(T_c - T_{air}) - T_{wet}}{T_{dry} - T_{wet}} \tag{1}$$

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) and Costa et al. (2020). Thus, T_{wet} and T_{dry} corresponded to the minimum and the maximum difference between T_c and T_{air}, respectively. The CWSI obtained with this methodology is called the 'Observed CWSI'.

2.2.3. 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 mol^{-1} CO_2 in the leaf chamber and the photon flux density photosynthetic active (PPFD) 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 the flag leaves in each plot with three technical repetitions at the end of the water stress periods.

2.2.4. Soil and plant analyzer development (SPAD index)

SPAD values were calculated by averaging five readings per plot using a portable, non-destructive chlorophyll meter, CFL1030 (Falker, Porto Alegre, Brazil). 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).

2.2.5. Leaf water potential (LWP)

Leaf water potential was measured before dawn 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 water stress periods. These samples were placed in appropriate containers with ice for transportation to the laboratory to be processed in the chamber as soon as possible.

2.2.6. Yield, Yield Components, Harvest Index and Water Use Efficiency

The plants were harvested at maturity. For plant 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 then weighted. 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, filled grain rate, 1000-grain weight, and grain yield per ha were calculated.

Water Use Efficiency (WUE) (kg m^{-3}) was calculated as the ratio of grain yield to the amount of total water input (Equation 2). The Harvest Index (HI) was computed as the ratio of grain yield and total dry matter (Equation 3).

$$WUE = \frac{Grain\ yield\ (kg)}{Water\ consumption\ (m^3)} \tag{2}$$

$$HI = \frac{Grain\ yield\ (kg)}{Total\ aerial\ dry\ matter\ (kg)} \tag{3}$$

2.2.7. Statistical analysis

All the statistical analyses were performed with the R software (http://www.r-project.org). Two-way ANOVA was done for grain yield, grain yield components, harvest index, and water use efficiency, and the means were compared by the Fisher's Least Significant Difference (LSD) test at the 5% probability level using the package 'ExpDes' (Ferreira et al., 2013). 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 with the Tukey test, which was applied using the package 'emmeans' (Russell, 2021). Principal component analysis (PCA) was performed to correlate grain yield, grain yield components, water use efficiency and physiological traits independently for each upland rice cultivar using the R package 'mdatools' (Kucheryavskiy, 2020).

2.3. Results

2.3.1. Weather conditions and water demand

Weather data at the experimental site during the trial period are shown in Table 2. The measured mean maximum and minimum air temperatures were 35.5 and 18.7 °C, respectively. 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}.

Table 2. 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

Water consumption of the upland rice cultivars is presented in Fig. 2. The average potential water demand ranged from 792 mm in BRS Serra Dourada to 1148 mm in Rio Paraguai. Water reductions for M2, M3, M4 and M5 were on average 58, 121, 51 and 103 mm, respectively, compared to well-irrigated management (M1).
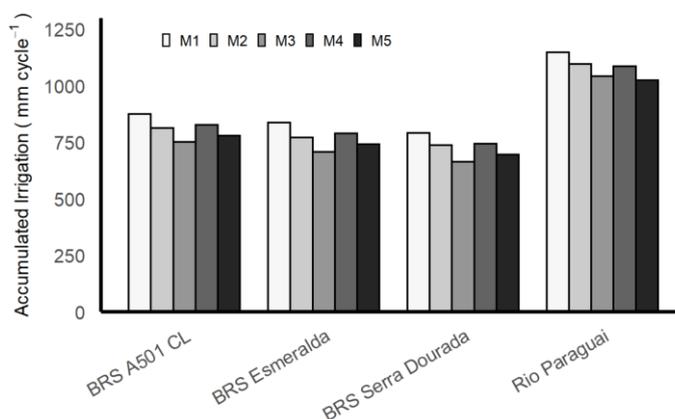

Fig. 2. Irrigation water accumulated during the crop cycle in four upland rice cultivars

2.3.2. Effects of drought stress on morphoagronomic traits, water use efficiency and harvest index.

The two-way ANOVA revealed a significant effect of the irrigation management and cultivar interaction on the filled grain (FG), 1000-grain weight (TGW), grain yield (GY), water use efficiency (WUE), and harvest index (HI) (Table 3). In addition, the source of variation management was significant for the number of panicles per plant (PP) and spikelets per panicle (SPN); and the source of variation cultivar was significant for LW, PP, and SPN.

Table 3. Analysis of variance (F-values) for grain yield, yield components, water use efficiency and harvest index among managements and cultivars.

Source of variation	df	LW	PP	SPN	FG	TGW	GY	WUE	HI
Irrigation management (M)	4	0.7ns	4.5*	14.6**	39.8**	17.6**	66.2**	59.1**	54.7**
Cultivar (C)	3	156.4**	73.4**	30.0**	23.8**	96.1**	38.1**	54.9**	44.8**
M x C	12	1.4ns	1.4ns	0.6ns	2.0*	2.1*	2.0*	2.4*	2.3*

Degrees of freedom, df; leaf weight, LW; panicles per plant, PP; spikelets per panicle, SPN; filled grain, FG; 1000-grain weight, TGW; grain yield, GY; water use efficiency, WUE; harvest index, HI. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level. nsNo significant.

The grain yield potential (M1) was similar in the modern cultivars, ranging between 8.3 and 9.3 Mg ha-1, while the traditional cultivar reached 5.2 Mg ha-1 (Table 4). The cultivars had a lower grain yield (GY) as irrigation levels were reduced at flowering and grain-filling stages. Moderate stress at the flowering stage (M2) decreased significantly GY for all cultivars except BRS Serra Dourada. In BRS A501 CL, BRS Esmeralda, and Rio Paraguai, the GY reductions were 28, 34, and 77%, respectively, while severe stress at this stage (M3) decreased GY for all cultivars, with reductions ranging between 46% in BRS A501 CL and 94% in Rio Paraguai (Table 4). Moderate stress at the grain-filling stage (M4) decreased significantly GY only for BRS A501 CL by 19%, while severe stress at this stage (M5) decreased GY for all cultivars with reductions ranging between 49.5% in BRS A501 CL and 66.3% in BRS Esmeralda (Table 4).

Table 4. Grain yield (Mg ha-1) for four upland rice cultivars subjected to five irrigation managements.

Irrigation management[1]	Upland rice cultivars[2]				
	BRS A501 CL	BRS Esmeralda	BRS Serra Dourada	Rio Paraguai	Mean
M1	9.3aA	8.3aA	9.2aA	5.2aB	8.0
M2	6.7 (28.0)bcAB	5.5 (33.7)bB	7.7 (16.3)aA	1.2 (76.9)bC	5.3 (33.8)
M3	5.0 (46.2)cdA	3.2 (61.4)cB	2.1 (77.2)bBC	0.3 (94.2)bC	2.7 (66.3)
M4	7.5 (19.4)bA	6.7 (19.3)abA	7.9 (14.1)aA	3.8 (26.9)aB	6.5 (18.8)
M5	4.7 (49.5)dA	2.8 (66.3)cB	3.4 (63.0)bAB	1.8 (65.4)bB	3.2 (60.0)
Mean	6.7	5.3	6.1	2.5	

Means followed by distinct lowercase letters within a column[1] and distinct capital letters within a row[2] are different by the LSD test at 0.05 significance. Values in the parentheses are the reduced percent compared to the M1 irrigation management.

After exposure to drought stress, all the irrigation managements showed similar leaf weight (Additional file 2). Under moderate stress, the number of panicles per plant (PP) was not affected, neither at flowering nor at grain-

filling (M2 and M4), whereas severe stress reduced PP by 14% at both stages (Table 5). Moderate and severe stress at flowering reduced the number of spikelets per panicle (SPN) by 19 and 24%, respectively, while SPN under irrigation deficits at grain-filling was not reduced. The filled grain rate (FG) under moderate stress managements was not different from well-watered conditions at flowering and grain-filling for all cultivars, except for BRS Esmeralda and Rio Paraguai at flowering, with decreases of 52 and 30%, respectively (Table 5). Severe water stress (M3 and M5) caused a huge reduction in FG for all cultivars. BRS A501 CL, BRS Esmeralda, BRS Serra Dourada, and Rio Paraguai decreased FG to 54, 36, 27, and 5% at the flowering stage and to 56, 33, 37, and 30% at the grain-filling stage, respectively (Table 5). Drought stress had no effect on 1000-grain weight (TGW) for BRS Esmeralda and BRS Serra Dourada, whereas severe stress at the grain-filling stage (M5) decreased TGW by 23% for BRS A501 CL and Rio Paraguai (Table 5).

Table 5. Yield components for four upland rice cultivars subjected to five irrigation managements

| Yield component | Irrigation management[1] | Upland rice cultivars[2] | | | | |
		BRS A501 CL	BRS Esmeralda	BRS Serra Dourada	Rio Paraguai	Mean
Panicles per plant	M1	3.5	2.7	3.2	2.4	3.0a
	M2	3.7	2.7	3.2	1.9	2.9a
	M3	3.3	2.5	3.1	1.4	2.6b
	M4	3.3	2.6	3	2.2	2.8ab
	M5	3.2	2.4	3.1	1.6	2.5b
	Mean	3.4A	2.6C	3.1B	1.9D	
Spikelets per panicle	M1	122.7	165	158.2	106.9	138.2a
	M2	105.5	139.2	142	63.2	112.5b
	M3	110	140.6	109.4	62.9	105.7b
	M4	126.3	156.5	157.1	94	133.5a
	M5	124.3	157.8	135.4	101.1	129.7a
	Mean	117.7B	151.8A	140.4A	85.6C	
Filled grain rate (%)	M1	78.8aA	69.8aA	78.4aA	59.5aA	71.6
	M2	64.5abcAB	52.2bcB	73.6aA	30.4bC	55.2
	M3	54.0cA	36.1cdB	26.9bB	6.0cC	30.7
	M4	71.4abA	63.3abAB	74.5aA	50.2aB	64.8
	M5	56.0bcA	32.5dB	37.1bB	30.1bB	38.9
	Mean	64.9	50.8	58.1	35.2	
1000-grain weight	M1	23.1aB	22.2aB	19.5aC	30.0aA	23.7
	M2	22.5aB	22.7aB	19.0aC	25.9bA	22.5
	M3	21.5aB	21.0aB	18.2aC	28.1abA	21.8
	M4	20.9aBC	22.0aB	18.9aC	28.7aA	22.6
	M5	17.7bB	20.0aB	17.9aB	23.4cA	19.7
	Mean	21.2	21.6	18.7	27.1	

Means followed by distinct lowercase letters whitin a column[1] and distinct capital letters within a row[2] are different by the LSD test at 0.05 significance.

Water Use Efficiency (WUE) based on the entire growing season varied from 0.16 to 1.75 kg m^{-3} (Fig. 3A). Higher values were observed in M1 of each cultivar. The WUE of modern cultivars under M1 ranged from 1.5 kg m^{-3} in BRS Esmeralda to 1.75 kg m^{-3} in BRS Serra Dourada, while WUE in Rio Paraguai reached 0.72 kg m^{-3}. Moderate stress at flowering (M2) reduced WUE in BRS A501 CL, BRS Esmeralda and Rio Paraguai by 22, 29 and 76%, respectively, while at grain-filling (M4), WUE was similar to M1 in all cultivars (Fig. 3A). Severe stress decreased WUE

at flowering and grain-filling stages for all cultivars, with the greatest reduction in Rio Paraguai by 94% at flowering and the lowest reduction in BRS A501 CL by 43% at grain-filling (Fig. 3A). The Harvest Index (HI) varied from 0.07 to 0.50 (Fig. 3B). Compared with M1, the HI of moderate stress at flowering and grain-filling (M2 and M5) was similar for BRS A501 CL and BRS Serra Dourada. In contrast, moderate stress at flowering (M2) reduced HI in BRS Esmeralda and Rio Paraguai by 26 and 72%, respectively. Severe stress at flowering and grain filling reduced HI in all cultivars (Fig. 3B), with the greatest reduction in Rio Paraguai by 92% at flowering and the lowest reduction in BRS A501 CL by 33% at grain filling. The average values of WUE and HI revealed that modern cultivars BRS A501 CL, BRS Esmeralda and BRS Serra Dourada differed widely from the traditional cultivar Rio Paraguai (Fig. 3).
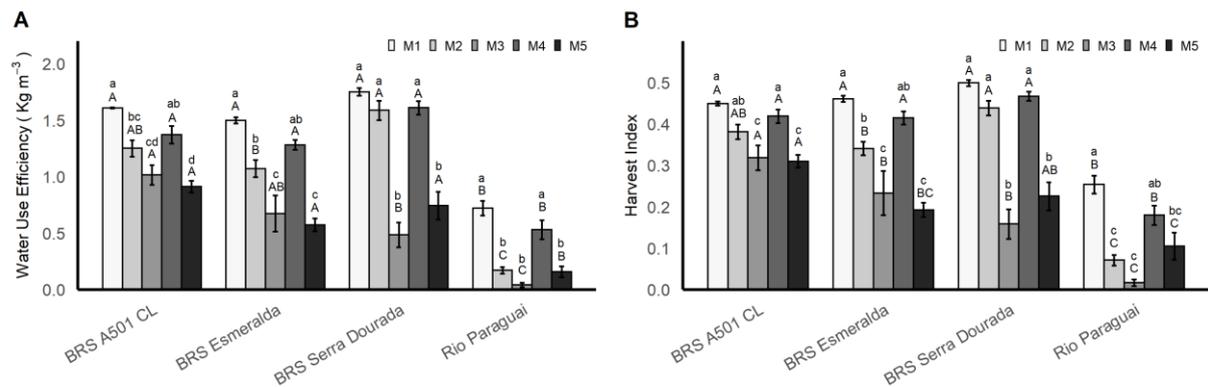

Fig. 3. Water Use Efficiency and Harvest Index of four upland rice cultivars subjected to five irrigation managements. Data indicate Mean ± SE (n = 4). Distinct lowercase letters within a variety and distinct capital letters within an irrigation management are different by the LSD test at 0.05 significance.

2.3.3. The physiological response to drought stress

The three-way ANOVA for physiological traits indicated significant differences for the double interaction irrigation management x cultivar for net photosynthesis rate (A), transpiration (E), leaf water potential (LWP) and soil and plant analyzer development (SPAD) (Table 6). Furthermore, for sources of variation irrigation management and cultivar, all physiological traits had significant differences, whereas developmental stage was significant only for A and SPAD (Table 6).

Table 6. Analysis of variance (F-values) for physiological traits among irrigation managements and cultivars.

Source of variation	df	A	gs	E	iWUE	LWP	CWSI	SPAD
Developmental stage (T)	1	17.99*	1.59ns	0.27ns	0.08ns	0.25ns	5.19ns	8.63**
Irrigation management(M)	4	64.7**	13.52**	32.70**	6.89**	29.32**	3.79**	2.69*
Cultivar (C)	3	17.17**	6.42**	8.12**	2.96*	7.00**	10.91**	113.28**
T x M	4	211.57**	65.91**	144.29**	17.74**	21.68**	38.23**	2.22ns
T x C	3	6.51**	7.61**	26.97**	9.06**	1.89ns	23.33**	0.77ns
M x C	12	3.88**	1.37ns	1.91*	1.35ns	2.61**	1.39ns	1.85*

Degrees of freedom, df; net photosynthesis rate, A; stomatal conductance, gs; transpiration, E; intrinsic water use efficiency, iWUE; leaf water potential, LWP; crop water stress index, CWSI; and soil and plant analizer development, SPAD. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level. nsNo significant.

The physiological traits A, gs, and E decreased under moderate and severe water stress at the flowering stage (M2 and M3) for all cultivars, except for Rio Paraguai, which had low gs even with M1 (Fig. 3A, 3C, 3E). At this stage, A, gs and E for BRS Serra Dourada under moderate water stress had slight decreases of 31, 55 and 41%, while under severe water stress, this cultivar had greater reductions of 92, 90 and 85%, respectively (Fig. 3A, 3C, 3E). At the grain-filling stage, the values of A, gs, and E under moderate water stress (M4) were similar to those under 100% FC management (M1) in BRS Esmeralda and Rio Paraguai (Fig. 3B, 3D, 3F), whereas all cultivars had significant decreases under severe stress. At this phenological stage, BRS A501 CL was the most affected by the two levels of water stress (Fig. 3B, 3D, 3F). Thus, this cultivar reduced A, gs, and E by 63, 65, and 48% under moderate stress and by 85, 91, and 85% under severe stress, respectively. iWUE increased under moderate stress at flowering in BRS A501 CL, BRS Esmeralda and Rio Paraguai, as well as at grain-filling in BRS Serra Dourada. Furthermore, iWUE increased under severe stress at grain-filling in BRS A501 CL and BRS Esmeralda (Fig. 3G, 3H).
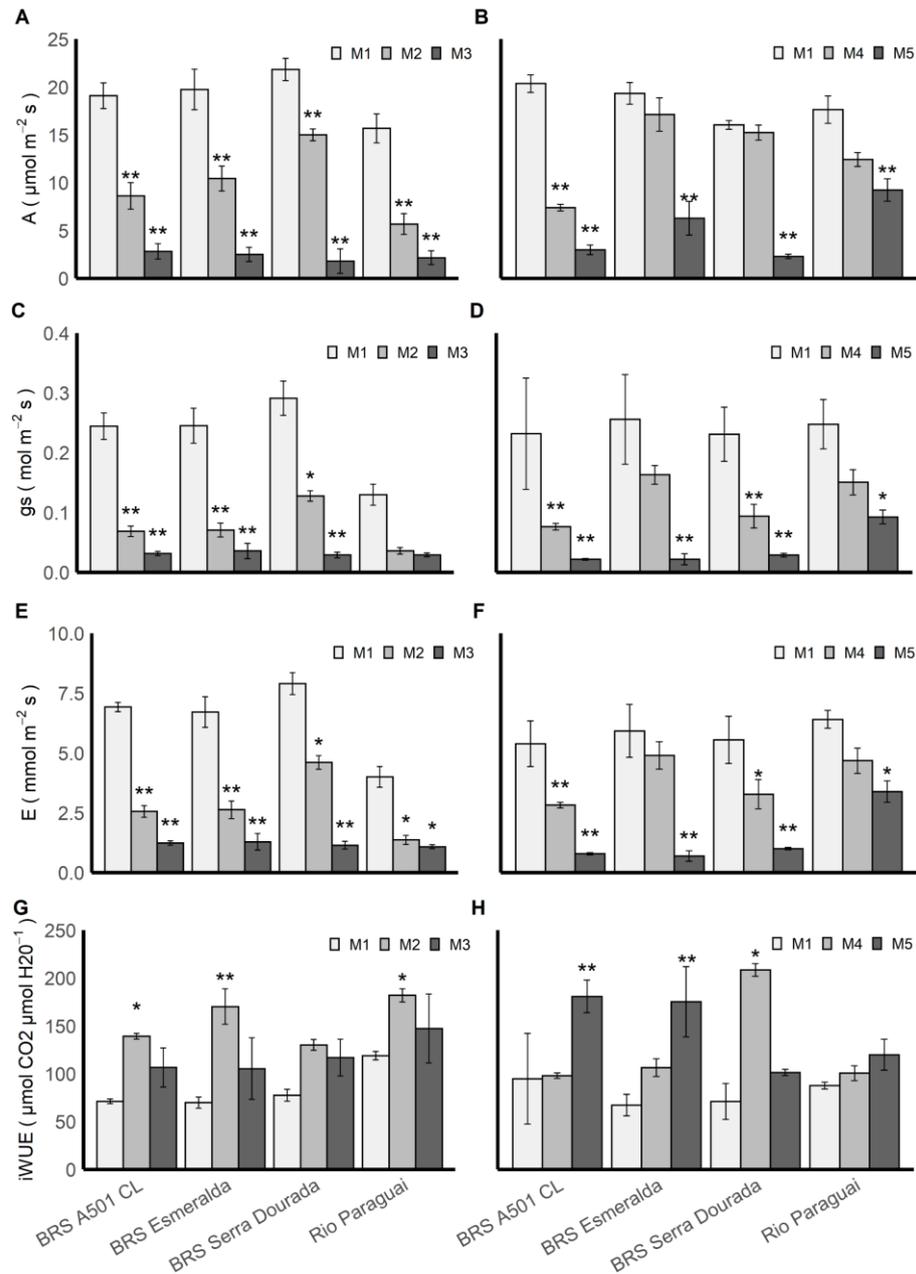
Fig. 4. Gas exchange traits of four upland rice cultivars subjected to five irrigation managements. Data indicate Mean ± SE (n = 4). A, Photosynthetic rate (A) at flowering stage. B, A at grain-filling stage. C, Stomatal conductance (gs) at flowering stage. D, gs at grain-filling stage. E, Transpiration (E) at flowering stage. F, E at grain-filling stage. * and ** indicate significant differences from 100% FC (M1) at the 0.05 and 0.01 levels within cultivars, respectively.

The Leaf Water Potential (LWP) of the four cultivars showed lower values under severe drought stress at flowering and grain-filling stages (M3 and M5) compared with M1 (Fig. 5A, 5B). The LWP ranged between -1.0 MPa in BRS Esmeralda and -1.4 MPa in Rio Paraguai under M3 and ranged between -1.4 MPa in BRS A501 CL to -1.9 MPa in Rio Paraguai under M5. Compared with M1, the LWP of moderate stress at flowering (M2) was similar for all cultivars, except for Rio Paraguai (Fig. 5A). At grain-filling under moderate stress (M4), LWP was not different from 100% FC (M1) in all cultivars (Fig. 5B). Under well-watered conditions (M1), LWP varied from -0.5 MPa in BRS Esmeralda to -0.8 MPa in BRS A501 CL at flowering and varied from -0.70 MPa in BRS A501 CL to -1.23 MPa in Rio Paraguai at grain-filling (Fig. 5).
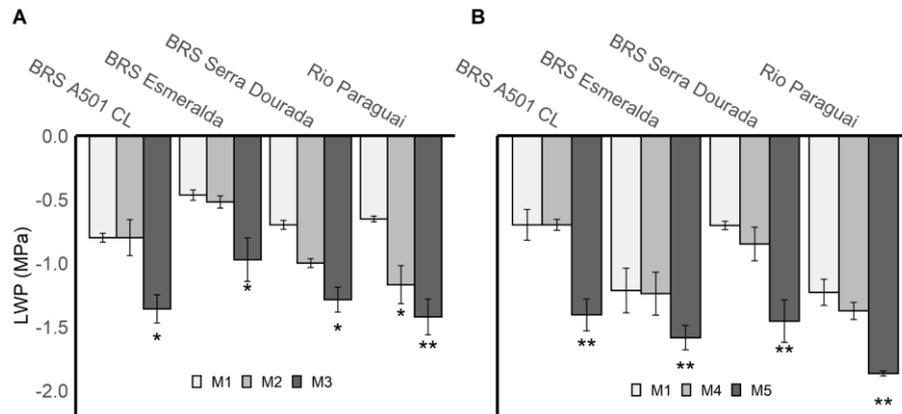
Fig. 5. Leaf water potential (LWP) of four upland rice cultivars subjected to five irrigation managements. Data indicate Mean ± SE (n = 4). A, LWP at flowering stage. B, LWP at grain-filling stage. * and ** indicate significant differences from 100% FC (M1) at the 0.05 and 0.01 levels within cultivars, respectively.

The Crop Water Stress Index (CWSI) ranges from 0 (no water stress) to 1 (extreme water stress). As shown in Fig. 6, moderate water stress at flowering and grain-filling stages (M2 and M4) was no different from the reference management (M1). During flowering, BRS A501 CL and BRS Serra Dourada under severe water stress differed from M1 (Fig. 6A). At this stage, BRS Esmeralda had the lowest value of CWSI, which ranged between 0.26 in M1 and 0.49 in M3. During the grain-filling stage, the CWSI of BRS Esmeralda and BRS Serra Dourada under severe stress had a huge difference compared to M1 (Fig. 6B). At this stage, CWSI ranged from 0.35 in BRS Esmeralda to 0.65 in BRS A501 CL under well-watered conditions (M1), ranged from 0.49 in BRS Esmeralda to 0.80 in BRS A501 CL under moderate water stress (M4), and varied from 0.57 in Rio Paraguai to 0.83 in Serra Dourada under severe water stress (M5).
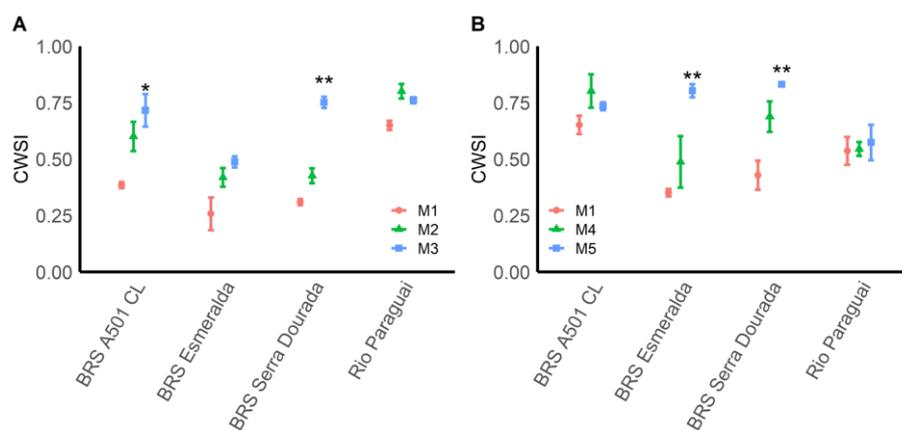

Fig. 6. Crop water stress index (CWSI) of four upland rice cultivars subjected to five irrigation managements. Data indicate Mean ± SE (n = 4). A, CWSI at flowering stage. B, CWSI at grain-filling stage. * and ** indicate significant differences from 100% FC (M1) at the 0.05 and 0.01 levels within cultivars, respectively.

Drought stress during the flowering stage had a slightly non-significant reduction in BRS Esmeralda and BRS A501 CL (Fig. 7A). In contrast, at the grain-filling stage, SPAD values for BRS A501 CL and BRS Esmeralda under severe drought stress decreased significantly by 8% in both cultivars (Fig. 7B). At both phenological stages, there was a non-significant slight increase in SPAD values with moderate stress in BRS Serra Dourada compared with a well-watered condition (Fig. 7).
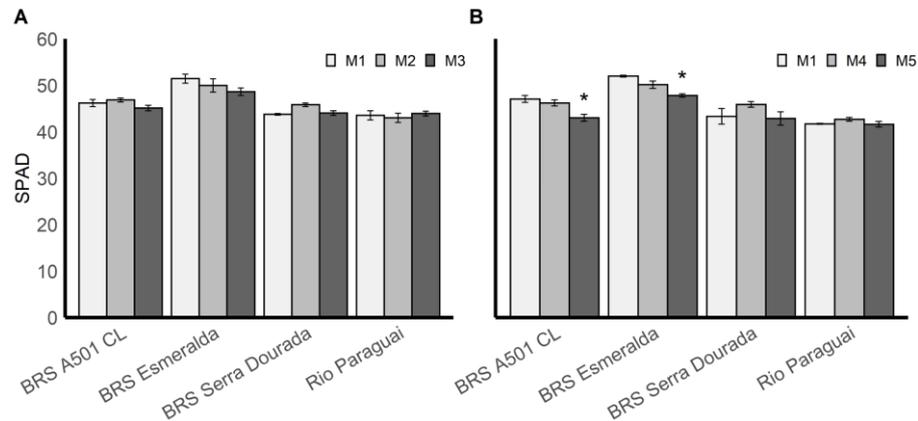
Fig. 7. SPAD of four upland rice cultivars subjected to five irrigation managements. Data indicate Mean $\pm$ SE (n = 4). A, SPAD at flowering stage. B, SPAD at grain-filling stage. * and ** indicate significant differences from 100% FC (M1) at the 0.05 and 0.01 levels within cultivars, respectively.

2.3.4. Principal Component Analysis

PCA was used on each cultivar to examine the relationship between water use efficiency, grain yield, grain yield components, and physiological traits. The first and second components explained 64.04%, 65.50%, 72.9% and 65.57% of the variance for BRS A501CL, BRS Esmeralda, BRS Serra Dourada and Rio Paraguai, respectively (Fig. 8). The sharp angle means positive correlation, the obtuse angle shows negative relations and the right angle shows no correlation between different traits. Thus, for all cultivars, gas exchange traits were highly positively correlated and the GY was positively correlated with WUE, FG, and TGW (Fig. 8). Moreover, A, gs, E, WUE, GY, FG and TGW had a positive correlation between them. All these traits were negatively correlated with CWSI (Fig. 8). Traits like SPAD, LWP, iWUE, SPN and PP had a particular behavior in each cultivar (Fig. 8). Thus, SPAD showed positive correlations with GY, FG, TGW, A, gs, E, for BRS Esmeralda (Fig. 8B). iWUE had a significant correlation with LWP and CWSI in Rio Paraguai (Fig. 8D). LWP had positive correlations with gas exchange traits, FG, TGW, and GY in BRS A501 CL and BRS Serra Dourada (Fig. 8A). PP had significant correlations with all traits except SPAD and SPN in the traditional cultivar, Rio Paraguai (Fig. 8D). SPN showed positive correlations with gas exchange traits, GY and FG in BRS Esmeralda and Rio Paraguai (Fig. 8B, 8D). These results suggested that some physiological traits of upland rice were closely related to yield responses and these relations varied between cultivars.
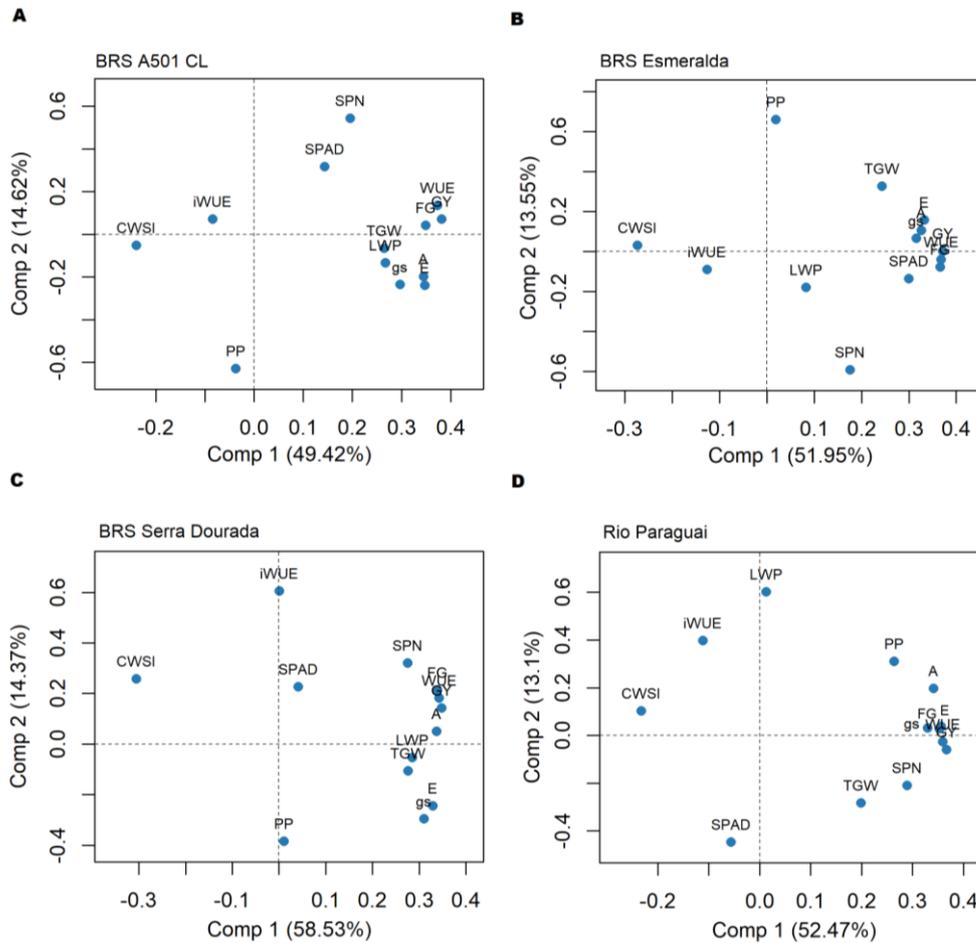
Fig. 8. Principal component analysis (PCA). Site score plots of the studied variables in the drought stress managements, for the four upland rice cultivars. A, BRS A501 CL. B, BRS Esmeralda. C, BRS Serra Dourada. D, Rio Paraguai. PCA included these variables: water use efficiency, WUE; grain yield, GY; panicles per plant, PP; spikelets per panicle, SPN; filled grain, FG; thousand grain weight, TGW; net photosynthesis rate, A; stomatal conductance, gs; transpiration, E; intrinsic water use efficiency, iWUE; leaf water potential, LWP; crop water stress index, CWSI; and soil and plant analyzer development, SPAD.

2.4. Discussion

High-efficient irrigation technologies allow the smart control of water requirements of crops (Kang et al., 2017). These technologies lead to maximization of available soil moisture for transpiration and can be considered as a strategy for genetic improvement (Blum, 2009). Furthermore, new cultivars are expected to be drought tolerant and water efficient (Ali and Hussain, 2021). In addition to the knowledge about water-saving technology in upland rice, this study can provide fundamental information of certain traits to identify drought-tolerant cultivars.

Water depletion had no effect on leaf weight with any deficit irrigation management (Additional file 3). This could be attributed to the fact that in rice, no new leaves are produced during and after flowering (Alou et al., 2018), and the duration of water stress was insufficient to reduce leaf weight (Vijayaraghavareddy et al., 2020). Water reduction affects grain productivity, but each genotype had different responses to drought. For example, under severe stress at flowering, Rio Paraguai was 48% lower than BRS A501 CL. These differences were expected since traditional cultivars limit grain yield by early stomatal closure (Heinemann et al., 2011) and modern cultivars may present favorable alleles for drought tolerance (Lanna et al., 2020). In addition, modern cultivars present differences in GY among them when subject to water stress (Table 4), which could be explained by the different genetic constitutions of the parents. Grain

yield potential (M1) had a mean of 8.0 Mg ha^{-1} for all upland rice cultivars. These results are similar to those obtained in several trials of upland rice irrigated by sprinkler systems in Japan (Kato and Katsura, 2014), but they are different from the grain yield reported for Central Brazil, which is 5.0 Mg ha^{-1} when subjected to supplementary irrigation (Pinheiro et al., 2006).

Consistent with previous studies, drought stress at flowering affected grain yield (Alou et al., 2018; Vijayaraghavareddy et al., 2020). In this trial, M3 (40% FC) and M2 (70% FC) reduced grain yield by 67% and 34%, respectively (Table 4). During flowering, drought had the greatest impact on the percentage of filled grains and the number of spikelets per plant (Table 5). According to Barnabás (2008), 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. In addition, high percentages of spikelet sterility may be caused by temperature (Table 2), as reported by Shah et al. (2011) and Sharma et al. (2018), who indicated critical temperatures for rice during flowering above 33 °C. Previous results indicated low grain yield resulting from water stress at grain-filling (Boonjung and Fukai, 1996). In this study, M5 (40% FC) and M4 (70% FC) affected grain yield, reducing it by 60% and 20%, respectively (Table 4). At this stage, water stress had the greatest impact on the filled grain rate and 1000-grain weight (Table 5). 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.

WUE based on the entire growing season was not improved by moderate stress during flowering and grain filling (Fig. 2A). Similar responses were reported by Alou et al. (2018), with slight reductions in WUE with short-term treatments at specific stages. This response shows that the crop was less efficient as water inputs were reduced. On the other hand, severe stress had the highest reduction in WUE. This response was expected since water savings ranged from 9% to 16% (Fig. 1) and grain yield loss was significant (Table 4). In contrast, Kato et al. (2006) and Kumar et al. (2017) found higher WUE under water stress compared to well-irrigated management, but these trials were subjected to long-term irrigation reduction treatments and progressive soil drying. The disagreement with these results probably stems from the high frequency of irrigation of plants under water restrictions. Under 100% FC management, WUE for modern cultivars (Fig. 2A) was higher than WUE values reported in previous field experiments, which ranged between 0.40 and 0.85 kg m^{-3} under favorable conditions (Bouman et al., 2005; Kato et al., 2009; Alou et al., 2018; Froes de Borja Reis et al., 2018). Overall, WUE response showed that, under certain conditions of precision irrigation, genotypes can express their maximum yield potential while saving water, and under different degrees of drought stress, it provides clear information about drought-resistant cultivars.

The HI under well-watered conditions was 0.25 for Rio Paraguai and ranged between 0.45 and 0.50 for BRS A501 CL, BRS Serra Dourada and BRS Esmeralda. The values found in this study were similar to those found by Alvarez et al. (2005), who indicated values of 0.25 for traditional cultivars and 0.50 for modern cultivars. Under severe water stress, HI was reduced for all cultivars (Fig. 2B). This reduction in HI under severe stress suggests a great accumulation of biomass (stems and leaves) combined with a lower grain yield. This finding was supported by a previous study which reported that a high percentage reduction in yield (above 70%) reduced significantly HI (Bernier et al., 2007). Moderate stress reduced HI in Rio Paraguai during flowering and grain filling, as well as in the modern cultivar BRS Esmeralda during flowering. Kumar et al. (2017), Biju et al. (2018) and Zhao et al. (2021) for rice, lentil and oat, respectively, found that under moderate water stress, each genotype had a different decrease in HI. Thus, the

significant reductions in HI in Rio Paraguai and BRS Esmeralda may be associated with the genetic characteristics of each one.

The present study showed that moderate and severe stresses caused reductions in photosynthesis (A), stomatal conductance (gs) and transpiration (Fig. 3). The reduction of gas exchange traits due to drought stress has been reported in lowland and upland rice (Dingkuhn et al., 1989; Yang et al., 2019; Lanna et al., 2020; Vijayaraghavareddy et al., 2020). Decreases in the rate of photosynthesis in drought-stressed plants can be caused by stomatal closure, or/and photochemical reactions such as reduction in NADPH, or/and biochemical reactions such as reduction in RuBP regeneration (Tezara et al., 1999; Flexas et al., 2010). The strong relationship between A and gs indicates that the reduction in A was mostly regulated by stomatal closure (Siddique et al., 1999). Thus, the high coefficient of correlation between A and gs for the four cultivars leads to thoughts of a predominance of stomatal closure for the reduction of photosynthetic rate. Furthermore, this experiment had short-term drought managements and high frequency irrigation. Zhou et al. (2019) argued that non-stomatal effects on photosynthesis come to dominate under high intensity and long duration of drought. Lower rates of decreases in A, gs, and E were observed at grain-filling than at flowering under moderate stress. However, although higher yields were reported with stress treatments at grain-filling compared to flowering, it is not possible to infer that this is due to improved gas exchange rates. It needs further validation since a previous study indicated that plants at the later phenological stage could use carbohydrates that were built up during pre-anthesis when reducing the photosynthetic rate under stress (Jagadish et al., 2015; Sehgal et al., 2018). Overall, BRS A501 CL had the worst performance in the gas exchange traits under water stress. Despite this, this cultivar was the most productive under severe drought stress. Lanna et al. (2020) found similar responses by testing elite upland rice cultivars. Furthermore, Alvarez et al. (2015) reported that gas exchange rates did not explain the differences in productivity of three Brazilian modern upland rice cultivars.

Intrinsic water use efficiency (iWUE) measured with IRGA at leaf level in this experiment is contradictory with the approach proposed by Yang et al. (2019), which attributes drought-tolerance to cultivars with higher iWUE. These differences could be caused by morpho-physiological mechanisms and spatio-temporal effects (Blum, 2009; Medrano et al., 2015). However, because of the complexity of the processes involved, water use efficiency at the leaf level in upland rice could be another topic of interest. Despite this, iWUE was included in this study with the purpose of finding a relationship with WUE since Flexas et al. (2010) reported evidence of the association between these variables. Nevertheless, all cultivars showed no correlation between WUE and iWUE (Fig. 7). Medrano et al. (2015) concluded that there is no correspondence between WUE at leaf level and WUE at crop level due to the influence of light interception, night transpiration, and respiration losses. Thus, iWUE may not be used as a method for estimating WUE.

In this study, LWP under moderate stress at flowering differed between the traditional cultivar and the modern cultivars (Fig. 4). This could be because traditional cultivars work with their stomata more closed than modern cultivars under moderate decreases in soil moisture (Heinemann et al., 2011). Thus, the Brazilian breeding program is carrying out an effective improvement for moderate drought stress. On the other hand, severe drought stress decreased LWP for all cultivars at flowering and grain-filling stages. Kumar et al. (2017) observed the same effect in lowland rice genotypes. Therefore, severe drought may affect LWP independently of its genetic constitution. Overall, the LWP of BRS A501 CL decreased the least when subjected to irrigation withholding. According to Guimarães (2019), lower LWP reduction indicates better water uptake. In addition, evidence has been found that high leaf water potential may contribute to reduced spikelet sterility (Jongdee et al., 2002; Serraj et al., 2009). Thus, a high grain filling rate was observed in A501CL for all managements. However, only BRS A501 CL and BRS Serra Dourada showed a significant

relationship between FG and LWP (Fig. 7). For this reason, Yang et al. (2019) suggest further validation of the effect of LWP on FG.

The Crop Water Stress Index (CWSI) has been used as a selection criteria to improve tolerance to drought stress in plants (Biju et al., 2018; Banerjee et al., 2020). In this study, the index was not significantly separated from the moderate stress of well-watered managements and had different responses between flowering and grain-filling. In any case, the CWSI increased as the irrigation level decreased (Fig. 5). Biju et al. (2018) reported that susceptible-drought genotypes had a high CWSI. Thus, following that approach, BRS Esmeralda at flowering can be considered drought-tolerant under severe stress. In addition, it was expected to have a reliable response at the two phenological stages because Zia et al. (2013) observed a suitable phenotypic response to drought with thermal-information even at the grain-filling stage. Nevertheless, during grain-filling, measurements of canopy temperature could be affected by leaf senescence because the sensor may get temperatures outside the canopy. Although canopy temperature obtained with portable infrared sensors has been used by researchers to separate cultivars, Serraj et al. (2009) recommend using thermal imagery.

SPAD is frequently used to evaluate drought tolerance since plants under environmental stress lose their green chlorophyll tissues (Swapna and Shylaraj, 2017; Vijayaraghavareddy et al., 2020). In the present study, modern cultivars BRS A501 CL and BRS Esmeralda had reductions in SPAD, while Rio Paraguai and BRS Serra Dourada maintained their SPAD values (Fig. 6). According to Singh et al. (2017), genotypes that have a substantial reduction in stomatal conductance might show high values of SPAD under drought. Therefore, as a result of their early stomatal closure, traditional cultivars may have a greater tendency to maintain SPAD values under water stress.

Plant breeders mainly use direct selection (grain yield) as the principal criterion for selection (Guimarães et al., 2019). However, it is helpful to have physiological indicators of drought-tolerant characteristics (Kumar et al., 2017). In the present study, the correlations between physiological traits and grain yield indicated that CWSI and gas exchange traits A, E, and gs could be used as a reliable reference in the selection of drought-tolerant cultivars (Fig. 7).

2.5. Conclusions

Under well-watered conditions, water use efficiency (WUE) measured along the entire growing season, ranged from 1.50 to 1.75 kg m^{-3} in modern cultivars, higher than reference values found in the literature for this crop. The results highlight the importance of precision water management to fully bring out genetic potential by reducing applied water amounts under drip irrigation conditions. However, WUE was not improved under controlled deficit irrigation compared to the reference treatment (100% field capacity). In addition, there were confirmed genetic gains in relation to saving water when comparing modern cultivars with traditional cultivars.

The grain yield potential of tested cultivars ranged between 5.5 and 9.3 Mg ha^{-1} over the current values found in the literature for central Brazil for rainfed conditions. Yield reductions at flowering and grain-filling stages are associated mainly with spikelet sterility. Moreover, it was confirmed that the grain yield penalty depends on the stage and level of water stress and the genetic constitution. Thus, for example, grain yield at the flowering stage under severe stress was reduced by 46 and 95% in BRS A501CL and Rio Paraguai, respectively.

Gas exchange traits A, gs, and E explained the effects caused by water depletion, but not necessarily high values are reflected in the improvement of grain yield or grain yield components. Unfortunately, the intrinsic water use efficiency (iWUE), at leaf level for all cultivars, showed no correlation with WUE at the crop level, thus, iWUE may

not be used as a method for estimating WUE at specific stages of phenotyping tests in the field. In addition, LWP and SPAD had different responses to drought depending on the cultivar.
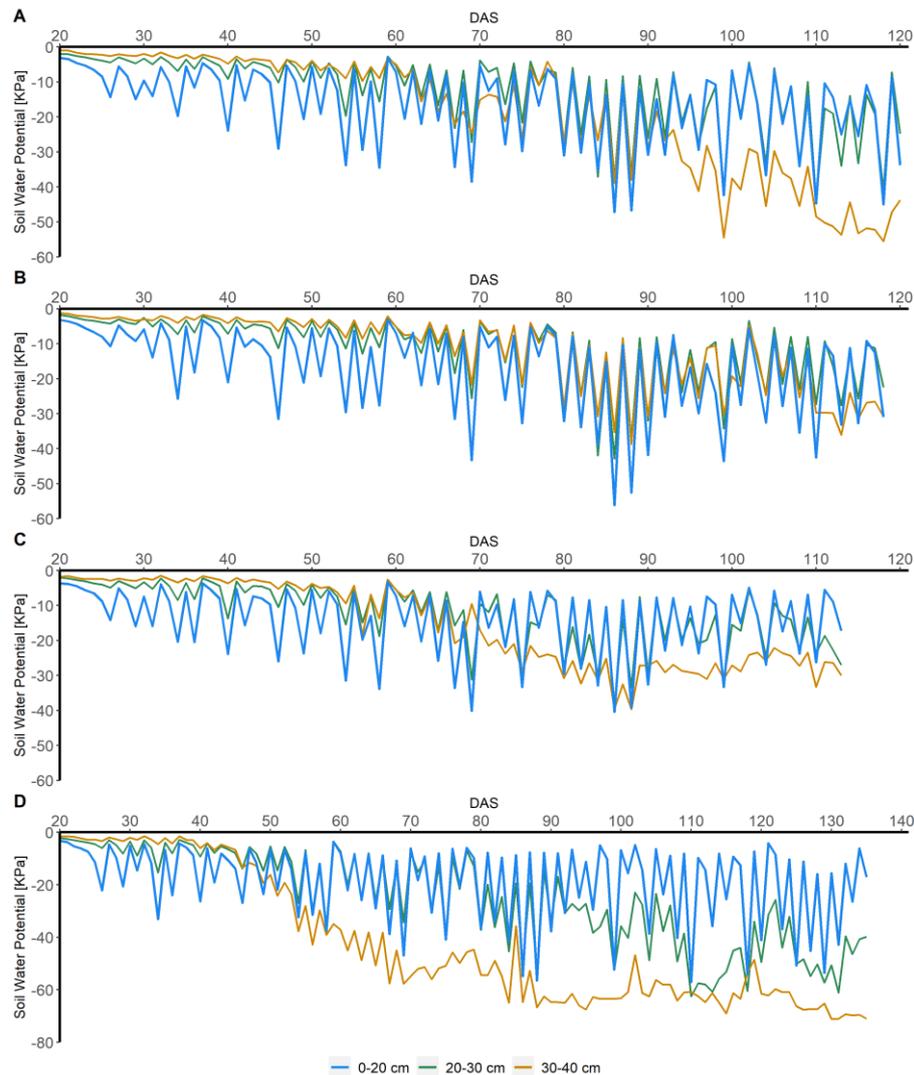

Additional Files

Additional file 1. Description of four upland rice cultivars used in this trial.

Cultivar	Year released/origin	Description
BRS A501 CL	2018/BASF-EMBRAPA	Long grain, mid-season, clearfield variety, tolerant to Kifix herbicide, flowering at 77 days, plant height of 107 cm.
BRS Esmeralda	2012/EMBRAPA	Long grain, mid-season conventional variety, flowering at 77 days, camping-resistant, pant height of 103 cm.
BRS Serra Dourada	2009/EMBRAPA	Long grain, mid-season conventional variety, flowering at 76 days, Pyricularia oryzae resistant, plant height of 98 cm.
Rio Paraguai	1992/EMBRAPA	Long grain, mid-season conventional variety, flowering at 84 days, hardiness to common diseases, plant height of 114 cm.

EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária. BASF, Badische Anilin & Soda Fabrik.

Additional file 2. Soil water potential of the 100% FC management (M1) along the crop cycle for four upland rice cultivars at three measured depths. A, BRS A501 CL. B, BRS Esmeralda. C, BRS Serra Dourada. D, Rio Paraguai.

Additional file 3. Leaf weight (Mg ha^{-1}) of four upland rice cultivars subjected to five irrigation managements.

| Irrigation management[1] | Upland rice cultivars[2] | | | | |
	BRS A501 CL	BRS Esmeralda	BRS Serra Dourada	Rio Paraguai	Mean
M1	10.66	8.64	8.49	14.94	10.68
M2	9.99	9.02	9.09	15.44	10.88
M3	9.78	8.82	10.05	15.55	11.05
M4	9.4	8.28	8.19	15.64	10.38
M5	9.18	9.68	9.47	14.25	10.65
Mean	9.80B	8.89C	9.06C	15.16A	

Means followed by distinct lowercase letters within a column[1] and distinct capital letters within a row[2] are different by the LSD test at 0.05 significance.

Additional file 4. Highlights of the study.

- High-precision irrigation in upland rice improves water use efficiency.

- The higher the upland rice yield, the better water use efficiency.

- The cultivar with high yield under moderate stress decreases under severe stress.

- Cultivars with low decrease in photosynthetic rate do not reflect in productivity.

- Leaf water use efficiency does not scale up to crop water use efficiency.

Additional file 5. Images of the activities in the experiment along the crop cycle. Installation, management, and variable measurement are all included.
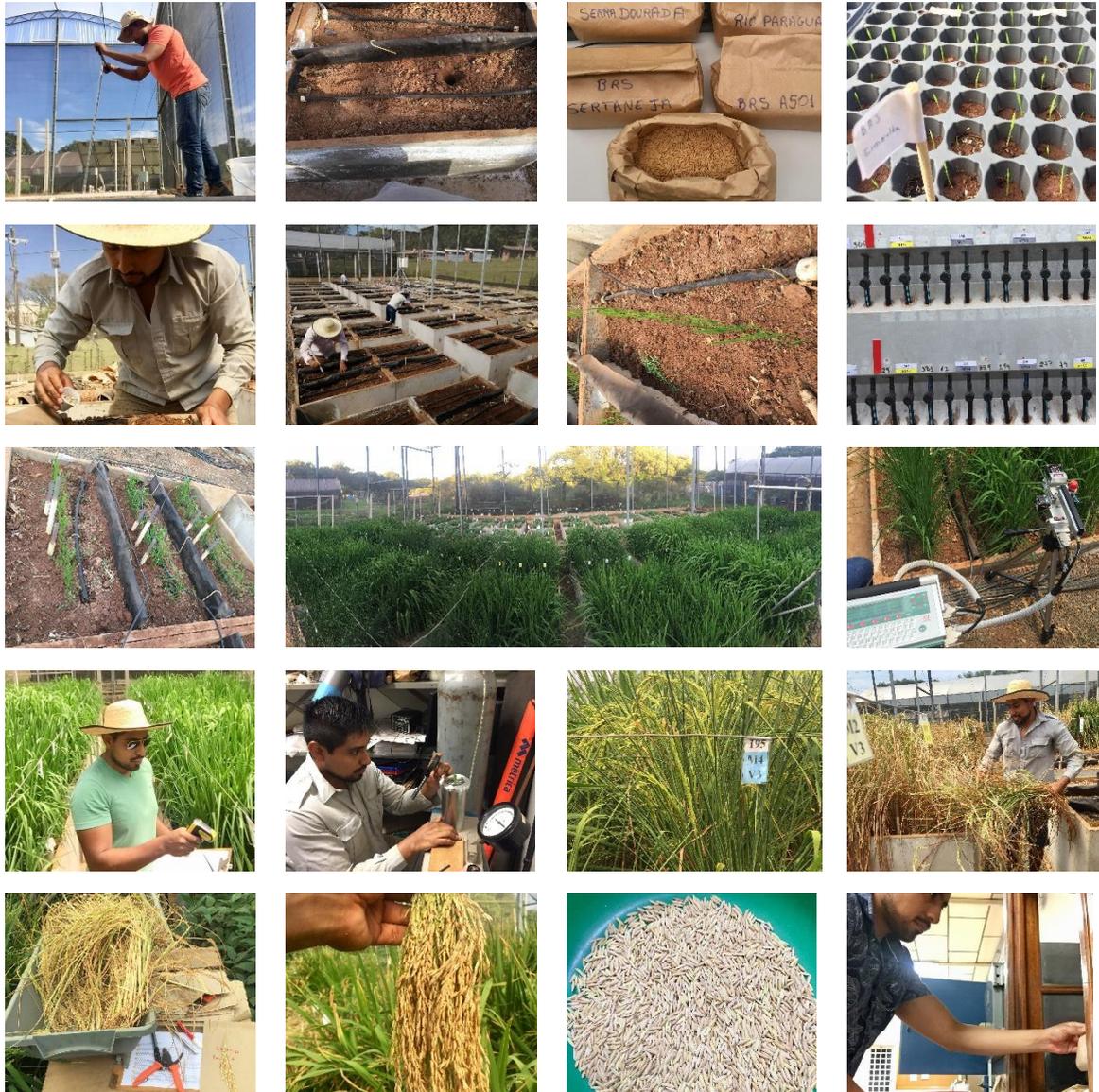
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3. WATER USE EFFICIENCY OF COMMON BEAN SUBJECTED TO WATER STRESS UNDER PRECISION DRIP IRRIGATION

ABSTRACT

Alternatives to improve water use efficiency are an important pathway to cope with water shortages that affect common bean cultivation areas. This paper aims to quantify the water use efficiency of common bean subjected to water stress under precision drip irrigation, as well as the morphological and physiological responses. A rain shelter experiment was conducted using a completely randomized design with five replications. The indeterminate growth cultivar TAA Dama was subjected to seven irrigation treatments: 100% of the field capacity (FC), 75 and 50% FC from 20 DAS until the last irrigation, 75 and 50% FC at the vegetative stage, and 75 and 50% FC at the flowering stage. The water use efficiency ranged between 1.10 and 1.55 kg m^{-3}. Mild and moderate water stress produced the opposite effects on water use efficiency when compared between long-term and short-term treatments. Grain yield potential was 4625 kg ha^{-1}, and only long-term moderate stress reduced yield productivity by 42%. Grain yield reductions were caused principally by decreases in the number of pods and grains per plant. Photosynthetic rate, stomatal conductance, and transpiration were reduced under moderate stress, whereas plants under rewatering recovered photosynthetic capacity. The intracellular concentration of CO_2 and SPAD index did not change under irrigation withholding. Water use efficiency at the leaf level did not correspond with water use efficiency at the crop level, and only photosynthetic rate and stomatal conductance had a relationship with grain yield. In conclusion, water use efficiency in common bean can be maintained under certain withholding irrigation strategies without significant yield losses.

Keywords: *Phaseolus vulgaris,* Grain yield, Water saving, Photosynthesis, Physiological mechanisms, Principal component analysis

3.1. Introduction

Phaseolus vulgaris L. is a widely cultivated grain legume that contributes with protein, complex carbohydrates, and micronutrients to the human diet (Dipp et al., 2017). In Brazil, the expected bean production for the 2020/2021 harvest is around 3.10 million tons (CONAB, 2021). The main issue with bean production in Brazil is that approximately 93% of the crop is grown on rainfed systems susceptible to abiotic stress, including water shortages (Heinemann et al., 2016). Rosales et al. (2012) reported reductions in grain yield of common bean by up to 80% as a consequence of drought stress. The yield reductions caused by drought depend on intensity, characteristics of the genotype, and developmental stage (Geerts and Raes, 2009).

Water content reduction in the plant leads to decreases in stomatal conductance, decreases in cell enlargement, and diminished leaf water potential, which results in several modifications in different plant processes (Bhattacharya, 2021). These problems may occur rapidly in beans because the root system is relatively shallow, has poor nodulation and consequently requires frequent amounts of water entering the soil profile (Calvache et al., 1997). Despite this limitation, withholding water during the vegetative period has a low impact on final yield (Mathobo et al., 2017). For this reason, research efforts for this crop have concentrated on long-term and short-term irrigation withholdings (Calvache et al., 1997; Webber et al., 2006; Mathobo et al., 2017). However, there is a lack of knowledge about the effects of drought intensity.

Cultivars with an indeterminate growth habit have a great capacity to produce new leaves when subjected to rewatering after a period of water withholding (Calvache et al., 1997). However, despite the importance of leaves as the mechanism for conducting phothosyntesys in plants, the response to rewatering in indeterminate growth cultivars has been poorly studied. In addition, under water stress, the roots are mediated by chemical signals traveling to the guard cells via the xylem stomatal closure (Webber et al., 2006), which reduces CO_2 availability and, as a result, lowers the photosynthetic rate and the response varies depending on drought intensity (Flexas and Medrano, 2002). Inclusion of gas exchange traits could explain how the cultivar used in this trial reacts to different water depletion strategies.

One of the alternatives for evaluating drought response is water use efficiency, which is defined as the ratio of dry matter production to water use (Geerts and Raes, 2009). Improved water use efficiency in common beans is important for leading to a rational use of resources without adverse effects on production (Webber et al., 2006; Mathobo et al., 2017). The approach to increasing water use efficiency could be made by adopting technologies that increase the proportion of water that is transpired by the crop (Blum, 2009), and increasing the crop's capacity to produce biomass and yield per unit of water transpired (Coelho, 2021). However, information about new technologies such as precision irrigation in common beans is still limited.

The hypothesis of this work is that irrigation withholding strategies improve water use efficiency without significant reductions in grain yield. Thus, this paper aims to quantify the water use efficiency of *Phaseolus vulgaris* L submitted to water stress under precision drip irrigation. In addition, to know the effects of long-term and short-term water stress treatments, morphological and physiological traits were included.

3.2. Materials and Methods

The experiment was carried out under rain shelter conditions at the Biosystems Engineering Department (LEB), Sao Paulo University (ESALQ), Piracicaba – SP, Brazil (22° 42' 32" S, 47° 37' 45" W and 548 m altitude). In a useful area of 130 m², 35 plots were sowed, each with a soil volume of 0.33 m³ and dimensions of 1.04 x 0.41 x 0.76 m (length, width and depth) filled with soil classified as red-yellow latosol with a sandy-loam texture. The physical water-retention characteristics of the soil are given in Table 1.

Table 1. Physical and hydrological characteristics of the soil used in the experiment.

| Layer | θ_{fc} | θ_{wp} | AWC | Ds | Sp | Texture | | |
| | | | | | | Sand | Silt | Clay |
m	cm³ cm⁻³		mm	g cm⁻³	%	%		
0.00-0.20	0.224	0.161	22.10	1.61	40.10	72.29	8.00	19.71
0.20-0.40	0.226	0.163	19.62	1.58	41.20	72.03	8.04	19.93
0.40-0.60	0.229	0.166	18.49	1.54	42.70	72.03	7.69	20.28

θFC: moisture at field capacity (corresponding to a matric potential (ψm) of -4.85 kPa for drip irrigation); θWP: moisture at the wilting point (corresponding to a matric potential (ψm) of −1500 kPa); AWC: available water capacity; Ds: soil bulk density; Sp: total soil porosity.

Common bean cultivar TAA Dama was sowed on March 4, 2020 using 16 seeds per meter. Thining was done 12 days after emergence (DAE) and 10 plants per meter were maintained in each plot. Nutritional management was carried out according to van Raij (1997) recommendations, based on macro and micronutrient soil analysis.

Pesticide applications were made when necessary and weed control was conducted manually throughout the experiment cycle.

The treatments were distributed completely at random, with five replications. Seven irrigation regimes were evaluated (Figure 1), 100% of field capacity (FC) (M1), 75% and 50% FC from 20 DAS until the last irrigation (M2 and M3), 75% and 50% FC at the vegetative stage (M4 and M5), and 75% and 50% FC at the flowering stage (M6 and M7).
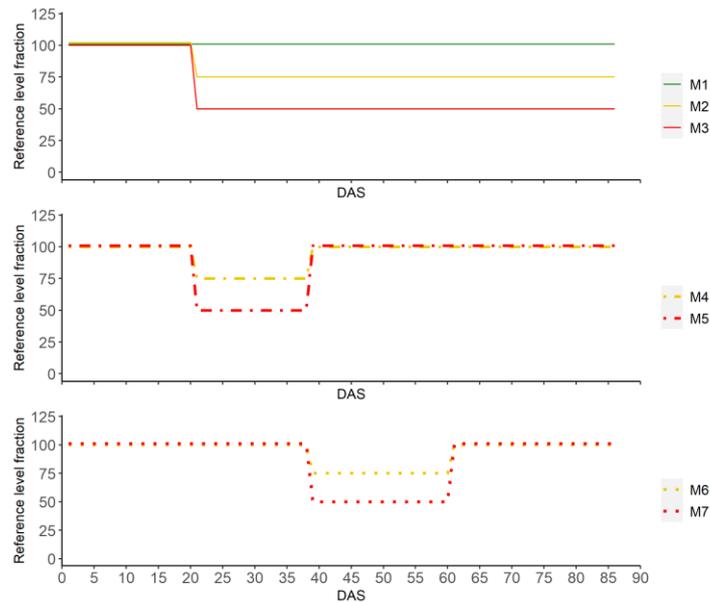

Fig. 1. Graphical representation of irrigation levels of each treatment imposed during all growing cycle; DAS – days after sowing.

A drip irrigation system was used in this experiment, with self-compensating emitters, anti-siphons and anti-drainage. A small drip line (1 m) 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 had individualized irrigation, controlled by micro-registers installed on a control panel. Irrigation was managed according to soil matric potential, monitored in four replications of a well-irrigated treatment (M1). Soil tension data was obtained from four tensimeter batteries, each battery with 3 tensiometers installed at 0.10, 0.30, and 0.50 m depths providing measurement in the center of three soil layers: 0.0-0.20, 0.20-0.40, and 0.40-0.60 m. Irrigation for the 100% FC level was computed by adding the water necessary to increase the soil water to field capacity (FC) for the three layers. The amount of soil water in each layer before irrigation was estimated from the soil matric potential using the van Genutchen soil water retention equation (van Genuchten, 1980).

Measurements of air temperature and relative air humidity were recorded with Vaissala sensor HMP45C-L12 (Campbell Scientific, Logan, Utah, USA) and global solar radiation with LP02-L12 pyranometer (Campbell Scientific, Logan, Utah, USA). The data were integrated every 10 minutes through an automatic station installed inside the greenhouse connected to a CR1000 data-logger (Campbell Scientific, Logan, Utah, USA). For the estimation of reference evapotranspiration (ETo), the method of Penman-Monteith was used (Allen et al., 1998). The meteorological data are shown in Figure 2.
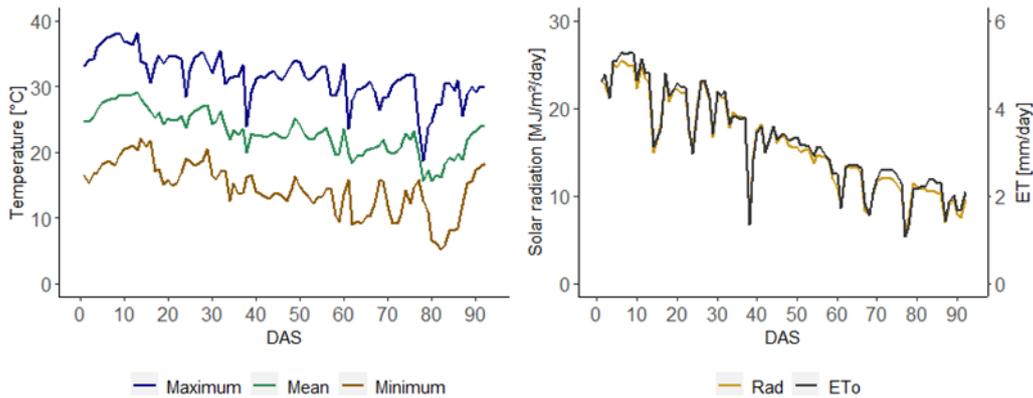
Fig. 2. Maximum, mean and minimum air temperature, global solar radiation and potential evapotranspiration inside greenhouse during the experiment; DAS – days after sowing.

Net photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci) and instantaneous water use efficiency (WUEi) were measured with a portable CO_2/H_2O infrared gas analyzer (IRGA) (LiCOR-Inc, Lincoln, Nebraska, USA), at a photon flux density of 1000 µmol m^{-2} s^{-1}. The leaf gas exchange measurements were performed between 9:00 am and 12:00 pm. The physiological characteristics related to total chlorophyll were evaluated using a portable, non-destructive chlorophyll meter CFL1030 (Falker, Porto Alegre, Brazil). All physiological traits were made in the fully expanded leaves exposed to the sun and located in the middle third of the plants 61 DAS.

All plots were harvested at R9 (physiological maturation) and were dried in a forced-ventilation oven at 60° C for 72 h. The number of pods per plant (PP), total number of grains per plant (TNG), number of grains per pod (NGP) and grain yield per plant (GY) (Kg ha^{-1}) were measured. Water use efficiency was also calculated for each irrigation treatment, which was calculated by the following formula:

$$WUE = \frac{Seed\ yield\ (kg)}{Water\ consumption\ (m^{-3})} \tag{1}$$

All the statistical analyses were performed with R software (http://www.r-project.org). For the analysis, outliers were eliminated since the variety used in the experiment had indeterminate growth and the plots suffered from contamination by overlapping stems and pods. One-way analyses of variance (ANOVA) were performed after testing the homogeneity of variances and normality of the residuals by the Levene and Shapiro-Wilks tests, respectively. The means were compared by the Tukey test at 5% probability. Pearson's linear correlation coefficient was performed for the studied traits. Additionally, principal component analysis (PCA) was used to reduce the dimensionality of the dataset.

3.3. Results and Discussion

Water consumption of the common bean along the crop cycle is presented in Table 2. The average potential water demand was 451 mm. Water reductions for M2, M3, M4, M5, M6 were 94, 157, 18, 35, 37, and 73 mm, respectively. The irrigation depths of this study were within the usual range for this crop, which ranges between 420 and 570 mm under field conditions (Calvache et al., 1997; Muñoz-Perea et al., 2007; Mathobo et al., 2017).

Table 2. Irrigation water accumulated during the crop cycle of common bean

Treatment	M1	M2	M3	M4	M5	M6	M7
Accumulated Irrigation (mm cycle^{-1})	450.64	356.59	293.89	433.15	415.66	414.03	377.43

Analysis of variance showed a significant effect of irrigation treatment ($p < 0.05$) on the morphoagronomic traits, GY, TNG and PP, also for the WUE (Table 3). NGP did not change when subjected to any level of soil drying.

Table 3. Analysis of variance, means and coefficient of experimental variation (CV) of morphoagronomic traits and Water Use Efficiency (WUE).

Source of Variation	Mean square of the Morphoagronomic Traits and WUE				
	GY	NGP	PP	TNG	WUE
Irrigation treatment	39.67*	0.08^{ns}	32.79**	725.30**	0.11*
Error	13.93	0.33	7.86	199.21	0.03
Mean	3202.29	4.53	16.06	72.06	1.30
CV %	19	11	15	17	12

Grain yield (GY); number of grains per pod (NGP); number of pods per plant (PP); total number of grains per plant (TNG); and water use efficiency (WUE); ^{ns}, * and ** are not significant, significant at 5 % probability by the F-test, significant at 1 % probability by the F-test, respectively.

WUE in this study ranged between 1.10 and 1.55 kg m^{-3}. In common bean, Webber et al. (2006) reported values that ranged between 0.20 and 0.60 kg m^{-3} under furrow irrigation, Calvache et al. (1997) reported values that varied between 0.45 and 0.75 kg m^{-3} under sprinkler irrigation, and Spurgeon and Yonts (2013) and Karimzadeh Soureshjani et al. (2019) found values that ranged between 0.60 and 0.70 kg m^{-3} under drip irrigation. This led to confirmation of the advantages of precision irrigation technology in common bean. The WUE for mild water stress in vegetative and flowering stages (M4 and M6) did not differ from the full irrigation treatment and decreased for moderate water stress treatments (M5 and M7) (Figure 4). In legumes, low WUE values were reported when drought occurred during the flowering stage and high WUE values when drought occurred during the vegetative stage (Calvache et al., 1997; Abd El-Wahed et al., 2017; Dipp et al., 2017). In this study, differences in WUE between tested stages were not found, which could be associated with the genotype and frequency or levels of irrigation, but these possibilities will be further tested. In long-term treatments, the opposite occurred than in short-term treatments (Figure 4). For example, moderate stress treatment (M3) presented similar WUE compared to well-watered treatment. Calvache et al. reported similar WUE responses when irrigation withholding occurs between vegetative and ripening stages. Thus, water use efficiency can be considered high due to the high reduction in the amount of water in these treatments (M2 and M3) (Table 2). On the other hand, WUE values were not superior under water stress treatments compared to reference treatment (M1). Webber et al. (2006) concluded that common bean invests photosynthetic resources for root production per unit water use in an attempt to extract more water under water stress conditions, though this strategy is insufficient to increase WUE for biomass and grain. Thus, under different strategies of irrigation withholding, WUE could hardly be improved.
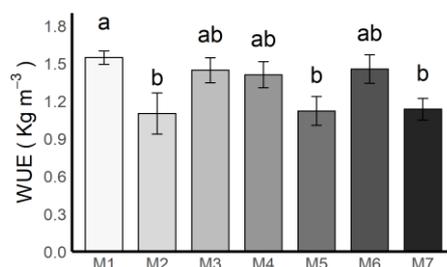
Fig. 4. WUE of common bean evaluated under seven irrigation treatments. Standard error of the means is shown as bars. Letters explain differences between means through Tukey test (p < 0.05).

In this study, the bean cultivar TAA Dama reached a grain yield potential of 4.62 Mg ha^{-1} (Table 4). Santis et al. (2019) reported a mean of 3.46 Mg ha^{-1} testing Carioca cultivars including TAA Dama under field conditions with supplementary irrigation. Moreover, da Silva et al. (2008) reported bean productivity ranged between 1.05 Mg ha^{-1} and 4.05 Mg ha^{-1} under rainfed conditions. In this way, the instability of grain yield due to drought stress and the importance of irrigation strategies can be appreciated. The decrease of GY for irrigation deficit treatments was caused by the reduction in the number of pods per plant and the low number of grains per plant (Table 4). However, only M3 showed significant differences for PP and TNG and the reductions were 40% and 43.5%, respectively. This was expected from previous studies that showed that the yield component most affected by water stress is the number of pods per plant (Nuñez Barrios et al., 2005), mainly by flower senescence and flower abortion (Mathobo et al., 2017). NGP per pod was constant across all irrigation treatments, with a mean of 4.53 seeds per pod. No changes in NGP suggest that the drought imposed in this trial did not disrupt the supply of the assimilates to the pods. In this way, previous studies confirm that NGP is not susceptible to drought stress (Acosta Gallegos and Kohashi Shibata, 1989; Calvache et al., 1997). M3 showed the lowest GY and their reduction was 42% compared to M1. Moreover, drought stress imposed during the vegetative and flowering stage did not reduce yield and yield components compared to the full irrigation treatment (Table 4). The reduction of GY is variable due to differences in the timing and intensity of drought stress (Acosta Gallegos and Kohashi Shibata, 1989; Mathobo et al., 2017). Thus, the reduction under long-term treatments was expected and the non-significant decreases in grain yield under short-term treatments may be associated with the levels and high frequency of irrigation employed in this experiment.

Table 4. Means of morphoagronomic traits of common bean evaluated under seven irrigation treatments. Letters explain differences between means through Tukey test (p < 0.05).

Treatment	GY (kg ha^{-1})	Pods plant^{-1}	Grains pod^{-1}	Grains plant^{-1}
M1	4624.98a	19.94a	4.65	92.23a
M2	3144.50ab	14.38ab	4.49	63.51ab
M3	2692.55b	11.98b	4.61	55.90b
M4	3547.86ab	18.87a	4.32	80.80ab
M5	3173.85ab	13.99ab	4.39	61.71ab
M6	3882.85ab	17.33ab	4.74	82.81ab
M7	3202.29ab	15.90ab	4.52	67.47ab

The analysis of variance indicated a significant effect of irrigation treatment (p < 0.05) on the physiological traits, A, gs, E, iWUE (Table 5). Ci and SPAD did not change when subjected to any level of soil drying.

Table 5. Analysis of variance, means and coefficient of experimental variation (CV) of physiological traits

Source of Variation	Mean square of the physiological traits					
	A	gs	E	Ci	iWUE	SPAD
Irrigation treatment	19.65**	0.015**	4.53**	1222ns	1783.80**	15.48ns
Error	3.43	0.01	0.97	1142	211.50	17.95
Mean	10.57	0.18	4.47	242	0.67	53.60
CV %	23	29	19	12	17	9

Net photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}); stomatal conductance (gs, mol m^{-2} s^{-1}); transpiration (E, mmol H_2O m^{-2} s^{-1}), intracellular concentration of CO_2 (Ci, μmol CO_2 mol^{-1}); intrinsic water use efficiency (iWUE, μmol CO_2 mol^{-1} H20); and SPAD; ns, * and ** are not significant, significant at 5 % probability by the F-test, significant at 1 % probability by the F-test, respectively.

Moderate drought stress treatments reduced photosynthesis for M3 and M7, with the exception of M5, which was measured after rewatering to 100% CC (Figure 5A). These results suggest that photosynthetic rate can be affected by moderate stress at any growth stage. Previous studies have demonstrated that common bean has shown a reduction in photosyntesys as a result of moderate drought stress (Lanna et al., 2016; Dipp et al., 2017; Mathobo et al., 2017). The net photosynthesis had slightly non-significant reductions under mild stress, which was similar to the results reported by Mansour (2021) in fabba bean. Moreover, a previous study showed differences between irrigation frequency treatments on net photosynthesis (Rodriguez-Ortega et al., 2017). Therefore, it could be assumed that for the mild stress treatments, due to the high frequency of irrigation, the available water in the root concentration zone influenced the photosynthetic response. Furthermore, Lanna et al. (2016) reported that each cultivar perceived water availability in the soil differently. On the other hand, treatments under rewatering recovered net photosynthesis capacity (Figure 5A). In a cotton experiment, Luo, Zhang and Zhang (2016) demonstrated that rewatering after moderate drought stress allowed for the same or superior value of net photosynthesis than a well-irrigated treatment. Thus, the recovery of net photosynthesis could indicate that the irrigation withholding of the treatments M4 and M5 did not cause irreversible damage to the photosynthetic apparatus.

The stomatal conductance (gs) and transpiration (E) decreased by 67% and 47% for moderate drought stress treatments M3 and M7, with the exception of M5, which was measured when the irrigation returned to 100% FC (Figure 5B). The decrease in gs might have resulted from stomatal closure, which helps to maintain high leaf water content (Bhattacharya, 2021). Significant reductions in gs and E under moderate stress have been reported for some cultivars of common bean (Dipp et al., 2017; Mathobo et al., 2017). For mild water stress treatments M2, M4 and M6, gs and E values were similar to the well-irrigated treatment. Castañeda et al. (2009) concluded that stomatal conductance is the most sensitive photosyntetic parameter to drought stress, even with a mild level of stress. However, low decreases in gs in this trial could be associated with the frequency of irrigation.

Intracellular concentration of CO_2 (Ci) was similar for all irrigation treatments (Figure 5D). The results were different from previous studies which indicated that moderate water stress increases Ci and mild water stress decreases Ci (Lawlor, 1995; Mathobo et al., 2017). However, there is evidence that for some legume genotypes, Ci values do not change when they are subjected to water stress (Dipp et al., 2017; Fávero et al., 2021). Furthermore, Flexas and Medrano (2002) demonstrated a specific dynamic of Ci in relation to stomatal closure. In this way, constant changes in the soil moisture could have contributed to a particular response of Ci in this trial.

Intrinsic water use efficiency (iWUE) under moderate stress treatments (M3 and M7) was higher than iWUE under well-watered conditions (M1) (Figure 5E). Mild stress treatments had similar behaviour, except for the long-

term treatment (M2). M5 had the lowest iWUE, in response to the rewatering effect after a period of moderate stress. In previous research, a cultivar with similar iWUE responses was classified as drought-tolerant since when subjected to moderate or severe water stress, it increased iWUE values (Dipp et al., 2017). Drought-tolerant cultivars may be able to overcome the limitation on CO_2 diffusion through stomata, most likely through more efficient CO2 mesophyl diffusion and effective CO_2 fixation (Rosales et al., 2012).

SPAD values were similar for all irrigation treatments, indicating that plants did not change in leaf color (Figure 5F). Based on a large data set, Shah, Houborg and McCabe (2017) concluded that SPAD is a non-destructive method to determinate with some degree of confidence relative values of chlorophyll content. However, SPAD values could be doubtful for drought stress in common bean (Rosales-Serna et al., 2004; Gonçalves et al., 2019).
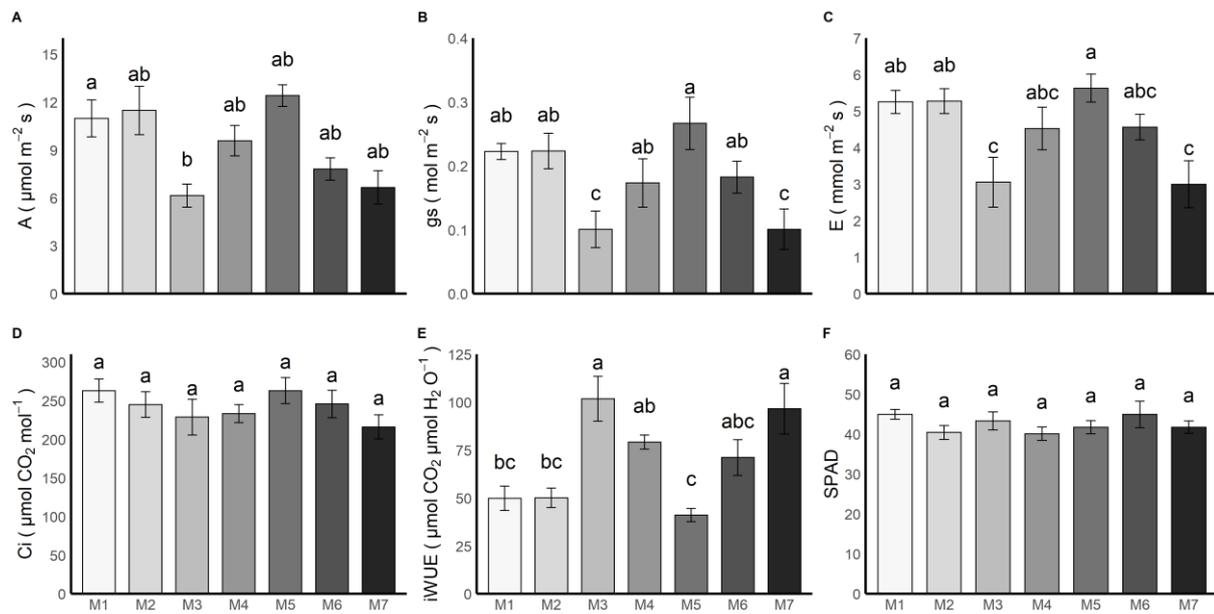

Fig. 5. Means of physiological traits of common bean evaluated under seven irrigation treatments. Standard error of the means is shown as bars. Letters explain differences between means through Tukey test (p < 0.05).

Pearson correlation analysis between physiological and morphological traits is shown in Figure 6. Morphological traits were correlated among them, except NGP with PP and TNG. The correlations between morphological traits were similar to those observed by Androcioli et al. (2020). The strongest correlation observed between GY with PP and NGP revealed the influence of the grain components on productivity. Furthermore, the same authors found the strongest correlation between GY and NGP analyzing drought-stress trials. Thus, NGP could be the yield component most affected by drought stress, even under mild stress. In addition, the correlation between WUE and GY may be attributed to the influence of different water availability levels on plant growth and grain yield.

A, gs and E were strongly positive correlated among them, whereas iWUE maintained a significant negative correlation with all physiological traits except A. The positive significant correlations found between physiological traits A, gs, and E, could be explained because high rates of A are associated with an increase in gs and E (Flexas and Medrano, 2002; Bhattacharya, 2021). On the other hand, Flexas et al. (2013) examined five species and concluded that iWUE has a negative relationship with A and gs in non-stressed conditions and a positive correlation under drought stress. The negative relationship between iWUE with gs and E could be explained because the correlation analysis was

done for all irrigation treatments. Thus, M1 (well-watered treatment), M4 and M5 (measured after rewatering) and mild stress treatments could influence the result.

A and gs had a relationship with GY. Previous studies reported that the reduction in photosynthetic capacity influenced grain yield (Mansour et al., 2021). Furthermore, the grain yield components analyzed did not have correlation with physiological traits. These results suggest the importance of GY as a criterion for evaluation of drought stress in common bean. On the other hand, iWUE did not have a relationship with WUE. Medrano et al. (2015) reported that water use efficiency at canopy level does not scale to water use efficiency at crop level because there are several factors that affect the correlation. Some of the above-mentioned effects that coincide with those found in this trial are: leaf position since the cultivar used had indeterminate growth; and data-set analyzed because significant relations could exist in non-irrigated treatments.
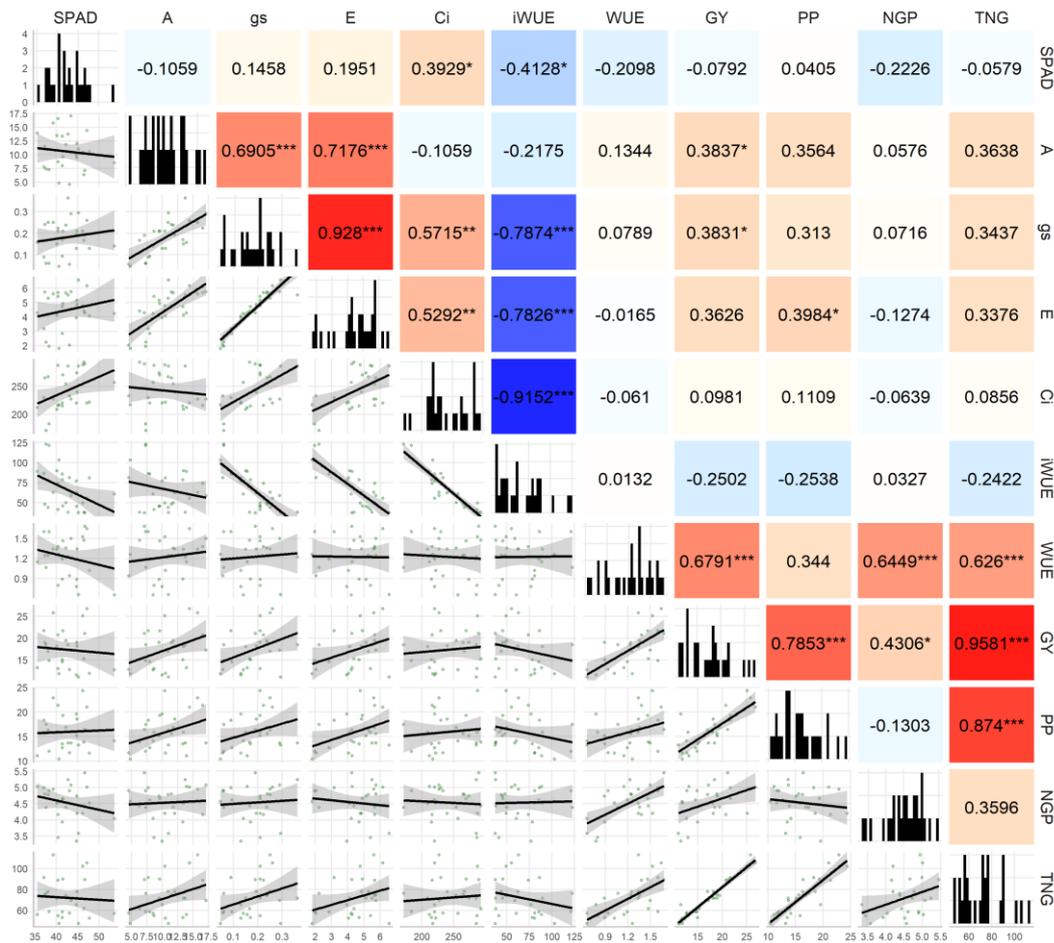
Fig. 6. Scatterplot, correlation matrix and heatmap of the studied morphoagronomic and physiological traits of common bean under seven treatments of irrigation. In the upper panel, red and blue boxes indicate positive and negative correlations, respectively, with increasing color intensity reflects a higher coefficient. The diagonal panel indicates the distribution histogram of correlated traits. The lower panel indicates a scatterplot and trendline of the correlated traits. *, ** and *** indicate significant at p < 0.05, p < 0.01 and p < 0.001. Physiological traits: net photosynthesis rate (A); stomatal conductance (gs); transpiration (E), intracellular concentration of CO2 (Ci); intrinsic water use efficiency (iWUE); and SPAD. Morphoagronomic traits: water use efficiency (WUE); grain yield (GY); number of grains per pod (NGP); number of pods per plant (PP); total number of grains per plant (TNG).

PCA-biplot showed that PC1 and PC2 exhibited about 67% of the total variability (PC1=40.80%; PC2=25.9%). PCA-biplot also indicated the cluster centroids and the approximation of distances between them (Figure 7). The analysis was successful in separating the irrigation treatments, so that short-term mild stress remained close to the well-irrigated treatment and moderate stress treatments remained separate, with the exception of M5, which was under rewatering. Moreover, moderate stress treatments M3 and M7 were significantly correlated with iWUE (Figure 7). Similar responses were reported by Yang et al. (2019) in treatments under severe water-stress, suggesting that iWUE could be used as drought stress indicator.

In the PCA biplot, the angle between the trait vectors approximates the correlation between them, where an acute angle (<90°) represents positive correlation, and an angle of >90° indicates a negative correlation. Thus, for example, WUE and morphoagronomic traits TNG, GY and PP had a great positive correlation with A. PCA demonstrated clearly that correlations of a trait pair (Figure 6) were well coordinated with the approximation of the vector angles in the PCA biplot.
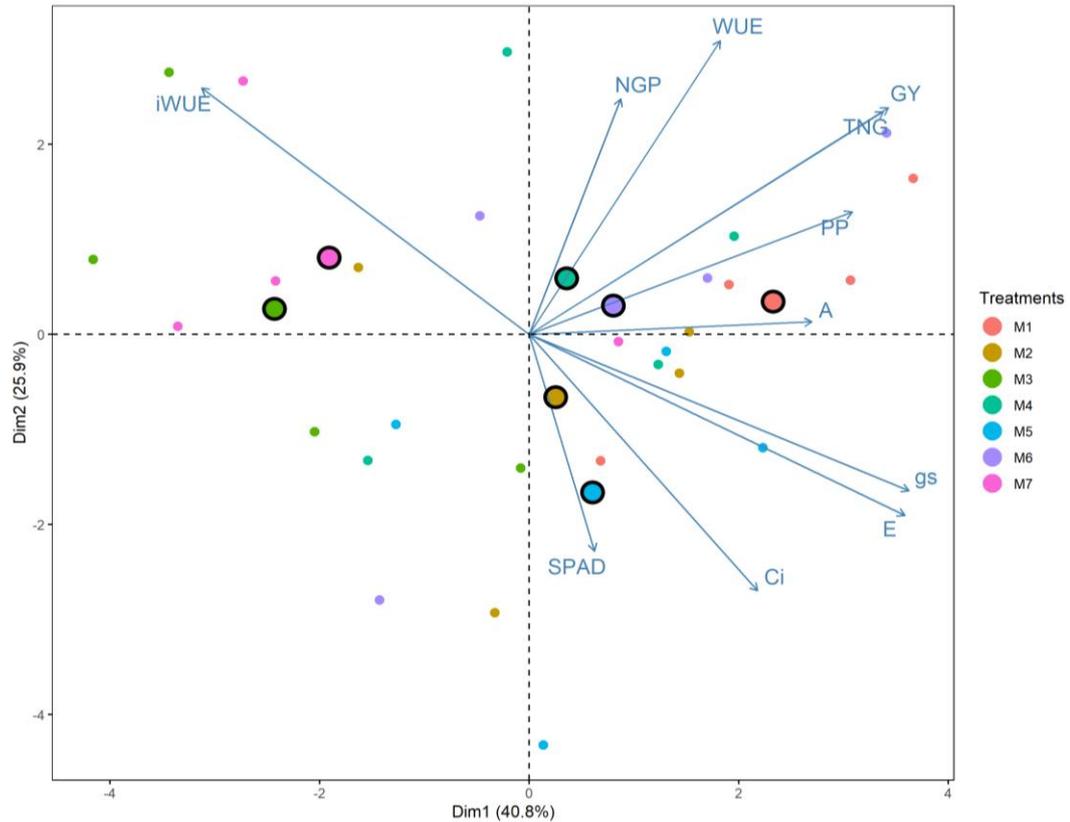
Fig. 7. PCA-Biplot of studied traits and treatments of irrigation. Treatments dispersed in different ordinates based on the dissimilarity among them. The angles between the vectors derived from the middle point of biplots exhibit positive or negative interactions of studied traits. Bigger circles indicate the centroid of the corresponding treatment. Net photosynthesis rate (A); stomatal conductance (gs); transpiration (E), intracellular concentration of CO_2 (Ci); intrinsic water use efficiency (iWUE); SPAD; grain yield (GY); number of grains per pod (NGP); number of pods per plant (PP); total number of grains per plant (TNG).

3.4. Conclusions

WUE under well-watered conditions was 1.55 kg m^{-3} and was similar to reference literature values for common bean under drip irrigation. WUE of long-term moderate drought treatment (M3) and short-term mild drought treatments (M4 and M6) were similar to the reference treatment.

The introduction of moderate drought stress in long-term treatment produced a serious reduction in yield by 42%, while all short-term drought treatments had slight reductions in yield. The well-irrigated treatment reached a grain yield of 4.6 Mg ha^{-1}, slightly superior to traditional values for this cultivar grown under supplementary irrigation in field conditions.

Mild water stress did not affect gas exchange traits. Furthermore, plants of treatments under rewatering recovered their photosynthetic capacity. Correlations among studied traits revealed that stomatal conductance could be used as a reliable reference to evaluate drought stress in common bean. In addition, water use efficiency derived from instantaneous measurements at leaf level did not reflect the water use efficiency measured at crop level.

This document provides information on different strategies to save water without significant losses in grain yield. However, further research is needed to examine smaller levels of irrigation to know the response of common bean to severe water stress.

Additional Files

Additional file 1. Images of the activities in the experiment along the crop cycle. Installation, management, and variable measurement are all included.

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