BIOSCIENCE JOURNAL

PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES OF TWO BEANS COMMON GENOTYPE TO WATER STRESS AT DIFFERENT PHENOLOGICAL STAGES

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How to cite: FOGAÇA, A.M., DE CASTRO, A.G. and BARBOSA, E.A.A. Physiological and morphological responses of two beans common genotype to water stress at different phenological stages. *Bioscience Journal*. 2023, **39**, e39053. https://doi.org/10.14393/BJ-v39n0a2023-59855

Abstract
Comprehension of the bean responses of beans common under to water deficit is an important tool in agricultural planning, like sowing time, and deficit irrigation management strategies. The study aimed to understand the morpho-physiological responses and yield attributes of two common bean genotypes submitted to water stress at different phenological stages. The study was carried out in a greenhouse, in randomized block scheme with five repetitions. To achieve the objectives deficit irrigation of 25% of crop evapotranspiration was practiced during vegetative (DI-V), flowering (DI-F), and pod filling (DI-PF) stages. A non-deficit irrigated (NDI) and deficit irrigated through vegetative to pod filling stages (DI-VP) treatments were added for comparison. The following morphophysiological responses and yield attributes were evaluated: net assimilation of CO_2, stomatal conductance, and leaf transpiration, chlorophyll index, number of trifoliate leaves, chlorophyll index, leaf area, number of grains per plant, number of grains per pod, number of pods per plant, the mass of thousand grains, harvest index, and water use efficiency. The beans genotype under DI-V exhibited acclimation, observed by the relative increment with NDI of 195%, 759%, and 231% of net assimilation of CO_2, stomatal conductance, and leaf transpiration, respectively. Plants under treatment DI-PF experienced dis-stress and plastic responses as leaf losses and exhaustion of gas exchanges. Treatment DI-V received 11% less water than NDI and exhibited equal yield, resulting in higher water use efficiency. Yield attributes correlations indicated that yield penalty might be related to pods abortion, which not occurred to plants under DI-V.

Keywords: *Phaseolus vulgaris* L. Physiological traits. Stage-based deficit irrigation. Water use efficiency. Yield attributes.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is worldwide cultivated, its direct consumption provides micronutrients and proteins, which is crucial for emerging countries (Broughton et al. 2003; Bitocchi et al. 2017). However, it is estimated that 60% of bean production regions are affected by drought, reducing yield by water stress (Beebe et al. 2012). To close yield gaps, breeding programs screen for adapted genotypes that shows superior efficiency in the water use (Darkwa et al. 2016).

In Brazil, cultivation comprises smalls and large-scale agricultural systems that part of them adopt irrigation. To overcome water stress constraints and to expand the possibilities to produce dry beans when prices are high, center-pivot irrigation has become more common, mainly in the central region of the country (Beebe et al. 2012). The total irrigated area in 2020 covers 6.95 million hectares and it is expected an expansion of 4.2 million hectares by 2040 (ANA 2021). The growing water demand for agriculture requires rational and efficient water use since ANA (2021) estimates that 46% of withdrawn water from water bodies are used for irrigation. Highlight, that expansion of agriculture irrigated will intensify conflicts over the multiple water use, as well as its availability for irrigated crops.

Irrigation management strategies will be essential to ensure high levels of productivity. Among the strategies to mitigate the effect of low water availability for irrigation, there is regulated deficit irrigation (RDI) is the technique of irrigation that water depth is reduced based on plant responsiveness to mild water stress in order to increase water use efficiency (WUE) with minor penalties to productivity. An approach of RDI is the stage-based deficit irrigation (SBDI) that is the reduction of water depth during a non-critical growth stage of plants (Kirda 2002). For this purpose, a thorough comprehension of morpho-physiological responses of plants to water deficit stress is crucial (Kumar et al. 2017).

To strategize SBDI, many factors that affect plant responses shall be taken into account as the species, genotype, agronomic management, climate, and soil conditions (Chai et al. 2016). Moreover, it is difficult to isolate water deficit effects in the field due to the superimposition of other abiotic and biotic stresses, resulting in variability among seasons as observed by Webber et al. (2006). Therefore, experiments in greenhouses, where environmental, water supply and pest control are superior, addresses the demand for investigations of plant responses to irrigation shortage (Chaves et al. 2002).

Water deficit promotes several physiological disturbances, such as reduced stomatal conductance (Soureshjani et al. 2019). Dioxide carbon is diffused to the substomatal cavity while stomata are open and enter into the photosynthetic pathway (Chaves et al. 2002). Plants under water stress commonly experience a restriction of gas exchanges by stomatal closure to regulate water losses, trading-off for carbon assimilation (Chai et al. 2016). Adaptive mechanisms are stimulated to cope with water limitations, resulting in eu-stress (elastic or reversible responses), which is indicated as a full recovery of gas exchange constraints when plants are rehydrated. This condition stimulates acclimation, which in turn is a series of complex and synergic adaptations that result in plants that are more resilient. Water stress occurrence during critical growth stages or highly severe water deficit may cause dis-stress that results in plant exhaustion and plastic or irreversible responses, e.g. local death and damage repair, ending in significant yield losses and reduction of WUE (Yordanov et al. 2000; Chaves 2008). However, much is needed to elucidate the physiological basis in relation to the effect of the application of water stress at different phenological stages (Chai et al. 2016); therefore, the trace of gas exchanges and leaf morphology may subsidize understanding the effects of SBDI.

Cultivar responses to induced water stress variate in accordance to its water stress tolerance, even for genotypes cultivated in the same geographical region (França et al. 2000; Darkwa et al. 2016; Soureshjani et al. 2019). The application of RDI on two Iranian cultivars to surpass limitations of water source exposed that genotypes increased WUE (Soureshjani et al. 2019). This result contrasts with the ones reported by Webber et al. (2006) for varieties in Uzbekistan. In Brazil, up-to-date information with current cultivars would encourage the technique to disseminate cooperating with deficit-irrigation expansion. To address this gap, the study aimed to understand the morpho-physiological responses and yield attributes of two common bean genotypes submitted to water stress at different phenological stages.

2. Material and Methods

The experiment was carried out in a greenhouse at Ponta Grossa State University, Paraná, Brazil (25°5'23.88" S, 50°6'8.25" W, and 975 m above sea level). According to Köppen and Geiger classification, the local climate is characterized as Cfb, mesothermic without dry season (Peel et al. 2007). The greenhouse was covered with EVA film of 150 microns and equipped with an evaporative cooling pad and four exhaust fans set to start a wind speed of 2.5 m s^{-1} whenever the temperature reaches a threshold of 25 °C. A thermo hygrometer HT 2000 (Perfect Prime, USA) and a Class A Pan was positioned in the center of the greenhouse in order to record the variation of air temperature, relative humidity, and reference evapotranspiration (*ETo*) during the experimental period. The mean air temperature following the standard deviation during the experiment was 26.0±5.5 °C and the absolutes maximum and minimum air temperature during the plant cycle were 44.2 and 14.50 °C, respectively. The *ETo* through the observational period is presented in Figure 1.

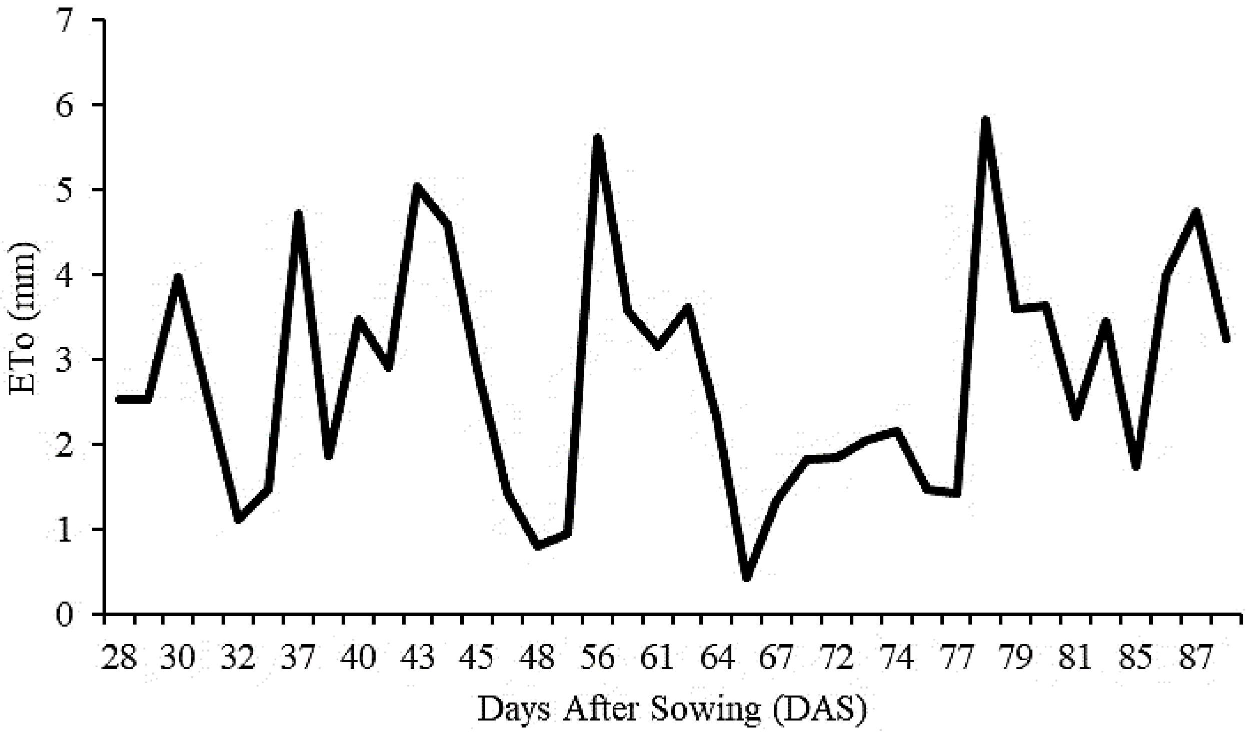

Figure 1. Reference evapotranspiration during the experimental period.

The growth medium was a Ferralsol sifted on 8-mm mesh, which showed the chemical and physical characteristics presented in Table 1. Based on the standardized recommendation for common bean cultivation in Brazil, it was incorporated 1.91 kg dm^{-3} of lime into the growth medium to reach 70% of base saturations three months before the experiment. Then, 10 dm^3 of soil was accommodated on a layer of 1.5 kg of gravel and 2 kg of sand, respectively in 12-liter pots. Lastly, the soil was saturated and left to drain in order to the water status of the soil to be near to the field capacity.

Table 1. Soil chemical and physical characteristics.

pH	H+Al	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	$CTC_{(pH\ 7,0)}$
		---------------------------------- cmol$_c$ dm^{-3} ----------------------------------				
4,9	6,69	0,1	3,2	1,7	0,29	11,88
P	C	Sand	Silte	Clay	V	m
mg dm^{-3}	------------------------ g kg^{-1} ------------------------				----------- % -----------	
6,5	33	158	302	540	43,7	1,9

pH in $CaCl_2$, P in Melich-1; C determined with methodology Walkley-Black; V and m: bases and aluminum saturation, respectively.

Two common-bean cultivars (*Phaseolus vulgaris* L.) extensively cultivated in central and southern Brazil cv. BRS Estilo and cv. IPR Campos Gerais were planted on 72 cell trays. The plants were sown on October 27 and emerged on November 5, 2017. When the primary leaves were fully expanded (September 11, 2017), two plants were transplanted on each pot. At this moment, the base fertilization composed of urea (46-00-00) and MAP+Zn (10-49-00) was applied at the doses of 360 and 40 g pot^{-1}, respectively. Topdressing fertilization was applied at the dose of 310 g pot^{-1} of urea 30 days after sowing (DAS). The more vigorous of the two plants transplanted was traced during the experiment.

Diseases and insects were controlled by treating seed with Standak® Top at 200 ml a.i. 100 kg $seeds^{-1}$. A preventive overhead spray with the fungicide Fox® at 0.5 L ha^{-1} was done when four trifoliates were fully expanded and three overhead sprays were done to control thrips and aphids with Pirate® and Engeo® Pleno at the doses 0.75 L a.i. ha^{-1} and 125 mL ha^{-1}, respectively.

The pots were grouped in five blocks and treatments were distributed in a factorial scheme of two factors. The first source of variation was the imposition of stage-based deficit irrigation (SBDI) by 25% of ETc during one of the three growth stages: vegetative (DI-V), flowering (DI-F), and pod filling (DI-PF). Additional treatments of no deficit irrigation (NDI) and deficit irrigation during vegetative to pod filling (DI-VP) were considered as controls. The value of 25% of ETc was applied because it promotes severe water stress for the crop, according to a previous study carried out with the genotypes in the same greenhouse (Fogaça and Barbosa, 2020). To take into account genetic diversity, the second source of variation was incorporated and was composed of the cultivars (Cv) IPR Campos Gerais and BRS Estilo, which show a life cycle of near to 90 days. The first treatment started when the fourth trifoliate was completely expanded. Subsequently, when 50% of plants bloomed, treatment DI-F took place. Lastly, the pod filling stage was considered when 50% of plants had at least one pod (Figure 2).

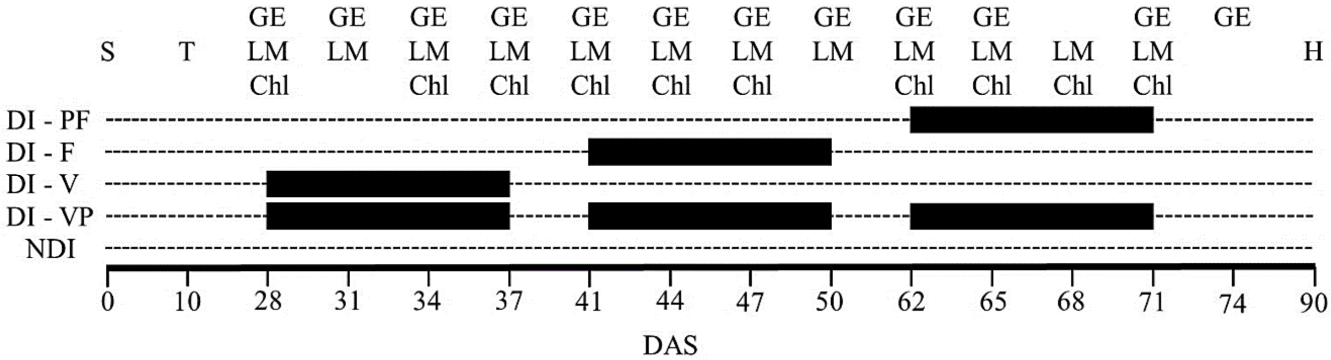

Figure 2. Experimental chronology. Black bars represent deficit irrigation during vegetative (DI-V), flowering (DI-F), and pod filling (DI-PF) stages. A treatment with no water deficit (NDI) and another with water deficit during the vegetative to the pod filling stages (DI-VP) were added for comparison. The sowing (S) represents day zero and transplantation (T) occurred 10 days after sowing (DAS). Evaluations of leaf gas exchanges (GE), leaf morphology (LM) and chlorophyll index (Chl) were done as the treatments were imposed. Plants were harvested (H) at 90 DAS.

Irrigation management was carried out based on the class A pan method. First, the reference evapotranspiration (*ETo*, Figure 1) was obtained by measuring the Class A pan evaporation (*Epan*) positioned in the center of the greenhouse. An empirical coefficient that takes account of the environment influence (*Kp*) was used in the expression *ETo = Epan Kp* with a value of 0.75. Then, the crop evapotranspiration (*ETc*) was calculated by expression ETc = ETo.Kc, being Kc the crop coefficient (Allen et al. 1998).

Kc values for beans common were adopted (Heinemann et al. 2009). This coefficient started by 0.5 at the sowing to the emergence of plants, raised to 1.4 linearly until flowering, and after pod filling decreased gradually until 1.1 at the end of the experiment. Finally, *ETc* was multiplied to the pot area to determine the water volume, which was measured with a graduated cylinder and applied uniformly to the soil.

Instantaneous leaf gas exchanges were measured with an infrared gas analyzer (IRGA) model LI-6400XT (LI-COR, USA) on the center leaf of the fully expanded trifoliate leaf, commonly the third trifoliolate counting from the apical meristem. The internal chamber temperature was set to 25°C, photosynthetic photon flux density set to 1200 µmol photons m^{-2} s^{-1}, CO_2 concentration set to 400 µmol mol^{-1}, and airflow set to 400 µmol s^{-1}. The parameters net assimilation of CO_2 (*A*, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}), and leaf transpiration (*E*, mmol H_2O m^{-2} s^{-1}) were used for further analysis. Measurements were performed between 10h and 12h.

Number of trifoliate leaves, chlorophyll index and leaf area, and of the young fully expanded trifoliolate leaf were recorded in order to understand the effects of water restriction on leaf morphology. Chlorophyll index was recorded with a chlorophyll meter model 1030 (Falker, BR). The third trait was estimated as suggested by Figueiredo et al. (2012) by measuring the length and width of individual leaflets with a pachymeter and applying the following equation:

$$LA= \sum\left(0.575\ (L\ W)\right) \qquad (1)$$

Where *LA* is the trifoliate leaf area (mm^2), *L* and *W* are the individual length and width (mm) of the leaflet, respectively. The equations features R^2 = 0.98.

At 90 DAS, the aerial part of the plants was harvested. The yield attributes: number of grains per plant (NGPl), number of grains per pod (NGPo), number of pods per plant (NPP) were recorded. The mass of thousand grains (MTG, g) was an extrapolation based on the ratio of the NGPl and the mass o grains per plant (MGP, g plant^{-1}) with humidity adjusted to 14%. Then, it was calculated the harvest index (HI, g g^{-1}) as a ratio of the MGP and total aerial biomass and the water use efficiency (WUE, g L^{-1}) as the ratio of MGP and total irrigated water.

All traits at all dates of analysis were subjected to the analysis of variance with stage-based deficit irrigation (SBDI) and cultivars (Cv) as factors. When the probability of F was p≤0.05, SBDI levels were compared by confidence intervals with t distribution (p=0.95) plotted as a time series to enhance visibility, and yield and yield components of cultivars were compared with the Tukey's Honestly Significant Difference test (p≤0.05). The yield attributes relationship was explored using Pearson correlation. All data analyses were performed in software R (R Core Team, 2018) and plotted using package ggplot2 (Wickham 2016).

3. Results

The inspection of factors over gas exchanges and leaf morphology revealed a predominance of single effects (Table 2). From the 71 variables analyzed, only *LA* on 47 DAS was significantly influenced by the interaction of factors at 0.01<p<0.05. Gas exchanges were predominantly influenced by water stress at different phenological stages, being *A* impacted in 69%, *gs* in 46%, and *E* in 61% of observations. The analysis of variance revealed that the Number of Trifoliates or *LA* variated mainly between cultivars, presenting 38% and 54% of observations influenced by Cv and 23% and 15% of observations influenced by SBDI, respectively. Chlorophyll index was mostly unresponsive to the sources of variation, presenting 8% and 15% of observations influenced by SBDI and Cv, respectively.

Table 2. Analysis of variance for gas exchanges and leaf morphology observed from 28 to 74 DAS of two common bean genotypes under stage-based deficit irrigation.

Growth Stage	DAS	A	gs	E	CI	NT	LA
Vegetative	28	NS	Cv*	Cv*	NS	Cv*	NS
	31	NS	NS	NS	NS	NS	Cv**
	34	Cv*, SBDI*	NS	Cv**, SBDI**	NS	Cv*	Cv*
	37	SBDI**	NS	SBDI**	NS	Cv**	Cv**
Flowering	41	NS	NS	NS	NS	Cv*	Cv*
	44	SBDI**	SBDI**	SBDI**	Cv*	NS	Cv**
	47	SBDI**	SBDI**	SBDI**	NS	NS	Cv x SBDI*
	50	Cv,** SBDI**	Cv*, SBDI**	Cv*, SBDI**	-	NS	Cv*
Pod Filling	62	Cv*, SBDI**	SBDI**	SBDI**	Cv*	NS	NS
	65	SBDI**	SBDI**	SBDI**	NS	SDBI*	NS
	68	-	-	-	SBDI**	Cv**, SBDI**	SBDI*
	71	Cv**, SBDI**	Cv**, SBDI**	Cv**, SBDI**	NS	SBDI**	NS
	74	SBDI**	NS	NS	-	-	-

Cv: cultivars; SBDI: stage-based deficit irrigation; Cv x SBDI: interaction of cultivars and stage-based deficit irrigation; -: no data; NS: non-significant; *: significant at 0.01<p<0.05 and ** at p<0.01. NT:Number of Trifoliates; *A*: net assimilation of CO_2; *gs*: stomatal conductance; *E*: leaf transpiration.

The responses of gas exchanges and leaf morphology for each SBDI are demonstrated in Figures 3 and 4. The practice of deficit irrigation during the vegetative growth stages affected plant gas exchanges, resulting in a highly significant suppression (p<0.01) of 32% of *A* and 40% of *E* when compared DI-V to control NDI on 37 DAS (Figure 3A and 3C). This effect was consistent with responses of control DI-VP, which received the same irrigation depth until that moment. Otherwise, water stress applied did not influence *gs* (Figure 3B) or leaf morphology on this growth stage (Figure 4).

Following to flowering, plants under DI-F suffered suppression comparable to DI-VP when taking NDI as reference. On 44 DAS, *A* was reduced in 90% and *E* in 68%; on 47 DAS, *A* was reduced in 72%, *gs* in 83% and *E* in 74%; and on 50 DAS, *A* was reduced in 77%, *gs* in 75% and *E* in 71% (Figure 3). It worth highlight the emergence of responses of *gs* that were highly significant ($p<0.01$), which were not detectable during the previous growth stage, while leaf morphology kept unresponsive (Figure 4). Treatment DI-V showed a compensative behavior verified by superior gas exchanges than control NDI at 44 and 50 DAS. The performance of such plants under this treatment reached values of 195%, 759%, and 231% higher than the means of its counterpart for *A*, *gs*, and *E*, respectively.

Lately, during the pod filling stage, the influence of water stress was detectable from the first observation date (Table 2), when treatment DI-PF exposed a reduction of 55% of *gs* and 58% of *E* compared to NDI (Figure 3). At 65 DAS, this treatment continued to show lower values of gas exchanges than control NDI of 22%, 37%, and 39% for *A*, *gs*, and *E*, respectively. A trend of defoliation started at this date for DI-PF, ending with 5 trifoliates on DAS 71, while other treatments showed a mean of 16 trifoliates (Figure 4, B). When it comes to leaf greenness, this treatment has not shown any reduction, whilst it was detected in treatment DI-VP of 16% on 68 DAS in comparison to NDI (Figure 4, A).

Cultivar BRS Estilo showed a 15% higher yield than IPR Campos Gerais with 17% higher NPP and 18% lower NGPo. These cultivars did not differ in NGPl, but MTG was 14% higher for BRS Estilo. The superior yield culminated in 16% higher WUE with no difference in HI (Table 3).

Table 3. Yield, yield atributes, HI and WUE of two common bean cultivars under stage-based deficit irrigation.

Source of Variation	Yield (g plant^{-1})	NGPl	NGPo	NPP	MTG (g)	HI (g g^{-1})	WUE (g L^{-1})
Cv							
BRS Estilo	6.01 a	20.58	2.67 b	7.78 a	292.40 a	0.43	0.43 a
IPR Campos Gerais	5.21 b	20.70	3.27 a	6.48 b	251.04 b	0.42	0.37 b
SBDI							
NDI	7.18 a	26.10 a	3.15 ab	8.4 a	276.90 ab	0.46 ab	0.46 ab
DI-VP	3.27 c	13.30 d	2.33 b	5.9 c	248.07 b	0.33 c	0.30 d
DI-V	6.90 a	25.05 ab	3.30 a	8.1 ab	278.39 ab	0.48 a	0.50 a
DI-F	5.51 b	20.35 bc	2.97 ab	7.2 abc	271.50 ab	0.42 b	0.40 bc
DI-PF	5.19 b	18.40 c	3.08 ab	6.2 bc	283.72 a	0.43 b	0.36 cd
F-value							
Cv	11.27**	0.01^{ns}	10.43**	9.94**	35.98**	0.97^{ns}	11.24**
SBDI	34.38**	19.46**	3.27*	5.84**	3.26*	22.04**	16.42 **
Cv x SBDI	0.70^{ns}	0.53^{ns}	0.83^{ns}	1.68^{ns}	1.02^{ns}	0.26^{ns}	0.64^{ns}
CV (%)	15.06	18.07	22.14	20.44	8.97	9.25	15.45

Different means indicated by the Tukey test are followed by different small letter in columns. Cv: Cultivars; SBDI: stage-based deficit irrigation; NGPl: number of grains per plant; NGPo: number of grains per pod; NPP: number of pods per plant; MTG: mass of thousand grains; HI: harvest index; WUE: water use efficiency; NDI: no deficit irrigation; DI-VP: deficit irrigation from vegetative to pod filling stages; DI-V: deficit irrigation during vegetative stage; DI-F: deficit irrigation during flowering stage; DI-PF: deficit irrigation during pod filling stage; CV: coefficient of variation; ^{ns}: non-significant difference ($p\geq0.05$); *: significant at $p\leq0.05$; **: significant at $p\leq0.01$. Values in parents are the relative difference to NDI.

Among SBDI, higher yields were of plants under NDI and DI-V treatments followed by DI-F and DI-PF, and the lowest yield was recorded for DI-VP. The yield attributes did not differ between NDI and DI-V. Contrasts of yield attributes were significant for DI-F and DI-PF relative to NDI, being greater for NGPl followed by NPP. Plants under DI-VP were the most affected in yield, with NGPl being the yield attributes mostly decreased. This yield attributes exhibited the strongest relationship with plant yield (Table 4). Other yield attributes were weak to moderated correlated with yield, ranging from 0.34 to 0.67. Screening HI and WUE, treatment DI-V stood as the most efficient in producing seeds per unit of water and unit of above ground biomass. This is due to the practiced irrigation on this treatment saved 1.73 L, resulting in a total of 13.78 L of water during the growth cycle, yet with productivity comparable to NDI.

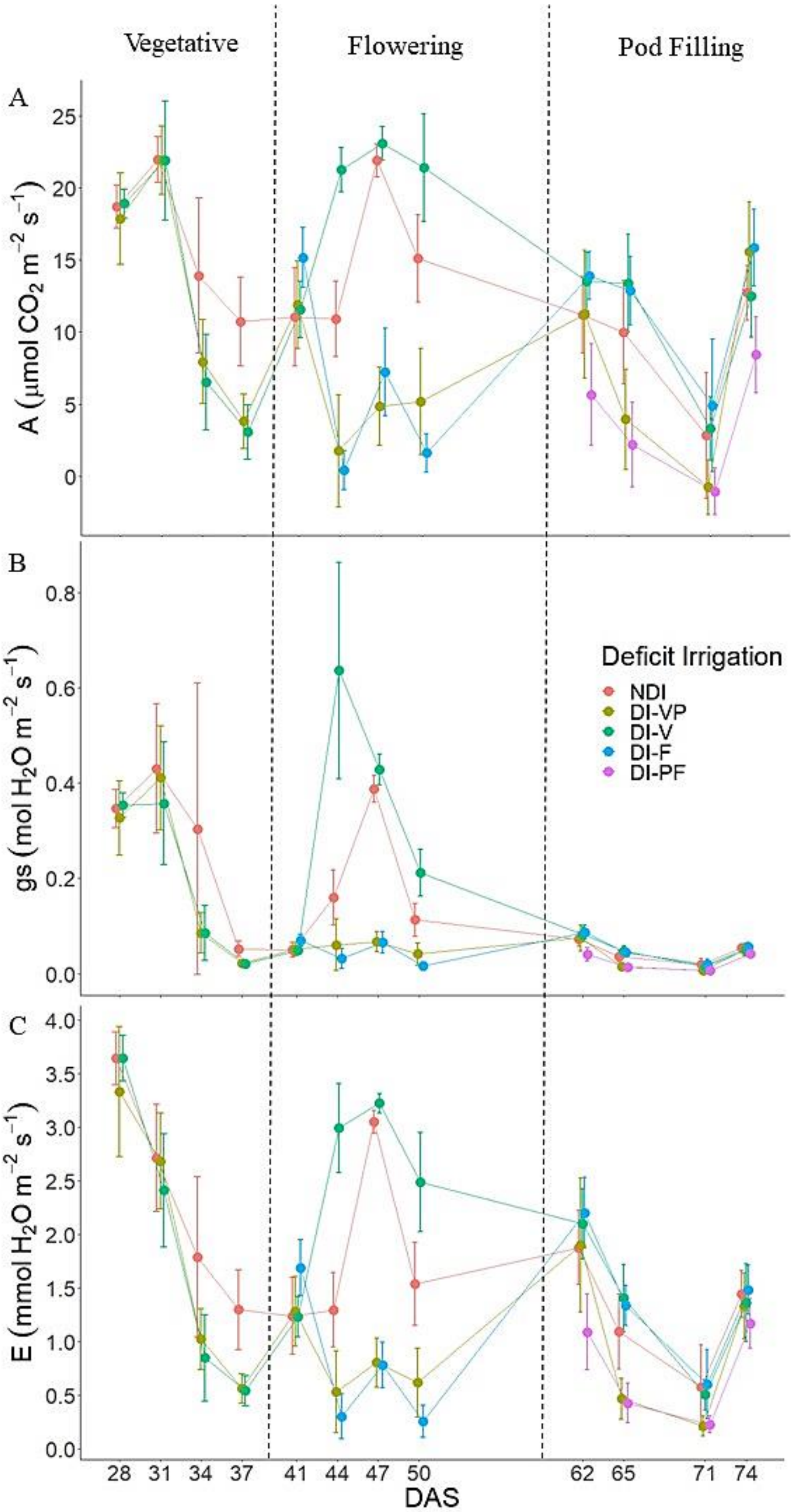

Figure 3. Effect of deficit irrigation during different growth stages on: A - instantaneous leaf net assimilation of CO_2, B - stomatal conductance and C- leaf transpiration observed along the days after sowing (DAS) of two common bean cultivars. Values are means of 10 observations and error bars represent 95% confidence intervals.

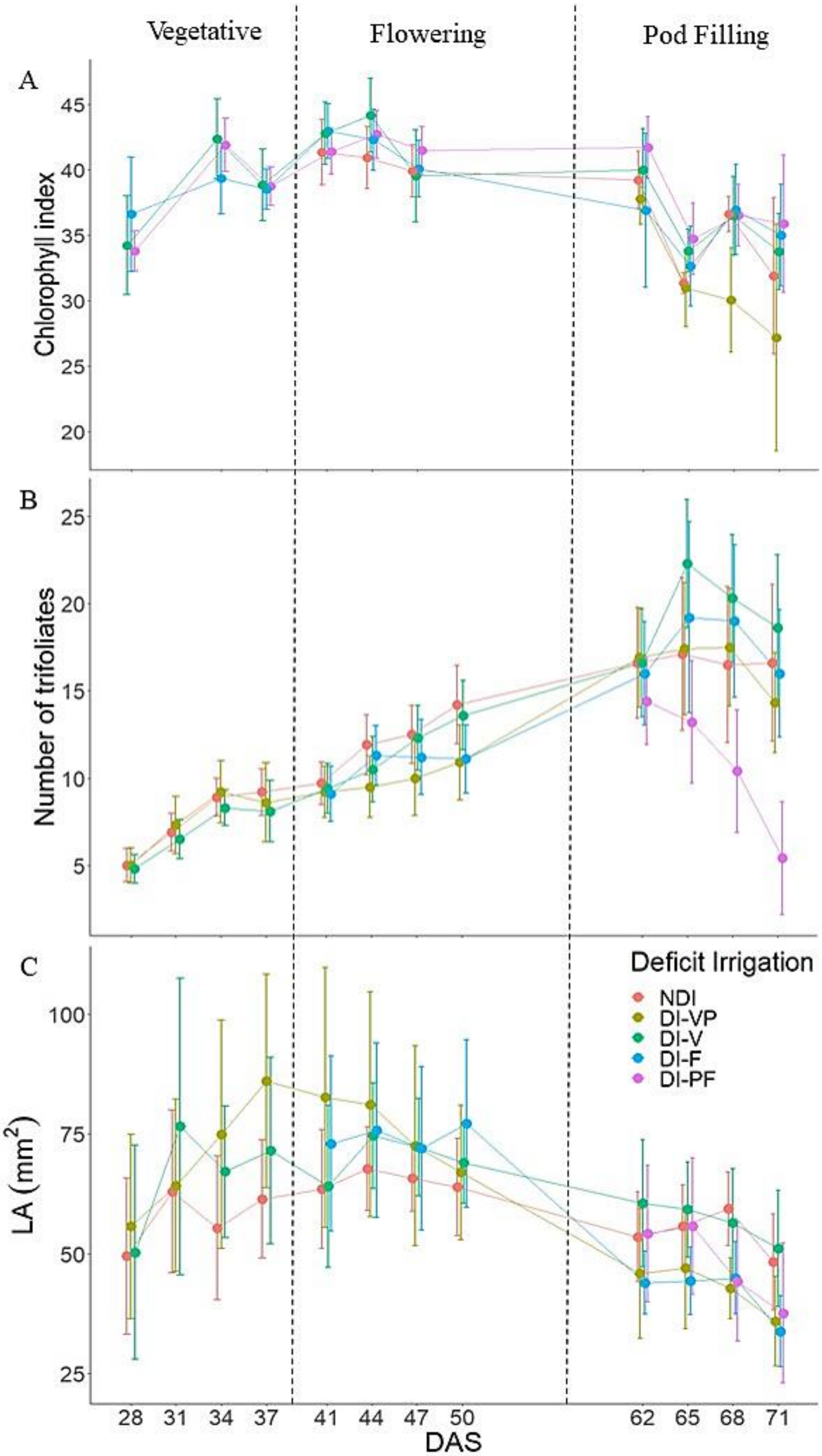

Figure 4. Effect of deficit irrigation during different growth stages on: A - chlorophyll index, B - number of trifoliates and C - young unfolded leaf area along the days after sowing (DAS) of two common bean cultivars. Values are means of 10 observations and error bars represent 95% confidence intervals.

Table 4. Pearson correlation matrix of yield attributes of common bean cultivars BRS Estilo and IPR Campos Gerais under stage-based deficit irrigation.

	Yield	NGPl	NGPo	NPP	MTG
Yield	1.00	0.93	0.34	0.67	0.37
NGPl		1.00	0.41	0.66	0.01
NGPo			1.00	-0.36	-0.05
NPP				1.00	0.11
MTG					1.00

NGPl: number of grains per plant; NGPo: number of grains per pod; NPP: number of pods per plant; MTG: mass of thousand grains.

4. Discussion

The purpose of this study is to provide quantitative data to supply the development of stage-based deficit irrigation based on genotypes responsiveness to water stress. The present study was carried out in a controlled environment to restrain abiotic and biotic stresses that could superimpose the effects of water stress. Diseases were prevented and herbivore insects were successfully controlled. Preventive management for abiotic stresses as toxic Al^{3+} or deficiency of nutrients were done with lime, base fertilization, and top dressing fertilization addition to the growth medium. On the other hand, in a few days, the pad and fan system were not sufficient to control the air temperature, reaching values near to 44 °C that might have affected the plants (Prasad et al. 2002). Although heat stress could not be fully controlled, the data generated is realistic comparing to the field conditions where water stress frequently occurs accompanied with high temperatures (Beebe et al. 2012) and water stress acclimation is suggested to be synergic to heat stress resistance (Yordanov et al. 2000; Chaves et al. 2002).

Cultivars BRS Estilo and IPR Campos Gerais, which feature growth habit type II of the group Carioca, displayed the same responses when under water stress applied during the vegetative, flowering, or pod filling stages, verified by negligible interactions in the analysis of variance (Table 2 and 3). Such evidence is consistent with our previous investigation (Fogaça and Barbosa, 2020). However, this outcome may differ if different cultivars are used, as demonstrate by França et al. (2000), Darkwa et al. (2016), and Soureshjani et al. (2019), or if the methodology of water reduction calculation is different, as observed by Webber et al. (2006) who used soil depletion to impose water deficit.

Contrasts of NGPo and NPP between genotypes indicated a compensative effect that resulted in similar NGPl. This effect corroborates with the observation of over 64 genotypes studied by Darkwa et al. (2016). Hence, the deduction of contrasts among yield attributes indicates that the superiority of BRS Estilo is a result of its higher MTG. This evaluation agrees with other studies that have exposed differences in yield between these two genotypes (Melo et al. 2010; Canizella et al. 2015; Fogaça and Barbosa 2020). With respect to its superior yield and WUE, cultivar BRS Estilo is advisable to be selected as a parent in improving breeding programs that search for plants that acclimatize under situations of deficit irrigation or drought.

Investigations of gas exchanges (Figure 3) revealed that treatments DI-V and DI-F passed through eu-stress evidenced by elastic responses when fully recovered from water shortage, verified on 41 DAS for DI-V and 62 DAS for DI-F. This magnitude of stress is suggested to stimulate plants to acclimate (Yordanov et al. 2000; Chaves et al. 2008). Acclimation was detected on plants under treatment DI-V when exhibited gas exchanges that exceeded the ones under control NDI on 44 and 50 DAS, but not detected on DI-F. This result corroborates with Boutraa and Sanders (2001) that reported detractive effects of water stress during flowering and pod filling.

All treatments undergo similar negative effects than control DI-VP when irrigation was shorted, saved for a dis-tress observed in *A* and number of trifoliates of treatment DI-PF that exhibited plastic responses when it did not recover its gas exchanges as rehydrated on 74 DAS and started a trend of defoliation on 65 DAS. This effect exposes that photosynthetic recovery might be dependent on the moment of water stress imposition and the previous stress memory of plants. This observation may contribute to the demand for investigations on the physiological basis of SBDI (Chaves et al. 2008; Chai et al. 2016).

Stomatal conductance is a known sensible trait for water deficit because is quicker than morphological adaptations against desiccation (França et al. 2000; Chaves et al. 2002), although it was unresponsive to water shortage during the vegetative stage. During flowering, this variable was the most responsive, reaching a relative difference of 759% comparing DI-V to control NDI. Analyzing the effect of SBDI on days with similar *ETo*, it was not possible to identify effects on *gs* on 37 DAS, while on 44 DAS the deficit irrigated treatment was inferior in relation to control (Figure 1 and 3). To the imposed degree of stress (25% of *ETc*), the genotypes tolerated the abiotic stimulus for stomatal closure when vegetating, but not tolerated when flowering or pod filling. This response might be related to the higher number of leaves that contribute to the plant global transpiration during these stages (Figure 4, B). The collected data gives evidence that the gas exchanges of plants are more resilient to water deficit during vegetative growth.

Leaf morphology assessed by chlorophyll index and *LA* were unresponsive to SBDI and the former was only reduced when plants were severely stressed as observed for those under treatment DI-VP on 65 DAS (Figure 4, A). This trait appeared to have a low relationship with gas exchanges and could not evidence the oxidative damage of reactive oxygen species to the photosynthetic apparatus, which agrees with our previous study (Fogaça and Barbosa 2020). Other studies report contrasts in responses of leaf greenness to water stress (Darkwa et al. 2016; Soureshjani et al. 2019). The lower leaf area index is suggested as a mechanism of reduction of transpiration (Soureshjani et al. 2019), but examining *LA* did not evidence water stress effects. When it comes to applying SBDI, the explored genotypes and the degree of stress imposed demonstrated that leaf greenness and *LA* might be insensitive to the effects of water deficit.

Yield and yield attributes of DI-V were statistically similar to NDI and some of them higher in percentage (Table 3). The inspections on gas exchanges (Figure 3) exposed that the resilience of yield could be related with its higher gas exchanges during flowering that compensates the suppression when under water deficit (Chaves et al. 2008). Although investigations revealed elastic responses of plants treated with DI-F, it was not detected acclimation, thus plants not compensated for the restrains of carbon assimilation and resulted in yield penalty. Leaf morphology data revealed plastic responses as defoliation for treatment DI-PF, which had a similar effect on yield and yield attributes with DI-F.

The correlations of yield attributes with yield (Table 4) suggested that yield is dependent on the number of grains per plant, which is moderately correlated with the number of pods per plant. Mass of grains and number of grains per plant displayed weak correlation with yield and exhibited stability even under severe stress. This result agrees with the previously reported by Boutraa and Sanders (2001) and Darkwa et al. (2016) and suggests that losses of pods by flower or pod abortion severely affect yield.

Biomass partitioning indicated by harvest index aids to reveal the SBDI treatment that stimulates plants to maximize grains production. Plants under DI-V produced a higher rate of grains per unit of aerial biomass, coping with yield resilience and increasing WUE. Plants under this treatment showed the highest efficiency in the use of ambient resources since it received 11% less water compared to the well-irrigated plants and had yield the resilience. Treatment DI-PF led to 8% less WUE in comparison to NDI than DI-F (Table 3). Thus, plastic responses might be more detractive to WUE than elastic responses, taking into account that treatment DI-PF was irrigated with a total of 14.32 L and DI-F with 13.88 L. Still, acclimation appears to be necessary to increase WUE, since the elastic response of plants under treatment DI-F was not sufficient to maintain grain production per unit of water.

The application of water deficit during the vegetative stage of common bean could contribute to the rational expansion of irrigated agriculture. If water savings reach 11% as demonstrated in the present experiment, a demand of 271 mm of ETc could be reduced to 241 mm, saving a total of ~30 mm or 30,000 L of water per hectare. This practice not only saves water but electric energy, reduces system wear and labor hours. However, future studies addressing variability among other genotypes, methodologies of water deficit (*ETc* reduction or soil water depletion), and field application could contribute to the expansion of the application of SBDI in Brazil.

5. Conclusions

The application of water stress in the vegetative stage of the genotypes IPR Campos Gerais and BRS Estilo, used in this study, increased water use efficiency. Plants under this condition demonstrated yield resilience attributed to elastic responses and acclimation.

The beans common genotype under the imposition of stage-based deficit irrigation by 25% of ETc in the vegetative stage exhibited acclimation, observed by the relative increment to net assimilation of CO2, stomatal conductance, leaf transpiration and similar morphological and yield attributes in relation to non-deficit irrigated.

The water restriction in the phenological phases flowering and pod filling resulting in yield great loss, thus the attempt to introduce deficit irrigation during flowering and pod filling is not advisable for the studied genotypes and the level of water deficit. In this way, if there is water restriction, producers must direct water to crops that are in the reproductive phase.

Authors' Contributions: FOGAÇA, A.M.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; CASTRO, A.G.: conception and design, acquisition of data, analysis and interpretation of data, and critical review of important intellectual content; BARBOSA, E.A.A.: conception and design, acquisition of data, analysis and interpretation of data, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

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Received: 16 March 2021 | **Accepted:** 16 November 2022 | **Published:** 6 April 2023