Physiological, biochemical and morphoagronomic characterization of drought-tolerant and drought-sensitive bean genotypes under water stress

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Abstract Water deficit is one of the main factors that reduce grain yield. A better understanding of the mechanisms related to this abiotic stress is a key aspect to design and act upon drought tolerance improvement in crop plants. Therefore, the major objective of this study was to investigate four common bean genotypes for drought tolerance and to establish their tolerance mechanisms. The experiment was carried out in a greenhouse, using the completely randomized design in a factorial arrangement (2×4), composed by 2 water conditions (well-watered and water deficit) and 4 cultivars, with six replicates per treatment. The four cultivars, two drought-sensitive (IAC Tybatã and BRS Pontal) and two drought-tolerant (IAPAR 81 and BAT 477), were evaluated for some physiological, biochemical and morphoagronomic traits. Drought promoted physiological and metabolic changes in the plants, reflecting on the morphoagronomic traits. Under water deficit, the genotype IAPAR 81 stood out from the others in terms of physiological characters, however, it presented a low efficiency concerning biochemical activities and a significant reduction in the morphoagronomic characters. The cultivar BAT 477 demonstrated to be drought-adapted

presenting more efficient biochemical and morphoagronomic adaptations and the genotype BRS Pontal obtained morphoagronomic values similar to BAT 477, thus it may be classified as moderately tolerant to drought.

Keywords *Phaseolus vulgaris* L. · Drought tolerance · Abiotic stress · Plant breeding

Introduction

The common bean (*Phaseolus vulgaris* L.) is considered one of the main legume species for human consumption and it is recognized as an important source of proteins, carbohydrates, fibers and minerals for more than 500 million people in Latin America and Africa (Petry et al. 2015; Blair et al. 2016). In these continents, beans are grown by small farmers, usually in marginal lands prone to abiotic stresses, mainly water deficit (Mukeshimana et al. 2014). Furthermore, approximately one-third of these areas is located in regions subjected to severe periods of drought (Andrade et al. 2016).

The effects of water deficit on beans have been extensively investigated and it has been demonstrated that they depend on the frequency, duration and intensity of the stress as well as on the growth phase of the crop. Drought affects root and shoot growth, reducing cell expansion, stomatal and root hydraulic conductance, photosynthesis and, consequently, dry matter accumulation. Moreover, it causes flower abortion, pod fall and seed weight reduction; it also decreases the number of days for the plant to reach maturity, phosphorus absorption and nitrogen fixation (Asfaw and Blair 2012).

Several studies have been carried out to identify sources of bean germplasm tolerant to drought, which are mainly

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found in the Mesoamerican gene pool in the Durango, Mesoamerica and Jalisco races (Terán and Singh 2002; Beebe et al. 2013). The races Durango, originated in the semiarid central and northern highlands of Mexico, and Mesoamerica, originated in the hot lowlands of Central America, are considered the main sources of genes for the development of drought-tolerant cultivars in tropical environments (Beebe et al. 2008; Asfaw and Blair 2012).

Drought tolerance in beans is a complex character, involving a multigenic regulation, and depending on the environment and on several morphophysiological characteristics (Ramirez-Vallejo and Kelly 1998). Traits related to deep root system, biomass accumulation, stored biomass translocation to the seed and harvest index have been indicated as important contributors to the stability of grain yield under water deficit (Mukeshimana et al. 2014). Regarding the physiological characteristics, photosynthetic efficiency, chlorophyll content, stomatal conductance, transpiration rate, leaf temperature and leaf water potential have been linked to drought tolerance in common bean (Beebe et al. 2013).

In addition to the morphophysiological characteristics, the accumulation of solutes has also been reported as a relevant trait related to the drought adaptation mechanism (Singh et al. 2015). Many plants accumulate osmoprotective solutes in response to the imposition of abiotic stresses that cause cellular dehydration. Among the osmolytes are amino acids, sugars, alcohols and quaternary ammonium compounds, produced to stabilize proteins and membranes, and to reduce the osmotic potential of membranes, preventing intracellular dehydration (Singh et al. 2015). In addition, the accumulation of solutes such as proline and glycine betaine has been described as a crucial source of drought response for some species (Ashraf and Foolad 2007).

Water deficit also leads to oxidative stress due to the formation of reactive oxygen species (ROS), which are partially reduced forms of atmospheric oxygen, such as superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) and oxygen singlet (O_2) (Gill and Tuteja 2010). The accumulation of ROS can damage

membranes and other cellular components causing cell death. In order to control the levels of ROS in the cell, a set of antioxidant enzymes is activated, including superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and catalase (CAT) as well as a wide variety of non-enzymatic antioxidants, as in the case of ascorbic acid (AA) and glutathione (GSH) (Choudhury et al. 2016).

Quantifying these components to get a better understanding of the physiological, biochemical and morphoagronomic mechanisms related to drought tolerance and how they are connected is of paramount importance to define strategies for breeding programs that aim at developing drought-tolerant cultivars. Therefore, the main purpose of this study was to evaluate four common bean genotypes for drought tolerance, analyzing some physiological, biochemical and morphoagronomic traits, and sought to clarify the tolerance mechanisms of the cultivars.

Materials and methods

Experiment conduction and plant material

The experiment was carried out in a greenhouse at the Agronomic Institute of Paraná (IAPAR) in Londrina, Paraná, Brazil (23°22'S, 51°10'W and 585 m altitude), using the completely randomized design in a factorial arrangement (2×4), composed by 2 water conditions (well-watered and water deficit) and 4 cultivars, with six replicates per treatment. Four genotypes of beans belonging to the Mesoamerican gene pool, described in Table 1, were evaluated. The cultivars IAC Tybatã, from the Agronomic Institute of Campinas (IAC), and BRS Pontal, developed at the Brazilian Agricultural Research Corporation (EMBRAPA) are considered drought-sensitive and the cultivars IAPAR 81 from IAPAR, and BAT 477 generated at the International Center for Tropical Agriculture (CIAT) are characterized as drought-tolerant. All the seeds used in the experiment were obtained from the IAPAR germplasm bank.

Table 1 Characteristics of the cultivars evaluated for drought tolerance

Cultivar	Origin ^a	Commercial group	Average lifecycle (days)	Reaction to drought
IAC Tybatã	IAC	Pinto	95	Sensitive (Aguiar et al. 2008)
BRS Pontal	EMBRAPA	Pinto	95	Sensitive (Paranzini 2007)
IAPAR 81	IAPAR	Pinto	92	Tolerant (Moda-Cirino et al. 2001)
BAT 477	CIAT	Brown	94	Tolerant (CIAT 1988)

^aIAC, Agronomic Institute of Campinas; EMBRAPA, Brazilian Agricultural Research Corporation; IAPAR, Agronomic Institute of Paraná; and CIAT, International Center for Tropical Agriculture

The cultivar IAC Tybatã, from the carioca commercial group, is derived from the cross between L933 $\times$ LM30630, and it presents pale straw-colored pods, elliptical shaped, semi full, cream and marbled cream-colored seeds with brown stripes and a light cream halo. The plants have a lifecycle with average growth duration, average weight of 100 seeds of 23.22 g and seed protein content of 24.11% (IAC 2001). The cultivar BRS Pontal, also from the carioca commercial group, comes from the crossing BZ3836 $\times$ FEB 166 $\times$ NA 910523, it exhibits indeterminate prostrate growth habit (type III) and has a lifecycle with average growth duration. The pods are straw and sandy yellow, the seeds are light beige with stripes and brown dots, the average weight of 100 seeds is 26.1 g and the protein content is 21.4% (Embrapa 2006).

The multiple crossing among the genotypes A 248, EMP 117, BAT 93, Carioca, Nebraska and Aroana originated the cultivar IAPAR 81. It has an indeterminate erect growth habit (type II) and a lifecycle with average growth duration. The pods are straw-colored and the seeds have a beige tegument with light brown stripes, the average weight of 100 seeds is 25.1 g and the average protein content is 22% (IAPAR 1997). BAT 477 belongs to the Mesoamerica race, it is a cream-colored, small-seeded (the weight of 100 seeds is 25 g) breeding line and it has an indeterminate prostrate type III growth habit (Briñez et al. 2017).

The seeds were pre-germinated on Styrofoam trays of 128 cells with the substrate Plantmax® and after the emission of the primary leaf (V2), the seedlings were transferred to pots containing 9 kg of substrate. The substrate was composed of 5.62 kg of soil (Red Latosol) and 3.38 kg of sand, which was sieved in a 3 mm mesh and received 50 g of the formulated fertilizer 4-30-10 (N-P₂O₅-K₂O). Two plants were transferred to each pot and when they presented the first trifoliate leaf fully expanded (V3), they were thinned to one seedling per pot.

Induction and monitoring of water deficit

The plants were cultivated under 80% of pot capacity, until the phenological stage R5 (CIAT 1983), appearance of the first floral bud, when water deficit was imposed on the plots under stress. The water regime of 30% of pot capacity was adopted for 19 days, while the other pots continued with sufficient water supply. This period was stipulated taking into account the day of maximum water deficit, in which the plants showed symptoms of severe wilting, high senescence and foliar abscission, being the threshold for the recovery of common bean after a period of drought, according to Boyer (1978).

Substrate moisture levels were monitored with a tensiometer and the variations of maximum, minimum and

average temperature inside the greenhouse and the relative humidity during treatments were measured using a thermohygrograph.

Physiological evaluations

The net photosynthesis (A), stomatal conductance (g_s), transpiration (T) and intercellular CO₂ concentration (iC) of the plants were measured in the early morning (between 8:00 a.m. and 10:00 a.m.) of the last day of stress treatment, in order to avoid maximum transpiration and stomatal closure. These were estimated using the portable system Photosynthesis LI-6400XT (LI-COR Biosciences, Lincoln, NE, USA). The values of the intrinsic water use efficiency ($iWUE$) were obtained by the ratio A/g_s and the carboxylation efficiency (CE) by the ratio A/iC . The central leaflets of the leaves in the middle third of each plant were placed in a measuring chamber 6400-02B of 6 cm², with photon flux density at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and airflow of 400 mL min⁻¹.

Biochemical evaluations

For the biochemical analysis, a leaflet of the central region of each plant replicate was collected on the last day of water-stressed condition, organized in bulk and diluted 1:5 in four different buffer solutions according to protocol (Sommano 2015). The samples were macerated for 5 min, and then centrifuged at 9000 $\times g$ for 30 min at 4 °C.

Regarding the polyphenol oxidase (PPO) analysis, the buffer solution used was composed of 0.2 M KH₂PO₄, 5% polyvinyl polypyrrolidone (PVPP) and 2% Triton at pH 6.8. The samples were then diluted 1: 100 with the same PVPP-free solution, followed by the addition of 250 μL of methylcatechol, homogenization with vortex and heating in a water bath at 30 °C for 30 min. The samples were read in a spectrophotometer at 410 nm. The PPO activity was calculated using the molar extinction coefficient of 2.47 mM⁻¹ cm⁻¹ and the results were expressed as μmol of methylcatechol min⁻¹ mg⁻¹ of plant material.

The buffer solution for glutathione peroxidase (GPX) and catalase (CAT) was 0.1 M KH₂PO₄ and 0.1 μM EDTA at pH 7. For the GPX analysis, the samples were diluted 1:100 with the same buffer solution, received 250 μL of guaiacol and 250 μL of H₂O₂, were vortex mixed, heated in a water bath at 30 °C for 30 min and finally read in a spectrophotometer at 470 nm. The GPX activity was calculated using the molar extinction coefficient of 26.6 mM⁻¹ cm⁻¹ and the results were expressed as μmol H₂O₂ min⁻¹ mg⁻¹ of plant material. During the CAT analysis, it was necessary to use different dilutions (1:20 and 1:80) for the spectrophotometric reading, and then 500 μL of H₂O₂ were added to the samples, which were

Table 2 Analysis of variance, means and estimates of the coefficients of variation of the physiological, biochemical and morphoagronomic characteristics of the bean genotypes evaluated in control and water deficit conditions

Variables	Mean square ^a				Mean ^b		CV%
	Stress (S)	Genotypes (G)	S × G	Error	C	WD	
Physiological characteristics							
A	1385.910**	19.51*	35.40**	5.31	16.25	3.09	23.83
<i>g_s</i>	0.923*	0.005 ^{ns}	0.009 ^{ns}	0.004	0.37	0.03	29.76
<i>i</i> WUE	11,640.200**	550.800**	4016.7**	646.9	45.86	84.00	39.17
T	166.250**	3.870**	3.64**	0.270	5.34	0.78	17.03
<i>i</i> C	9117.30*	12,860.10**	9666.0**	1582.9	305.68	271.93	13.78
CE	0.012**	0.0005**	0.0007**	0.0001	0.05	0.01	30.49
Biochemical characteristics							
GPX	0.217**	0.076**	0.046**	0.002	0.23	0.42	13.49
APX	0.418**	0.091*	0.123**	0.021	1.11	0.85	14.80
PPO	2.562 ^{ns}	1.909 ^{ns}	1.207 ^{ns}	0.606	6.57	5.92	12.46
PAL	0.023**	0.0004 ^{ns}	0.003*	0.0007	0.12	0.19	16.88
Protein	910.52**	4.18 ^{ns}	19.51**	3.36	17.12	4.80	16.73
Proline	0.149**	0.121**	0.020*	0.005	0.84	0.99	7.43
Morphoagronomic characteristics							
NN	0.682 ^{ns}	39.493*	21.397 ^{ns}	12.082	13.54	13.78	25.45
PP	736.71**	69.21*	25.89 ^{ns}	18.66	17.48	8.90	32.74
SP	30,622.54**	2145.38*	1078.36 ^{ns}	620.51	97.86	42.52	35.49
LL	10.332 ^{ns}	0.436 ^{ns}	8.225 ^{ns}	3.743	11.70	10.72	17.67
LW	4.586 ^{ns}	1.654 ^{ns}	2.717 ^{ns}	2.671	7.99	8.27	20.17
TB	2866.88**	96.33 ^{ns}	109.90 ^{ns}	131.72	37.67	21.07	38.35

^aA, net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); *g_s*, stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$); *i*WUE, intrinsic water use efficiency ($\mu\text{mol CO}_2 \mu\text{mol H}_2\text{O}^{-1}$); T, transpiration ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); *i*C, intercellular CO₂ concentration ($\mu\text{mol mol}^{-1}$); CE, carboxylation efficiency ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$); GPX, glutathione peroxidase (activity per minute); APX, ascorbate peroxidase (activity per minute); PPO, polyphenol oxidase (activity per minute); PAL, phenylalanine ammonia lyase (activity per minute); NN, number of nodes; PP, pods per plant; SP, seeds per plant; LL, leaflet length (cm); LW, leaflet width (cm); TB, total biomass (mg)

^bC, control (well-watered); WD, water deficit; ^{ns}, not significant; and **, * significant by F test at 1 and 5% of probability, respectively

Fig. 1 Common bean plants with different vegetative performances in control and water deficit conditions

homogenized and heated in a water bath at 30 °C for 30 min. The reading was performed at 240 nm. The CAT activity was calculated from the absorbance reading divided by the time of 30 min using the molar extinction coefficient of $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ and the results were expressed as $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1}$ of plant material.

For ascorbate peroxidase (APX), the buffer solution used was 0.05 M KH_2PO_4 and 0.1 μM EDTA at pH 6. The plant extract was diluted 1:100, mixed with 500 μL of ascorbate and heated in a water bath at 30 °C for 30 min and the reading was taken at 290 nm. The APX activity was calculated using the molar extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ and the results were expressed as $\mu\text{mol ascorbate min}^{-1} \text{ mg}^{-1}$ of plant material.

The analysis of phenylalanine ammonia-lyase (PAL) and total proteins were performed using the same buffer solution, which was composed of 50 μM sodium borate, 0.005 M dithiothreitol and 5% PVPP, at pH 8.5. For the

Fig. 2 Comparison of means using the Tukey test ($P < 0.05$) for physiological characters of bean genotypes, evaluated in control and water deficit conditions. * Uppercase letters refer to the water conditions, while lowercase letters refer to the genotypes

analysis of PAL, the samples were diluted 40 times, 350 μL of phenylalanine were added; the solution was heated in a water bath at 40 $^{\circ}\text{C}$ for 1 h and read at 290 nm. The PAL activity was calculated using the molar extinction coefficient of 30.5 $\text{mM}^{-1} \text{cm}^{-1}$ and the results were expressed as μmol phenylalanine $\text{min}^{-1} \text{mg}^{-1}$ of plant material. The protein samples were diluted 200 times, mixed with 1000 μL of Bradford's solution, homogenized and heated at 30 $^{\circ}\text{C}$ for 5 min. The protein content was

calculated based on a standard curve with 40–400 μg of bovine serum albumin and the results were expressed as mg of protein g^{-1} of plant material.

The plant extract obtained with the buffer solution of the PAL analysis was used for the proline analysis. The samples were diluted 50-fold in 70 $^{\circ}\text{GL}$ ethanol. A reactive solution containing 150 mL of 60% glacial acetic acid, 1.5 g of nihydrine and 30 mL of 70 $^{\circ}\text{GL}$ ethanol was prepared. A volume of 700 μL of the sample and 1400 μL

of reactive solution were pipetted, homogenized and heated in a water bath at 95 °C for 20 min and then the reading was performed at 520 nm. The proline concentration was calculated based on a standard curve with 1.15–1200 mg L⁻¹ of proline and the results were expressed in µg of proline g⁻¹ of plant material.

Morphoagronomic evaluations

At the end of the crop cycle, morphoagronomic evaluations were carried out, including number of nodes, pod and seed number per plant and total biomass (root, stem, leaves and pods).

Data analysis

Analysis of variance was undertaken after checking the data for homogeneity of variance and normality. The means were compared using the Tukey test at a significance level of 5% of probability. A cluster analysis heatmap was also generated. The software R (<http://www.r-project.org>) and the ExpDes package were used for all analysis.

Results and discussion

The joint analysis of variance showed significant effect of stress for the majority of traits investigated (Table 2). Moreover, water deficit (WD) promoted a reduction in agronomic, physiological (except for intrinsic water use efficiency—*i*WUE) and some biochemical characters. In contrast, an increase was observed for glutathione peroxidase (GPX), phenylalanine ammonia lyase (PAL) and proline, indicating that the stress promoted changes in the physiological and metabolic activities of the plants, reflecting on the morphoagronomic characteristics. One of

the main effects of drought is the reduction of the vegetative and productive growth, which is in accordance with the results of the experiment that showed a 44% reduction on plant biomass (TB), as can be seen from Fig. 1.

Concerning the variation source genotypes, significant effects were observed for number of nodes (NN), seeds per plant (SP), GPX, APX, proline, and for all physiological characters, except stomatal conductance (*g_s*). In the interaction stress × genotypes (S × G), significant effects were verified for most of the physiological and biochemical traits studied, indicating a differentiated behavior of the genotypes regarding environment (Table 2). However, for the morphoagronomic variables, no significant effect for the S × G interaction was detected.

The decomposition of the S × G interaction was performed for all variables, and no significant effect was observed for some characters. According to Barbin (2013), although the interaction may not be significant, it is often advisable to decompose the degrees of freedom, since there is a possibility of detecting a significant effect that was hidden. Thus, the decomposition revealed effects that were significant for PP, SP, TB and *g_s*.

For gas exchange measurements (net photosynthesis—A, transpiration—T and *g_s*), significant reductions were verified for all genotypes under WD (Fig. 2). One of the first reactions of the plant in response to water deficit is stomatal closure, induced to avoid water loss through transpiration, also causing the reduction of the availability of CO₂ inside the leaf, and, consequently, leading to a decrease in the photosynthetic rate (Androcioli et al. 2016).

The reduction of net photosynthesis as a consequence of WD was lower in tolerant genotypes when compared to the sensitive ones, and the tolerant plants presented the highest values for this trait under WD (Fig. 2). For T and *g_s*, the tolerant and sensitive genotypes did not differ under water restriction. However, despite the negative impact that WD had on the gas exchange, the tolerant cultivars IAPAR 81

Table 3 Comparison of means using the Tukey test ($P < 0.05$) for biochemical traits of bean genotypes evaluated in control and water deficit condition

Genotypes	GPX ^a		APX		PAL		Protein		Proline	
	Control	WD ^b	Control	WD	Control	WD	Control	WD	Control	WD
BAT 477	0.345Ba	0.608Aa	1.289Aa	0.784Bab	0.117Ba	0.197Aab	18.853Aa	4.379Ba	0.643Bc	0.957Ab
IAPAR 81	0.233Ab	0.248Ab	1.004Aab	0.623Bb	0.142Aa	0.146Ab	19.538Aa	3.626Ba	0.887Ab	0.843Ab
BRS Pontal	0.199Bb	0.281Ab	1.260Aa	0.942Bab	0.138Ba	0.190Aab	13.780Ab	5.906Ba	1.069Aa	1.177Aa
IAC Tybatã	0.156Bb	0.556Aa	0.897Ab	1.045Aa	0.099Ba	0.212Aa	16.302Aab	5.287Ba	0.793Bbc	0.843Ab

^aGPX, glutathione peroxidase (activity per minute); APX, ascorbate peroxidase (activity per minute); PAL, phenylalanine ammonia lyase (activity per minute); Protein, mg g⁻¹ of plant material; Proline, mM g⁻¹ of plant material

^bWD, water deficit. Means followed by the same lowercase letter in the column and upper case in the row do not differ by the Tukey test, at a significance level of 5% of probability

and BAT 477 presented an increase in *i*WUE of 143.42 and 166.76%, respectively, when compared to the plants under the control condition. According to Rosales et al. (2012), the tolerant genotypes are able to overcome the limitation in CO₂ diffusion through stomata by mesophyll diffusion of the gas and by having a more effective carbon fixation.

The intercellular CO₂ concentration (*i*C) was reduced for the tolerant genotypes under WD, which presented the lowest values in this condition. In plants under dry conditions, stomatal closure limits CO₂ diffusion from the external source to the leaf mesophyll, resulting in a reduction of internal CO₂ (Chaves and Oliveira 2004). One of the consequences of this reduction is the increase in the oxygenase activity of the enzyme Rubisco leading to the production of 3-phosphoglycerate and phosphoglycolate, and therefore, stimulating photorespiration. This process reduces the efficiency of photosynthesis, since it deviates part of the reducing power from light-dependent reactions without generating any ATP (Taiz and Zeiger 2009). Furthermore, CO₂ limitation may result in ROS accumulation, causing indiscriminate damage to the photosynthetic apparatus (Bhargava and Sawant 2013).

The sensitive genotypes showed a drastic reduction in carboxylation efficiency (CE) when compared to the tolerant ones under WD. The genotype IAPAR 81 obtained the highest CE under water deficit, indicating a greater efficiency for this trait. This cultivar was released by IAPAR in 1997, it is still in use by farmers, and it has shown wide adaptation and high tolerance levels to heat and drought (Moda-Cirino et al. 2001, 2012).

In the biochemical analysis, it was observed that WD promoted oxidative stress in the plants, leading to accumulation of GPX, PAL and proline, along with a decrease in protein levels in most genotypes (Table 3). Several publications have associated ROS level to the activity of antioxidant enzymes involved in stress signalling and defence processes, including responses to water stress, salinity and extreme temperatures. The lack of water reduces the photosynthetic rate and increases plant respiration, generating an excessive production of ROS, capable of causing oxidative damages to lipids, proteins and DNA (Apel and Hirt 2004; Moller et al. 2007). In order to minimize the toxic effects of ROS, plants activate a set of enzymatic and non-enzymatic systems, which begin with an enzymatic cascade to avoid the formation of free radicals, to sequester or to degrade them, preventing cell damage (Serkedjieva 2011).

The greatest values of GPX under WD condition were obtained for the genotypes BAT 477 and IAC Tybatã which showed an increase of 76 and 256% in this solute concentration when compared to the control treatment. For PAL, BAT 477, BRS Pontal and IAC Tybatã presented the highest accumulation with increments of 68, 37 and 114%, respectively, in relation to control plants, indicating the

influence of these enzymes on the control of oxidative stress triggered by drought in beans. For proline, increases in its concentration were observed in the genotypes BAT 477 and IAC Tybatã when cultivated under drought. For the genotype IAPAR 81, no alteration of GPX, PAL and proline was observed when WD was imposed.

Proline accumulation has been related to several functions such as osmotic adjustment, carbon and nitrogen reserve used for restoration after stress, ammonia excess detoxification, protein and membrane stabilization and ROS elimination (Kavi-Kishor et al. 2005). However, the use of proline as an indicator of drought tolerance, on its own, should be cautious, due to the complex network of changes that occurs in plants when subjected to dry conditions. Some authors suggest that the accumulation of proline in drought-sensitive cultivars could be a symptom of stress and not necessarily a result of osmotic adjustment (Sánchez-Rodríguez et al. 2010; Rosales et al. 2012).

Considering the morphoagronomic characters, the genotype BAT 477 was the one that obtained the lowest biomass (TB) reduction when subjected to water stress (Table 4). No significant differences were detected for genotypes regarding control and water deficit conditions. The same pattern was observed for pods per plant (PP) and seeds per pod (SP), which means a smaller decrease for BAT 477 (32 and 44%, respectively) and BRS Pontal (37 and 43%, respectively). Asfaw and Blair (2012) observed that BAT 477 presented a larger root system in volume and better rooting capacity when compared to other genotypes evaluated in the same study, besides expressing a greater potential in water extraction in non-irrigated soil. A more efficient water absorption and translocation of biomass to the grains are important characteristics that contribute to the stability of grain yield under WD (Mukeshimana et al. 2014).

When evaluating the behavior of the physiological, biochemical and morphoagronomic traits by cluster heat-map analysis, the genotypes were separated when submitted to control and water deficit conditions, as can be seen in Fig. 3. Under WD, three groups were formed, with the genotype IAPAR 81 being the most distant. This genotype showed the highest physiological efficiency (*i*WUE, CE and A); however, for the biochemical characteristics, a low efficiency in the activities of peroxidases (APX and GPX) and proline was observed, which is related to the osmotic adjustment and, consequently, it is a means to combat the oxidative stress caused by water deficit.

The second group consisted of the genotype BAT 477, which obtained a good physiological adjustment when compared to the drought-sensitive genotypes, showing a smaller decrease in the morphoagronomic traits when cultivated under WD. The two drought-sensitive genotypes were placed in the third group, presenting low *i*WUE, CE

Table 4 Comparison of means using the Tukey test ($P < 0.05$) for morphoagronomic characters of bean genotypes evaluated in control and water deficit conditions

Genotypes	PP ^a		SP		TB	
	Control ^b	WD	Control ^b	WD	Control ^b	WD
BAT 477	20.40Aa	13.80Ba	117.27Aa	66.04Ba	38.80Aa	23.80Ba
IAPAR 81	17.40Aa	7.40Bab	91.62Aa	35.65Bb	35.40Aa	17.20Ba
BRS Pontal	14.33Ab	9.00Bab	74.87Ab	43.00Bab	41.00Aa	24.20Ba
IAC Tybatã	17.80Aa	5.40Bb	107.68Aa	25.40Bb	38.20Aa	19.10Ba

^aPP, pods per plant; SP, seeds per plant; TB, total biomass (mg)

^bWD, water deficit. Means followed by the same lowercase letter in the column and upper case in the row do not differ by the Tukey test, at a significance level of 5% of probability

Fig. 3 Heatmap representation of the values of physiological, biochemical and morphoagronomic characteristics of common bean genotypes in control and water deficit conditions

and A values. The data gathered about the four genotypes evaluated, contribute for a better understanding of the mechanisms related to drought tolerance in plants, which can be used to guide breeders in the process of defining the traits to be analyzed in order to select superior parental genotypes to initiate the development of drought-tolerant bean cultivars.

Conclusions

From the research that has been conducted, it is possible to conclude that the genotype BRS Pontal may be considered partially tolerant to drought. Moreover, under water deficit,

the genotype BAT 477 presented more efficient biochemical and morphoagronomic adaptations, while IAPAR 81 exhibited interesting results related to physiological adaptations.

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