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Drought stress tolerance in common bean: what about highly cultivated Brazilian genotypes?

C. C. Dipp · J. A. Marchese · L. G. Woyann · M. A. Bosse · M. H. Roman · D. R. Gobatto · F. Paludo · K. Fedrigo · K. K. Kovali · T. Finatto

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Abstract Drought stress on reproductive stages constitute a major problem for common bean (*Phaseolus vulgaris* L.) production because it affects flowering and pod-filling processes which are highly drought-sensitive. In this study, we used a greenhouse experiment to evaluate the response to drought stress in ten highly cultivated Brazilian genotypes in response to moderate intermittent drought during flowering and pod-filling periods (R7 and R8 stages). Morphological, biochemical, physiological and agronomic traits were used to identify tolerant cultivars and elucidate their strategies to cope this stress. The drought intensity index for the experiment reached 0.63. The cultivar IAC Imperador can be defined as a tolerant cultivar, presenting the lowest grain yield reduction (43%) and a reduced drought susceptibility index (0.65). This cultivar elevated their level of proline in roots under stress, which allowed the osmotic adjustment and the maintenance of an intermediate stomata closure during the day, which maintained the intrinsic WUE stable in NS and DS conditions. In addition, this cultivar was able to mobilize the assimilated carbon for the production of pods and grains, evidenced by the high harvest index and the high grain filling index. In this way, IAC Imperador can be used as a check in breeding programs to identify and select lineages with drought tolerance in common bean.

Keywords *Phaseolus vulgaris* L. · Intermittent drought stress · Drought susceptibility index · Proline · Harvest index · Physiological parameters

Abbreviations

100GW	100 grains dry weight
ABA	Abscisic acid
A_N	Net CO_2 assimilation rate
C_i	Leaf internal CO_2 concentration
CO_2	Carbon dioxide
DAE	Days after emergency
DII	Drought intensity index
DS	Drought-stressed
DSI	Drought susceptibility index
DTI	Drought tolerance index

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C. C. Dipp · J. A. Marchese · L. G. Woyann · M. A. Bosse · M. H. Roman · D. R. Gobatto · F. Paludo · K. Fedrigo · K. K. Kovali · T. Finatto
Departamento Acadêmico de Ciências Agrárias, Universidade Tecnológica Federal do Paraná - Câmpus Pato Branco, Via do Conhecimento - Km 01, Pato Branco, Paraná 85503-390, Brazil

C. C. Dipp · J. A. Marchese · L. G. Woyann · K. Fedrigo · K. K. Kovali · T. Finatto (✉)
Programa de Pós-Graduação em Agronomia, Universidade Tecnológica Federal do Paraná - Câmpus Pato Branco, Via do Conhecimento - Km 01, Pato Branco, Paraná 85503-390, Brazil
e-mail: tfinatto@utfpr.edu.br

E	Transpiration rate
EUW	Effective use of water
FGP	Number of failed grains per pod
GFI	Grain filling index
GMP	Geometric mean productivity
g_S	Stomatal conductance
GY	Grain yield per plant
HI	Harvest index
Ψw	Water potential
iWUE	Intrinsic water use efficiency
LSR	Leaf stem ratio
MP	Mean productivity
NS	Non-stressed
NGP	Number of grains per pod
NPP	Number of pods per plant
RCBD	Randomized complete block design
WUE	Water use efficiency
WUEinst	Instantaneous WUE
YRR	Yield reduction rate
YSI	Yield stability index

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume in the human diet. It provides protein, complex carbohydrates, and valuable micronutrients for more than 300 million people in the tropics (CGIAR 2016). Due to the importance of pulses such as common beans in worldwide human nutrition, the 68th General Assembly of the United Nations (UN) declared 2016 as the "International Year of Pulses" with the theme "Nutritious seeds for a sustainable future". According to the FAO (2015), dried legumes (beans, peas, chickpeas, and lentils) are a cheaper alternative source of protein than meat and are essential components of a healthy diet.

The common bean is a crop of paramount importance in Brazil, being consumed daily by most of the population. In this sense, Brazil stands out as the world's third largest producer, with approximately 3.3 million tons (FAOSTAT 2014). The national yield average was 886 kg ha^{-1} for the 2015/16 crop season, with large differences in productivity between the different regions of Brazil. The south-central region obtained an average productivity of more than 1500 kg ha^{-1}, which is far less than the productive potential of the species, and in the northeast region, the productivities are generally below 500 kg ha^{-1} (CONAB 2015). Drought is a major cause of this productivity constraint (Beebe et al. 2013; Chai et al. 2016; Lanna et al. 2016; Polania et al. 2016a).

Drought affects nearly 60% of the common bean cultivated under rainfed conditions worldwide and can reduce grain yield up to 80% in some regions (Cuellar-Ortiz et al. 2008; Zadražnik et al. 2013). This stress can occur at any stage of development of this crop, but there are stages during which common beans are more sensitive to water restriction. One of these stages is the reproductive stage, which includes flowering and pod-filling, both of which are highly drought-sensitive processes (Farooq et al. 2009; Rosales et al. 2012; Daryanto et al. 2015). Drought stresses in this stage can be classified as terminal drought or intermittent drought (Pérez-Vega et al. 2011; Blair et al. 2012). Terminal drought consists of the restriction of water availability during the reproductive stages until physiological maturity, with no additional water supplied (Rosales et al. 2012, 2013; Beebe et al. 2013; Heinemann et al. 2016). In Brazil, terminal drought occurs in the northeastern region, which presents semiarid conditions (Singh et al. 2001). Intermittent drought consists of the reduction of water availability during the reproductive stages, with a posterior restoration of water levels (Beebe et al. 2013). This condition can affect all bean production regions in Brazil and can vary in frequency and duration in a single year and during multiple years (Oya et al. 2004; Omae et al. 2012).

Modulation of the carbohydrate partitioning towards seed filling has been a successful strategy to cope with drought during the pod-filling stage in drought-resistant cultivars, and efficient carbon mobilization towards the seeds is favored by a mechanism that implies more effective sucrose transport (Cuellar-Ortiz et al. 2008). Several physiological characters for common bean relevant to drought have been evaluated for their effectiveness in identifying drought-tolerant genotypes. Among these physiological characters are net CO_2 assimilation rate (A_N), water use efficiency (WUE), stomatal conductance (g_S), leaf internal CO_2 concentration (C_i) and transpiration rate (E) (Beebe et al. 2013). Photosynthesis is a fundamental physiological process affected by drought, resulting in the reduction of the CO_2 assimilation rate, which decreases the amount of assimilates and, consequently, the growth and grain yield (Rao and Chaitanya 2016). Blum (2009) noted that higher-yielding genotypes under drought stress have greater stomatal conductance and transpiration, allowing greater CO_2 fixation per unit area. The term "more crop per drop" was coined to explain the importance of WUE in drought resistance in different crops (Kijne et al. 2003). In recent years, the concept of effective use of water (EUW) has gained prominence (Blum 2009) to complement the concept of WUE and to address some critical points regarding WUE, e.g., genotypes with higher WUE do not always have higher grain yield; WUE is difficult to be phenotyped; and WUE values can vary within the plant, throughout the day and throughout the year (Medrano et al. 2015; Flexas 2016).

Several morphological characters have been studied and have proven to be effective in identifying drought-tolerant genotypes. Both shoot and root traits are morphological characters that can be used. Grain yield and yield components are of key importance since grains are the goal of production (Polania et al. 2016a). Dry weights and fresh weights of shoot components (leaves, stems and pods) at the mid-pod-filling stage and at harvest (Beebe et al. 2013; Lanna et al. 2016) are also important. Furthermore, there are several indexes that have been shown to be effective: the harvest index (HI) (White and Castillo 1992), grain filling index (GFI) (Beebe et al. 2013), drought intensity index (DII) (Fischer and Maurer 1978; Beebe et al. 2013) and drought susceptibility index (DSI) (Fischer and Maurer 1978; Beebe et al. 2013).

Plants can make use of different strategies to cope with drought stress. These mechanisms can be grouped into three categories: drought escape, drought avoidance, and drought tolerance (Levitt 1972; Beebe et al. 2013; Nezhadahmadi et al. 2013; Singh et al. 2016). Drought escape consists of an accelerated plant cycle, with early flowering and maturity. This mechanism depends on the plant capacity to rapidly relocate photosynthates to reproductive structures, in special for seed production (Beebe et al. 2013; Villordo-Pineda et al. 2015). Drought avoidance is the ability of the plant to maintain relatively high tissue water potential even when soil moisture decreases to non-optimal levels (Namugwanya et al. 2014). Deep rooting ability and reductions of hydraulic conductance, radiation absorption in leaves, water-loss area and evaporative surface are major components of the drought avoidance mechanism in common bean (Beebe et al. 2008; Villordo-Pineda et al. 2015; Assefa et al. 2015). Drought tolerance is the ability of the plant to resist to drought stress by adjusting cell osmosis, cell plasticity, and cell size (Blum 2009; Beebe et al. 2013; Villordo-Pineda et al. 2015). Plants can produce and accumulate many organic solutes in the cytoplasm, and this strategy can account for part of the drought tolerance mechanism (Szabados and Savouré 2010; Rao and Chaitanya 2016; Mwenye et al. 2016). Proline is one of these organic solutes and plays an important role in safeguarding cells from damage caused by drought stress (Rao and Chaitanya 2016; Andrade et al. 2016).

Identifying genotypes that can maintain their productivity under drought stress conditions is the most rational and economical strategy to address this stress and to avoid productivity and economic losses during climate change. In this study, we aimed to analyze the physiological, biochemical, morphological, and agronomic parameters of common beans in non-stressed conditions and under intermittent drought stress applied during the reproductive stage (beginning of the R7 stage) and maintained for 14 days in widely grown Brazilian cultivars. We assessed the dynamics of stomatal closure during the last day of stress and the proline content in the roots and shoots, and we performed the simultaneous analysis of gas exchange, biomass accumulation and yield components to identify drought-tolerant cultivars.

Materials and methods

Plant growth conditions

The experiment was conducted in a greenhouse, with the temperature controlled at 25 ± 5 °C, relative humidity at $70 \pm 20\%$ and a natural light/dark cycle. The experiment was conducted from mid-August until mid-November 2015. We used ten Brazilian common bean genotypes (ANFC9, ANFP110, BRS Esplendor, BRSMG Realce, IAC Imperador, IAC Milênio, IPR Siriri, IPR Tangará, IPR Tuiuiú and IPR Uirapuru), whose characteristics are described in Supplementary Table 1. The IPR Uirapuru cultivar has previously been described as a moderate drought-tolerant cultivar (Moda-Cirino et al. 2001; Molina et al. 2001), and it was used as a check cultivar in this study. All genotypes belong to the Mesoamerican gene pool, with the exception of cultivar BRSMG Realce, which represent the Andean gene pool.

The experiment was conducted as a 10 cultivar × 2 water treatment (non-stressed and drought-stressed) factorial, organized as a randomized complete block design (RCBD) with four replications. Each experimental unit was composed of twelve pots with two plants per pot. Each pot (6 L capacity) was filled with 5 kg of soil + sand, at a 3:1 ratio. The soil samples (0–20 cm) were classified as Hapludox (pH 6.2) and were sun dried and sieved (5-mm mesh). Chemical and physical analyses of the soil were performed, and the soil was fertilized according to common bean recommendations. The entire phytosanitary management was carried out preventively to avoid the occurrence of pests and diseases. Plants were maintained under ideal irrigation conditions using soil water potential sensors. Trickle irrigation was used to maintain the amount of water at 80% of field capacity. Drought stress was imposed for each cultivar when the R7 [beginning of pod formation, according to the CIAT phenological scale (Fernández et al. 1982)] stage was reached. To suppress the irrigation on the same day, sowing was performed at different times according to the cycle of the cultivars. Drought stress was imposed in the treatment condition for 14 days, and for the control condition, the irrigation was maintained (Supplementary Fig. 1). On the night before stress imposition, the pots were saturated with water. Early the next day, they were weighed. The drought level was measured every two days by measuring the percentage of water reduction in relation to field capacity (weight of pots in relation to the first day of stress) and reached 16% of field capacity on the last day of drought stress (day 14). After this, the plants were re-watered, and the soil was maintained at 80% of field capacity until physiological maturity. Water potential (Ψw) was also measured using a Scholander-type SAPS II pressure chamber (model 3115) [Soil Moisture, Santa Barbara, CA, USA (Scholander et al. 1965)]. Petioles of the trifoliate leaves located in the intermediate region of the bean plants were used. This procedure was always performed before sunrise, between 5:00 and 6:00 h.

Physiological and biochemical parameters

Gas exchange analysis

The gas exchange analysis, which includes A_N (μmol CO_2 m^{-2} s^{-1}), intrinsic water use efficiency (iWUE) (A_N/g_S) (μmol CO_2 mol^{-1} H_2O), instantaneous WUE (A_N/E) (WUEinst), g_S (mol H_2O m^{-2} s^{-1}), C_i (μmol CO_2 mol^{-1}) and E (mmol H_2O m^{-2} s^{-1}), occurred between 10:00 and 12:00 h on the last day of stress, i.e., before rehydration. The measurements were performed with a LI-6400XT model infrared gas analyzer (IRGA) (LI-COR, Lincoln, NE, USA) with an artificial source of red and blue light and a CO_2 injection system. The evaluations were performed on fully developed and healthy leaves from three plants per block on the last day before restoring irrigation. The microclimatic conditions in the chamber were kept constant at 25 °C, 1500 μmol m^{-2} s^{-1} of photosynthetic active radiation (PAR), and 400 ppm of CO_2 during all measurements. All genotypes had pods of the R8 stage (genotypes with an indeterminate growth habit had at least 50% of pods of this stage).

Dynamics of stomatal closure during the last day of drought stress

Evaluations of the stomatal closure during the last day of stress were made using three slides from the abaxial leaf epidermis for each genotype and in each treatment using the method described by Voleníková and Tichá (2001). In both conditions, 1 cm^2 of leaf epidermis of each cultivar was collected, with four replications. This analysis occurred at four different times: 9:00, 12:00, 15:00 and 18:00 h (local time). The stomata were analyzed by light microscopy at 400×. Stomata with an aperture more than 0.15 μm were considered opened, and the rest were considered closed. The number of stomata and the percentage of closed stomata at different times were collected.

Determination of proline concentration

The free proline concentration in the shoots and roots was determined on the last day of drought stress for both conditions. Levels of proline were measured according to Bates et al. (1973). Half a gram of roots or a mixture of leaves (composed of young and old leaves) was homogenized in 3% sulfosalicylic acid, and the homogenate was centrifuged at 3000*g* for 10 min. The supernatant was mixed with ninhydrin prepared in acetic acid and 6 M H_3PO_4 and incubated at 100 °C for 1 h. The reaction was stopped in an ice bath, after which the solute was extracted with toluene and shaken for 15 s. The toluene fraction absorbance was then measured at 520 nm using a spectrophotometer (Shimadzu UV-1700 UV–Vis) (Kyoto, Japan).

Morphological traits, yield components, and indexes

On the last day of drought stress, in non-stressed and drought-stressed conditions, the plants were collected to determine fresh and dry weights. We analyzed the fresh weights of the leaves, stems and pods that together composed the total weight of the fresh shoot biomass and the fresh root biomass. Afterward, the biomass was dried at 60 °C in a hot-air oven until constant weight, obtaining the dry weights of these traits. These traits were used to calculate the dry weight of the leaf stem ratio (LSR) and the HI in DS.

Harvest was carried out when the grains exhibited harvest maturity, and the grain yield was corrected to 13% humidity. The following traits were evaluated: number of pods per plant (NPP), 100 grains dry weight (100GW, in g), number of grains per pod (NGP) and number of failed grains per pod (FGP). The grain yield per plant (GY, in g) was calculated using the mean grain yield in every pot and then using the mean of every pot in each replication. Several of these characters are used in the indexes and thus are not presented and discussed individually.

Several indexes were calculated, such as the HI: [seed biomass dry weight at harvest in DS/total shoot biomass dry weight at mid-pod filling in DS × 100]; GFI: [100 grains dry weight under drought conditions/ 100 grains dry weight under non-stressed conditions × 100]; DII: $[1 - (X_{DS}/X_{NS})]$ (Fischer and Maurer 1978; Beebe et al., 2013) and ranges from 0 to 1; DSI: $[(1 - Y_{DS}/Y_{NS})/\mathrm{DII}]$ (Fischer and Maurer 1978; Beebe et al. 2013); drought tolerance index (DTI): $[(Y_{DS} \times Y_{NS})/(X_{DS})^2]$ (Darkwa et al. 2016); mean productivity (MP): $[(Y_{DS} + Y_{NS})/2]$ and geometric mean productivity (GMP): $[(Y_{DS} \times Y_{NS})^{0.5}]$; yield reduction rate (YRR, in %): $[(Y_{NS} - Y_{DS})/Y_{NS}) \times 100]$ (Rosielle and Hamblin 1981; Darkwa et al. 2016); and yield stability index (YSI): $[Y_{DS}/Y_{NS}]$ (Bouslama and Schapaugh 1984; Darkwa et al. 2016), where X_{DS} and X_{NS} are the means of all genotypes under the drought stress and no stress treatments, respectively, and Y_{DS} and Y_{NS} are the mean yield values per plant of a given genotype in the drought-stress and non-stress conditions, respectively.

Statistical analysis

The normality and homogeneity of variances were analyzed by the Lilliefors and Bartlett tests, respectively. The analysis of variance (ANOVA) for all variables was performed using Genes software (Cruz 2013). Some traits were analyzed in a factorial manner (cultivar x condition (non-stressed and drought-stressed)) because the conditions were compared. Traits belonging to this group were the 100GW, A_N, C_i, dry weights of root biomass, E, FGP, free proline concentration in the leaves, free proline concentration in the roots, NGP, GY, g_S, iWUE and WUEinst. Indexes (DSI, DTI, GMP, GFI, HI, LSR, MP, and YSI) were obtained using results from both non-stressed and drought-stressed conditions; therefore, ANOVA was performed to analyze the RCBD comparing the cultivars. Comparison of the means was performed using the Duncan test ($p < 0.05$). All effects were considered fixed. For the analysis of the number of closed stomata, the means and standard errors were plotted. A phenotypic correlation coefficient was obtained for traits in each condition (stressed and non-stressed) and for the indexes. The significance of correlations was analyzed using the t test.

Results

Analysis of variance

Results of the ANOVA for the traits analysed as a factorial are presented in Supplementary Table 2. Significant effects ($p < 0.01$) for the interaction cultivar x condition was obtained for all trait, with exception to dry weights of root biomass. ANOVA results for indexes are presented in Supplementary Table 3. Indexes LSR and YSI were significant at $p < 0.01$. DSI, DTI, GMP and HI presented significant effects ($p < 0.05$). Already, indexes GFI and MP were significant at $p < 0.1$.

Grain yield under non-stressed and drought-stressed conditions

The genotypes were subjected to moderate stress, where the DII reached 0.64. DII considered the grain yield of all cultivars in non-stressed and drought-stressed conditions. The smaller the grain yield was in

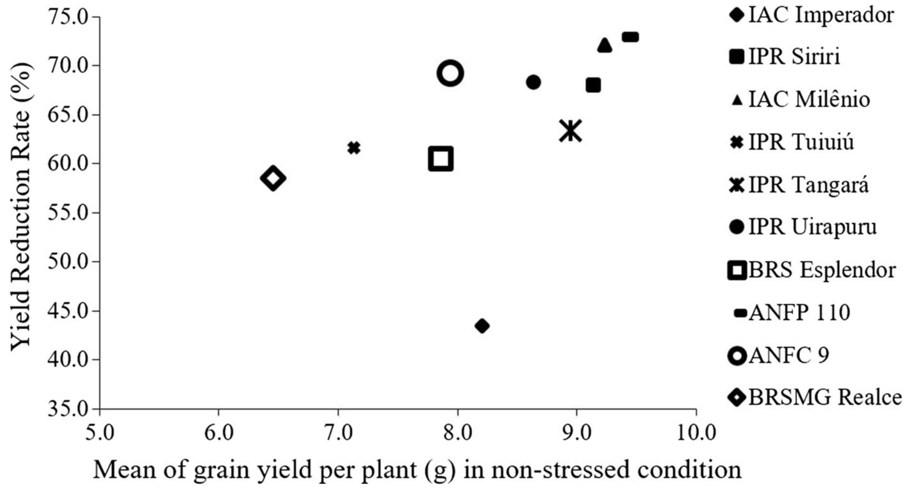

Fig. 1 Mean of grain yield per plant (g) of ten Brazilian common bean (*Phaseolus vulgaris* L.) cultivars evaluated in non-stress condition and yield reduction rate (YRR) under intermittent drought stress in reproductive stages (R7–R8). Drought intensity index (DII): 0.64

the drought-stressed condition compared with the control condition, the higher the DII was.

The results of the cultivar performance in non-stressed condition and the YRR under drought stress at the reproductive stages are presented in Fig. 1. The experiment showed an average GY in non-stressed conditions of 8.3 g and under intermittent drought stress of 3.0 g. In non-stressed conditions (Supplementary Table 4), the cultivars ANFP 110, IAC Milênio and IPR Siriri presented the highest GY, at 9.4, 9.2 and 9.1 g, respectively. At the same time, BRSMG Realce presented the lowest GY, at 6.5 g per plant. Despite the high grain yield in the control conditions, ANFP 110, IAC Milênio and IPR Siriri were the genotypes most sensitive to drought stress, with YRR values of 73, 72 and 68%, respectively. Additionally, IAC Imperador presented a yield close to the general mean yield of the cultivars, but after intermittent drought exposure, it presented the lowest YRR (43.42%), with a GY of 4.65 g per plant.

The mean value of the DSI was 0.95 (Fig. 2). The lower the value of the DSI, the less susceptible the cultivar was to drought stress. The cultivar IAC Imperador showed the lowest DSI (0.65), which did not differ from that of BRS Esplendor (0.87). At the same time, IAC Imperador differed from the other cultivars, presenting the highest DTI (4.55). The higher the DTI index, the higher the grain yield was in both non-stressed and drought-stressed conditions compared with the mean production of all cultivars under drought-stressed conditions. A low DTI indicates that the cultivar does not present high production in non-stressed conditions nor present a high YRR.

The GY and yield components, such as the NPP, 100GW, NGP and FGP, are presented in Table S2. Regarding the yield components, all cultivars significantly reduced their yield in response to drought. With exception of BRSMG Realce, all cultivars reduced the NPP under DS. Regarding the 100GW, the four cultivars ANFC9, IPR Tangará, IAC Imperador and BRS Esplendor did not present decreased values under DS compared with NS. The cultivars IAC Imperador, IAC Milênio and IPR Siriri did not significantly reduce their NGP and did not significantly increase their FGP.

When comparing the 100GW for the cultivars under NS conditions, the highest significant value was observed for BRSMG Realce; this was expected because it comes from the Andean gene pool. However, this was not observed under the DS conditions, in which the 100GW of this cultivar did not differ from that of ANFC9, IPR Tangará, IAC Imperador and IAC Milênio. The NPP did not differ among cultivars. However, regarding the FGP, the cultivars IPR Siriri and IAC Imperador presented the lowest values, but IAC Imperador compensated for productivity by maintaining heavier grains.

Indexes related to grain yield under non-stress and drought-stressed conditions

Several indexes are described in the literature that aim to measure the effects of drought stress in common beans. Cultivar BRSMG Realce had the highest LSR (Table 1). Regarding the HI, cultivars IAC Imperador, IPR Siriri and IPR Tangará showed the highest values of 91, 80 and 72,

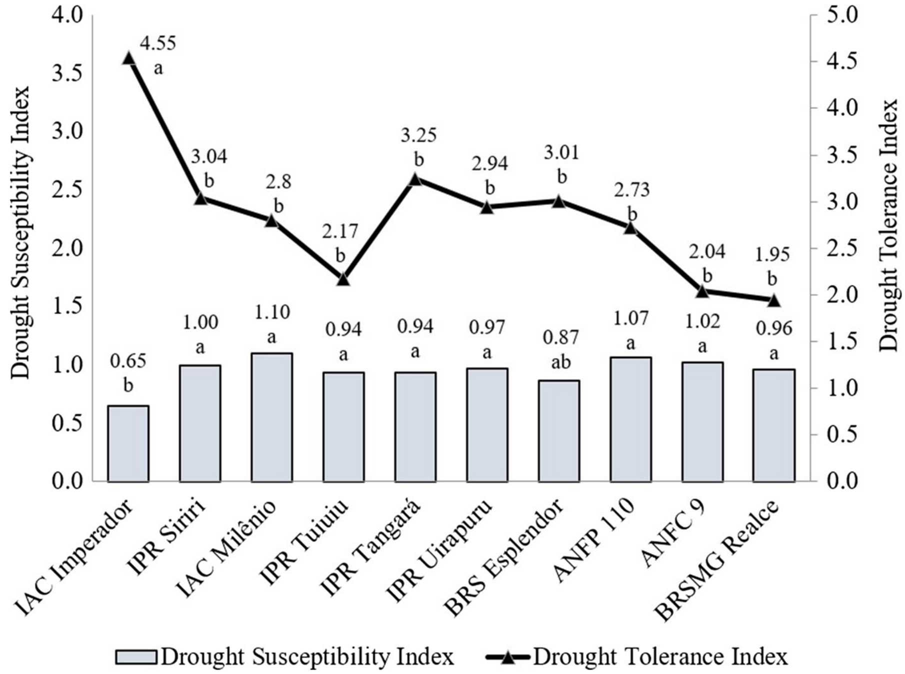

Fig. 2 Drought susceptibility index (DSI) and drought tolerance index (DTI) of ten Brazilian common bean (*Phaseolus vulgaris* L.) cultivars submitted to intermittent drought stress in reproductive stages (R7–R8). Means followed by the same letter do not differ significantly (Duncan's test, $p < 0.05$)

Table 1 Indexes for mensuration of drought tolerance of ten Brazilian common bean cultivars grown under non-stressed and drought-stressed conditions in reproductive stages (R7–R8)

Cultivar	LSR	HI	GFI	MP	GMP	YSI
IAC Imperador	0.55c	91a	109a	6.49a	6.21a	0.57a
IPR Siriri	0.82b	80ab	87ab	5.99ab	5.09bc	0.32b
IAC Milênio	0.71bc	56bc	86ab	5.99ab	4.90bc	0.27b
IPR Tuiuiú	0.71bc	52bc	96a	5.97ab	4.84bc	0.28b
IPR Tangará	0.78b	72abc	100a	5.91ab	5.24ab	0.37b
IPR Uirapuru	0.74bc	56bc	97a	5.79abc	5.02bc	0.35b
BRS Esplendor	0.67bc	56bc	108a	5.56abc	5.07bc	0.42b
ANFP 110	0.64bc	46c	94ab	5.05bc	4.31bc	0.36b
ANFC 9	0.54c	40c	88ab	4.87bc	4.17bc	0.32b
BRSMG Realce	1.02a	43c	68b	4.65c	4.06c	0.35b

Means followed by the same letter do not differ significantly (Duncan's test, $p < 0.05$)

LSR leaf stem ratio, *HI* harvest index, *GFI* grain filling index, *MP* mean productivity, *GMP* geometric mean productivity, *YSI* yield stability index

respectively. With respect to the GFI, cultivar BRSMG Realce presented the lowest index, which differed significantly from that of IPR Uirapuru, IPR Tuiuiú, IPR Tangará, IAC Imperador and BRS Esplendor.

Regarding the MP, cultivar IAC Imperador presented the highest value, which differed significantly from that of the cultivars ANFC 9, BRSMG Realce and ANFP 110. In addition, for GMP, cultivar IAC Imperador presented the highest value, differing from that of ANFC 9, BRSMG Realce, IPR Uirapuru, ANFP 110, IPR Tuiuiú, IAC Milênio, BRS Esplendor and IPR Siriri. Finally, cultivar IAC Imperador presented the highest value (0.57) for the YSI, which differed from that of all of the other cultivars.

Table 2 Intrinsic water use efficiency (WUE), instantaneous WUE, net CO_2 assimilation (A_N), stomatal conductance (g_S), leaf internal CO_2 concentration (C_i) and transpiration rate (E) for ten Brazilian common bean cultivars under non-stress and drought stress conditions in reproductive stages (R7–R8)

Cultivars	Intrinsic WUE A_N/g_S (μmol CO_2 mol^{-1} H_2O)		Instantaneous WUE A_N/E		A_N (μmol CO_2 m^{-2} s^{-1})	
	NS†	DS	NS	DS	NS	DS
IAC Imperador	72.63Ab	64.00Acd	3.06Aa	1.97Bcd	17.97Aa	3.45Bcde
IPR Siriri	68.97Ab	69.27Abc	2.85Aa	2.91Aab	8.46Ade	4.51Babcd
IAC Milênio	60.83Abc	65.57Acd	1.87Ab	1.68Ad	9.33Acd	4.81Bab
IPR Tuiuiú	35.67Bde	81.30Ab	1.47Bb	2.93Aab	12.40Ab	4.62Babc
IPR Tangará	96.67Aa	64.87Bcd	2.56Aa	2.27Acd	17.23Aa	3.77Bbcde
IPR Uirapuru	57.80Bbc	78.90Abc	2.49Ba	3.20Aa	12.62Ab	2.92Bef
BRS Esplendor	90.33Aa	64.67Bcd	2.76Aa	1.95Bcd	8.00Ae	3.39Bde
ANFP 110	52.77Ac	37.93Be	1.68Bb	2.53Abc	11.79Ab	1.88Bf
ANFC 9	50.67Bcd	122.50Aa	1.85Bb	3.20Aa	10.20Ac	5.45Ba
BRSMG Realce	27.00Be	53.63Ad	1.71Bb	2.37Abc	7.95Ae	2.11Bf

Cultivars	g_S (mol H_2O m^{-2} s^{-1})		C_i (μmol CO_2 mol^{-1})		E (mmol H_2O m^{-2} s^{-1})	
	NS	DS	NS	DS	NS	DS
IAC Imperador	0.25Ac	0.05Babc	227.7Bde	272.30Acde	5.88Ac	1.75Bbc
IPR Siriri	0.12Ag	0.07Bab	256.7Acd	244.18Ae	2.97Af	1.56Bbcde
IAC Milênio	0.16Af	0.08Ba	285.2Abc	255.20Bde	4.99Ade	2.91Ba
IPR Tuiuiú	0.35Aa	0.06Babc	298.4Ab	287.67Abc	8.58Aa	1.58Bbcde
IPR Tangará	0.18Aef	0.06Babc	198.3Be	280.90Abcd	6.79Ab	1.68Bbcd
IPR Uirapuru	0.22Acd	0.03Bc	270.9Abc	261.98Acde	5.15Acde	0.91Bcde
BRS Esplendor	0.09Ah	0.06Babc	253.0Bcd	280.68Abcd	2.91Af	1.76Bb
ANFP 110	0.23Acd	0.05Babc	284.6Abc	308.84Ab	7.02Ab	0.75Be
ANFC 9	0.20Ade	0.04Bbc	284.7Abc	262.73Acde	5.56Acd	1.72Bbcd
BRSMG Realce	0.30Ab	0.04Bbc	331.7Aa	350.78Aa	4.67Ae	0.89Bde

Means followed by the same capital letter in the line and the same lowercase letter in the column do not differ significantly (Duncan's test, $p < 0.05$)

NS non-stressed, *DS* drought stressed

Physiological parameters related to drought stress

Regarding the iWUE, IPR Tangará and BRS Esplendor presented the highest values under non-stressed conditions (Table 2). Under drought stress, cultivar ANFC 9 presented the highest value. Cultivars ANFC 9, BRSMG Realce, IPR Uirapuru and IPR Tuiuiú exhibited increases in their iWUE under drought stress, while the other genotypes exhibited reduced values. At the same time, with respect to WUEinst, the same cultivars plus ANFP 110 presented increased WUEinst values. Cultivars IPR Uirapuru, IPR Tangará, IAC Imperador, BRS Esplendor and IPR Siriri presented the highest values in the non-stressed conditions, while, in the drought-stressed conditions, cultivars ANFC 9, IPR Uirapuru, IPR Tuiuiú and IPR Siriri presented the highest values.

Cultivars IPR Tangará and IAC Imperador presented the highest values of net CO_2 assimilation (A_N) in the non-stress conditions. Under drought stress, cultivar ANFC 9 presented the highest value, while cultivars BRSMG Realce and ANFP 110 presented the lowest values. All cultivars exhibited a reduction in A_N under drought-stressed conditions.

Cultivar IPR Tuiuiú presented the highest value of g_S in the non-stressed conditions. Under drought stress, cultivar IAC Milênio presented the highest value, which differed from that of ANFC 9, BRSMG Realce and IPR Uirapuru. All cultivars exhibited reduced g_S values under stress.

Cultivar BRSMG Realce presented the highest value of C_i in both non-stressed and drought-stressed conditions. Cultivar IPR Tangará presented the lowest value in non-stressed conditions, while IPR Siriri presented the lowest value in the drought-stressed conditions. Cultivars IPR Tangará, IAC Imperador and BRS Esplendor exhibited increased C_i values under stress, while IAC Milênio and BRS Esplendor exhibited reductions.

Cultivar IPR Tuiuiú presented the highest *E* under non-stressed conditions, while IAC Milênio presented the highest value in drought-stress conditions. The lowest values were obtained by cultivars BRS Esplendor and IPR Siriri in the non-stressed conditions and by ANFP 110 in the drought-stressed conditions. All genotypes exhibited reductions *E* under stress.

Dynamics of stomatal closure during the day

Considerable variation was observed among the cultivars in both non-stressed and drought-stressed conditions. In non-stressed conditions (Fig. 3), the percentage of closed stomata ranged from 6% (IPR Uirapuru) to 76% (IPR Siriri) at 12:00 h. In the drought-stressed conditions, the percentage ranged from 11% (ANFP 110) to 87% (IPR Siriri) at 12:00 h. In general, a greater percentage of closed stomata were observed in both conditions at 12:00 h. We observed that most cultivars presented a more stable stomatal aperture in the day during drought stress. The difference between the lowest and the highest percentage of closed stomata during the day in the non-stressed conditions for the different cultivars was as follows: BRS Esplendor (7%), BRSMG Realce (13%), ANFP 110 (18%), ANFC 9 (22%), IPR Tangará (22%), IPR Tuiuiú (23), IAC Imperador (28%), IPR Uirapuru (35), IPR Siriri (38%) and IAC Milênio (46%). In contrast, in the drought-stressed conditions the ranking was IAC Imperador (7%), BRS Esplendor (10%), IPR Tuiuiú (12%), ANFC 9 (12%), IPR Uirapuru (14%), IPR Siriri (14%), ANFC 110 (16%), BRSMG Realce (25%), IPR Tangará (22%), and IAC Milênio (32%). In the drought-stressed conditions, the cultivars IPR Tangará and IAC Imperador presented a higher percentage of closed stomata at 15:00 h than in non-stressed conditions; the same behavior was observed in IAC Milênio at 09:00 h. However, among the three cultivars, IAC Imperador presented greater stability throughout the day, showing a variation of 7%, while IPR Tangará and IAC Milênio presented 22 and 32% variation, respectively.

Proline concentration in the shoots and roots

Proline levels were measured in the leaves and roots in non-stressed and drought stressed conditions. ANFC 9 presented the highest value in non-stress conditions (Table 3). Under stress, cultivars ANFP110, IAC Imperador and BRS Esplendor presented the highest levels of proline in leaves, which differed from those of BRSMG Realce, IPR Uirapuru, IPR Tuiuiú, IAC Milênio and IPR Siriri. Cultivar IPR Siriri presented an increase in proline content of more than 500% from non-stressed to drought-stressed conditions. This increase occurred because the proline level was very low in the non-stressed conditions. However, this cultivar presented the lowest content of proline both in stressed and non-stressed conditions. Regarding the roots, no differences were observed in the non-stressed conditions. Under drought stress, cultivars IAC Imperador and IPR Tuiuiú presented the highest values, which were not different from those of IPR Tangará. The highest percentage of increase in proline content in the roots was obtained by the cultivars IPR Tangará and IAC Imperador, at 537.67 and 499.63%, respectively.

Root biomass under intermittent drought stress in the reproductive stages

The dry weight of root biomass is an important trait that is related to drought stress in common bean. In non-stressed conditions, IPR Tuiuiú exhibited higher values that were not different from those of IPR Uirapuru, ANFP 110, IAC Milênio and IPR Siriri (Table 4). In contrast, BRSMG Realce had the lowest values for this trait, and the values were not different from those of ANFC 9 and BRS Esplendor. Already under drought stress, the genotypes presented less variability. Cultivars IPR Uirapuru, IPR Tuiuiú, BRS Esplendor and IPR Siriri presented the highest values, which did not differ from those of ANFP 110 and IPR Tangará. In general, the cultivars exhibited reductions in the dry weight of root biomass, but cultivars IPR Tuiuiú, ANFP 110, IAC Imperador, IAC Milênio and IPR Siriri exhibited significant reductions in their dry weight of root biomass. BRS Esplendor was the only cultivar that presented an increased root biomass dry weight under drought stress.

Coefficient of correlation between grain yield per plant and other traits in non-stressed and intermittent drought-stressed conditions

In the non-stressed conditions, only traits NPP and MP were significantly correlated with GY (Supplementary Table 5). In the intermittent drought stress

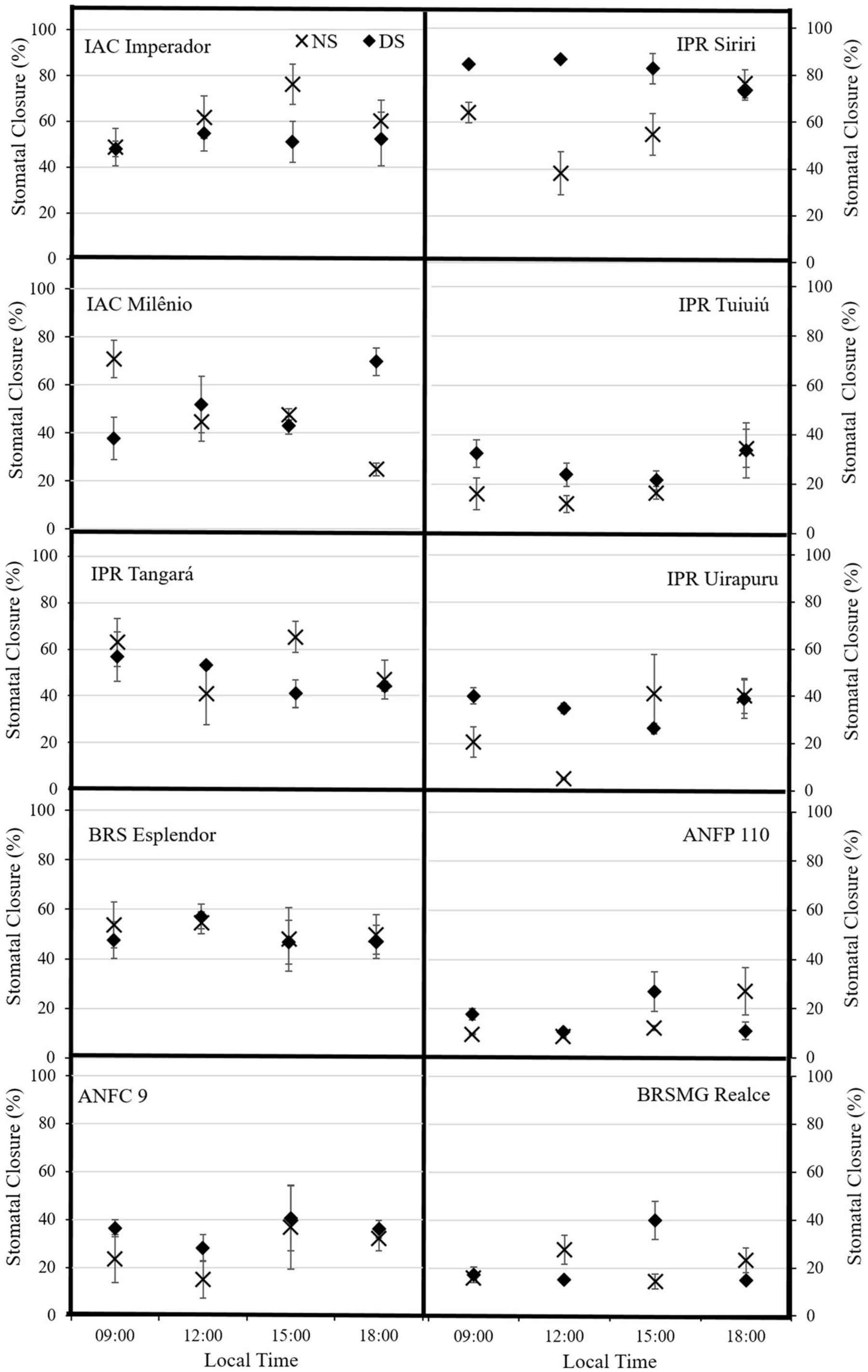

Fig. 3 Percentage of stomatal closure for ten Brazilian common bean cultivars under non-stress and drought stress conditions in reproductive stages (R7–R8). Values are averages of 4 replicates ± standard error (SE)

condition, a higher number of traits presented correlation with GY. Traits HI, DTI, GMP and YSI, and GFI and MP were positively correlated with GY at a 0.01 and 0.05 levels of probability. However, the ISS index was negatively correlated with GY at a 0.01 level of probability.

Table 3 Free proline concentration (μmol g^{-1} fresh weight) in leaves and roots of ten Brazilian common bean (*Phaseolus vulgaris* L.) cultivars submitted to drought stress and non-stressed condition in reproductive stages (R7–R8)

Cultivar	Leaves			Roots		
	NS	DS	% increase in leaves	NS	DS	% increase in roots
IAC Imperador	4.95Bab	16.96Aa	242.63	5.34Ba	32.02Aa	499.63
IPR Siriri	1.06Bc	6.60Ad	522.64	0.94Aa	2.63Ad	179.79
IAC Milênio	7.39Ba	12.35Abc	67.12	5.78Ba	16.95Ab	193.25
IPR Tuiuiú	4.03Babc	12.06Abc	199.26	6.53Ba	31.65Aa	384.69
IPR Tangará	4.45Babc	14.69Aab	230.11	3.69Ba	23.53Aab	537.67
IPR Uirapuru	2.43Bbc	8.65Acd	255.97	4.24Ba	21.74Ab	412.74
BRS Esplendor	5.29Bab	16.47Aa	211.34	0.51Aa	2.58Ad	405.88
ANFP110	2.84Bbc	17.09Aa	501.76	4.47Ba	15.28Abc	241.83
ANFC9	7.43Ba	14.44Aab	94.35	2.86Aa	6.98Acd	144.06
BRSMG Realce	4.61Babc	11.17Abc	142.30	4.06Aa	4.25Ad	4.68

Means followed by the same capital letter in the line and the same lowercase letter in the column do not differ significantly (Duncan's test, $p < 0.05$)

NS non-stressed, *DS* drought stressed

Table 4 Dry weights of root biomass (in g) of ten Brazilian common bean (*Phaseolus vulgaris* L.) cultivars submitted to intermittent drought stress and non-stressed condition in reproductive stages (R7–R8)

Cultivar	Non-stressed	Drought stressed
IAC Imperador	1.29Acd	0.65Bb
IPR Siriri	2.05Aab	1.41Ba
IAC Milênio	1.54Aabcd	0.64Bb
IPR Tuiuiú	2.15Aa	1.37Ba
IPR Tangará	1.47Abcd	1.20Aab
IPR Uirapuru	1.53Aabcd	1.43Aa
BRS Esplendor	1.09Ade	1.34Aa
ANFP 110	1.77Aabc	1.00Bab
ANFC 9	1.05Ade	0.57Ab
BRSMG Realce	0.57Ae	0.64Ab

Means followed by the same capital letter in the line and the same lowercase letter in the column do not differ significantly (Duncan's test, $p < 0.05$)

Discussion

Tolerance or sensitivity to water stress in common bean, as in other species, depends on the genotype, length and severity of water deficit and on the stage of development (Silvente et al. 2012). In this way, drought stress at flowering and at the pod-filling stage is the most important production factor worldwide that affects common bean, especially in the developing world (Darkwa et al. 2016). The shortness of the common bean cycle also influences the effects of drought stress because each stage of development lasts only a few days. The incidence of drought stress during the flowering and pod-filling periods can lead to flower and pod abortion, significantly affecting grain yield (Rao et al. 2013; Farooq et al. 2016; Polania et al. 2016a).

The drought stress imposed on the cultivars was measured by the DII, which reached 0.64. The DII indicates the reduction of grain yield under stress in relation to that of the well-watered controls; therefore, that the greater the reduction of grain yield is, the higher the value of DII and the greater the intensity of stress. A DII of 0.64 indicates a moderate water stress (Ramirez-Vallejo and Kelly 1998; Szilagyi 2003). When the DII reached 0.78, plants were considered to have been subjected to severe drought stress (Ramirez-Vallejo and Kelly 1998). Evaluating genotypes under moderate drought stress conditions is the most appropriate way to identify genetic differences between cultivars and is useful for genotypic selection applications (Darkwa et al. 2016). In contrast, severe drought stress causes an extreme reduction of grain yield that could prevent the identification of more tolerant genotypes among test materials, and insufficient levels of drought stress could result in selection of non-resistant genotypes (Asfaw and Blair 2014; Ambachew et al. 2015; Darkwa et al. 2016).

Other experiments described in the literature reported similar DII values. As such, Terán and Singh (2002) obtained a DII of 0.625, and the drought-stress tolerant cultivars BAT 477, SEA 5, SEA 9 and G 17341 presented DSI values of 1.0, 0.9, 0.8 and 0.7, respectively. In the same study, these four cultivars presented 63, 57, 53 and 41% reductions in seed yield due to drought stress, respectively. Several genotypes have been identified that are considered tolerant to drought and used as checks. Among these materials are BAT 477, SEA 5 and SEA 9. In another study performed by Muñoz-Perea et al. (2006), with a similar DII (0.62), they analyzed sixteen genotypes, including three landraces. The DSI values ranged from 0.5 (cultivar Othello) and 0.7 (Common Red Mexican) to 1.5 for the most susceptible genotype. Most of the genotypes presented DSI values close to 1.0, as observed in our experiment. The Brazilian cultivar IPR Uirapuru was previously described as presenting moderate tolerance to water deficit (Moda-Cirino et al. 2001; Molina et al. 2001). However, in this study, this cultivar did not exhibit this behavior. IPR Uirapuru presented a high DSI, high YRR, reduced HI, reduced YSI, reduced C_i, and reduced percentage of stomatal closure during the day, and this cultivar did not show tolerance to intermittent drought stress during the reproductive stages. This cultivar exhibited increased root weight under stress, but this strategy could be efficient under drought, corroborating the results obtained by Andrade et al. (2016).

The cultivar IAC Imperador was shown to be the only genotype in this study that presented tolerance to drought stress during the reproductive stages. This cultivar is distinguished from the others because it presented reduced values of YRR, DSI and LSR and high values of DTI, HI, GFI, MP and GMP, and YSI. In addition, this cultivar has high levels of proline in the roots and leaves and a stomatal closure rate near 50%, which remained stable throughout the day. This cultivar originated from the introduction of lineages to Brazil that were developed by CIAT with the goal of obtaining drought tolerance (Chiorato et al. 2012). After the evaluation of these lines and the identification of the best ones adapted, they were used in crossing blocks, ultimately leading to the production of IAC Imperador.

Genetic, physiological and morphological characteristics determine drought resistance of common bean genotypes (Müller et al. 2014; Polania et al. 2016b; Zadražnik et al. 2013). The genetic component determines if the physiological and morphological attributes benefit a genotype under drought conditions. Improvement in drought resistance can be a result of (1) superior capacity to acquire water by the root system, facilitating transpiration, (2) increased transpiration efficiency, resulting in increased biomass, and (3) increased HI, i.e., superior capacity to mobilize accumulated carbon to the harvestable economic product (Condon et al. 2004; Polania et al. 2016b). In this sense, the cultivar IAC Imperador seems to employ processes one and three.

The use of process one can be justified by the increased production of osmo-regulatory substances, such as proline. Proline can act as a molecular chaperone, capable of protecting protein integrity and enhancing the activity of different enzymes (Szabados and Savouré 2010). This osmoregulator can act on the osmotic potential of the plant so that the vital processes remain active, averting the loss of water and facilitating water uptake in this situation (Gupta et al. 2013; Kishor et al. 2014). For example, this allows the plant to maintain a medium but stable rate of stomatal closure throughout the day because proline is implicated in the regulation of stomatal responses to water deficit (Wilkinson and Davies 2010; Boyle et al. 2016). In this way, photosynthetic parameters including A_N, g_s, E, and C_i remain as intermediate values in relation to those of the other genotypes. Partial stomatal closure without limiting photosynthesis represents an important target for improving plant responses to reduced water availability (Boyle et al. 2016). According to Rosales et al. (2013), drought-tolerant genotypes present an increasing stomatal closure during the day, and attaining a higher relative water content (RWC) during the night can maximize carbon uptake and limit water loss upon drought. These factors together would lead to fine control of water balance. IAC Imperador showed the most stable stomata dynamics during drought stress, and it is worth mentioning that at 15:00 h this cultivar presented more closed stomata in non-stress conditions than in drought-stress conditions, which suggests that IAC Imperador uses the stomatal aperture as a strategy to maintain photosynthetic parameters during the day, avoiding opening at the hottest time (12:00 h). Under moderate water stress, this capacity becomes more evident, where the increase of proline levels can help this cultivar maintain open stomata.

IAC Imperador did not present the values of iWUE and WUEinst expected for drought stress-tolerant cultivars. The iWUE remained constant between plants of the control conditions compared with those of the drought-stress conditions. In addition, the WUEinst decreased in the drought-stressed conditions compared with those of the control. This indicates that this cultivar does not use the “more grain per drop” approach, indicating that it is also necessary to evaluate the EUW in selecting genotypes for drought stress tolerance. The EUW implies capture of the maximal soil moisture for transpiration, involving reduced non-stomatal transpiration and minimal loss of water by soil evaporation (Blum 2009). In addition, the WUE can be affected by several factors. The individual leaf WUE (both iWUE and WUEinst) is highly dependent on the leaf position in the plant, leaf angle, and canopy geometry. In this sense, only the evaluation of whole-plant water use efficiency (WUE_{WP}) can give a better image of the WUE of the plant (Medrano et al. 2015). IAC Imperador showed the most stable stomata dynamics during drought stress, and it is worth mentioning that at 15:00 h it presented more closed stomata in non-stressed conditions than in drought-stressed conditions, indicating possible evidence of insensitivity to abscisic acid (ABA), which significantly increases to maintain closed stomata during drought stress (Tombesi et al. 2016). The increase in ABA reprograms gene expression patterns, resulting in a cascade of genes that regulate various physiological and metabolic responses (Blum 2015). However, the effect of the message as it is perceived by molecular receptors and transcribed through the network depends on the ABA concentration and the sensitivity to ABA at the receiving end (Blum 2015).

Process three seems to be used by IAC Imperador because this cultivar had an elevated HI. This can be explained by the ability of the genotype to mobilize carbon that has accumulated in the vegetative parts to the reproductive structures—the pods and grain. As observed here, IAC Imperador presented the highest 100GW, and this cultivar did not exhibit a reduced NGP nor an increase in the FGP under stress. This can be verified by the reduction of canopy biomass, especially the leaf biomass and the reduction of root dry biomass in drought-stress conditions compared with those of the control. In this sense, process two does not appear to be present in this cultivar, since the *E* significantly decreased in drought-stress conditions with that of the control during the reproductive stages compared.

The cultivar BRS Realce differs from the other cultivars used in this study because it comes from the Andean gene pool. This cultivar showed the lowest GY in control conditions and among the smallest GY under drought stress. This genotype showed the highest LSR and had among the lowest HI, GFI, MP and GMP. In addition, it exhibited increased iWUE and WUEinst under stress. It also presented the highest C_i as well as increased proline levels in the shoots but not in the roots under drought stress. Polania et al. (2016b) indicated that in general, Andean genotypes present higher susceptibility to drought conditions with low grain production compared with the Mesoamerican genotypes. These authors also indicated that the sensitivity to drought stress in Andean genotypes results from poor performance of mobilization of photosynthates from vegetative growth to pod and grain production.

A single trait alone is usually not sufficient to indicate drought tolerance in common bean genotypes. In this way, Beebe et al. (2013) indicated some core traits to identify tolerant genotypes. These include characters of the shoot, root and yield components. Furthermore, different traits can be more efficient during different plant phases. This occurs because a genotype can use many strategies to address drought, and these strategies can be adopted in different magnitudes at different times. In this way, the evaluation of a set of characters is necessary to quantify the behavior of the genotypes.

In conclusion, we show that cultivar IAC Imperador presents tolerance to intermittent drought stress in reproductive stages. This tolerance results from increased proline levels in the root during drought, maintaining of photosynthetic parameters at appropriate levels, and maintaining of stomatal closure levels at different times of the day ($\sim$50%) as well as the ability to mobilize assimilated carbon to reproductive structures, represented by the harvest index and grain filling index. As such, this cultivar can be used as a source of drought stress tolerance in breeding programs aiming for the improvement of this trait.

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Author's contribution TF, JAM and CCD conceived the study. CCD, MAB, KKK, MHR, DRG, FP, KF, LGW and TF performed the experiment and collected data. CCD, MHR, LGW and TF analysed the data. CCD, TF and LGW wrote the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of conflict of interest.

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