

Physiological characterization of common bean (*Phaseolus vulgaris* L.) genotypes, water-stress induced with contrasting response towards drought**Anna Cristina Lanna¹, Suzy Taeko Mitsuzono², Thiago Gledson Rios Terra³, Rosana Pereira Vianello¹ and Milene Alves de Figueiredo Carvalho^{4*}**¹Embrapa Arroz e Feijão, Rodovia GO-462, km 12, Zona Rural, Caixa Postal 179, 75375-000, Santo Antônio de Goiás, GO, Brazil²Universidade Federal de Goiás, Campus Samambaia, Caixa Postal 131, 74001-970, Goiânia, GO, Brazil³Universidade Federal de Viçosa, Av. PH Rolfs sn, 36570-000, Viçosa, MG, Brazil⁴Embrapa Café, Parque Estação Biológica, Av. W3 Norte, Caixa Postal 40.315, 70770-90, Brasília, DF, Brazil***Corresponding author: milene.carvalho@embrapa.br****Abstract**

Among the three annual common bean seasons in Brazil, two of them are subject to drought. The objective of this study was to identify physiological traits in common bean under water restriction. The common beans genotypes, BAT 477 (tolerant to water-stress) and Perola (sensitive to water-stress), were grown under greenhouse conditions under two different watering regimes: irrigated (control treatment) and non-irrigated (stress treatment). The water deficit treatment consisted of suspension of irrigation to the plants at 10 days after emergence (DAE) (at the V2 physiological stage) for 30 days, followed by the irrigation's re-establishment at 40 DAE (at the R6-R7 physiological stage) up to the phase of physiological maturity of the grains for assessment of grain yield and production components. Under water-stress, BAT 477 showed less reduction in grain yield (33%), more capacity for osmotic adjustment (0.30 MPa) and superiority in the root system's development (~50%) compared to Perola that showed a 53% reduction in the grain yield and 0.06 MPa of osmotic adjustment. In this study, the robustness of the root system and osmotic adjustment are the main physiological indicators of tolerance to water deficit in common bean plants.

Keywords: indicators of tolerance to water deficit; low water availability; growth and development of plants; agronomic characteristics; physiological mechanisms; tropical conditions.

Abbreviations: ABA_ abscisic acid; CO₂_ carbon dioxide; DAE_ days after emergence; FC_ field capacity; LA_ leaf area; SDMB_ shoot dry matter biomass; A_ photosynthetic rate; E_ transpiration rate; gs_ stomatal conductance; RWC_ relative water content; Ψ_ osmotic potential; OA_ osmotic adjustment; WUE_ water use efficiency.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most popular legume of the Americas and of Eastern and Southern Africa (Asfaw and Blair, 2012). The world's largest producer and consumer of common beans is Latin America, especially Brazil, Mexico, the Andean Zone, Central America and the Caribbean (Akibode and Maredia, 2011). According to Rosales et al. (2012), 60% of the bean's production occurs in agricultural land prone to water deficit, without irrigation systems, where dry periods result in losses that may reach a yield reduction of up to 80%. Water deficit, caused by irregular rain distribution patterns, may occur one or more times during the common bean's life cycle, including crop development phases such as initial establishment of the seedlings, vegetative growth, flowering, and/or grain filling (Rao et al., 2013). In addition, climatic changes may cause temperature increase's and a greater evapotranspiration rate, which in combination with rainfall irregularity and reduction, may compromise the cultivation's of small farmers whom are the main producers of common bean in tropical regions (Asfaw et al., 2012).

According to Asfaw et al. (2012), different adaptive mechanisms of tolerance to water deficit in different common bean genotypes have already been identified, including (1) deepening of the root system with a suitable architecture that

increases soil moisture extraction at deeper layers; (2) maximization of water use efficiency for photosynthesis, growth, and development; (3) greater transport rate of photoassimilates to seeds under stress by means of an efficient nutrient remobilization rate; and (4) phenological plasticity, involving early maturity, drought avoidance and post-drought recovery. However, different phenotyping methods to identify the relevant characteristics of the diverse sources of tolerance are necessary since each genotype may have different response mechanisms (Beebe et al., 2013).

Up to now, the monitoring of yield stability (as the most important agronomic characteristic) has been the most adequate way to evaluate the adaptation of the common bean plant to water deficit. However, this property requires a time-consuming procedure that limits the efficiency of breeding programs (Beebe et al., 2008). In this context, secondary traits associated with tolerance to water deficit have been identified (Asfaw and Blair, 2012; Recchia et al., 2013); however, their applications are limited by the use of cultivars with different growth habits and the crops environmental diversity. Thus, to establish efficient strategies for the development of cultivars tolerant to water deficit and adapted to tropical conditions, the aim of this study was to identify and characterize physiological components in the common

bean (*P.vulgaris* L.) genotypes with contrasting responses towards drought tolerance in the presence of water-stress to help the breeding programs to efficiently select the more promising genotypes, with less cost and time.

Results

Water deficit and its effects on the growth of common bean plants

The effect of water deficit on common bean (*P.vulgaris* L.) plants, grown in a greenhouse, was assessed by the LA (leaf area) and the quantity of shoot dry matter biomass (SDMB) after 30 days of soil desiccation (Table 1). Under water deficit conditions, the sensitive cultivar, Perola, showed a 71% reduction in SDMB and a 70% decrease in LA; whereas in the tolerant genotype, BAT 477, the measured decreases detected were 50% and 41%, respectively (Table 1).

In relation to the root system, the first property assessed was the soil moisture profile in the columns with three common bean plants in order to analyze the impact of irrigation suspension (data not shown). In general, it was observed that the amount of water present in the soil that was cultivated with the Perola genotype was greater than in the soil with BAT 477, especially in the deepest layers of the column, regardless of the water regime applied. In addition, stress was applied through the lack of water as the soil contained in the columns of the control plants had a greater quantity of water than the soil contained in the columns of the stressed plants, especially in the first layers, regardless of the genotype (data not shown).

Together with the analysis of the soil moisture profile, the properties of the root system (length, surface area, and volume) of the common beans that were assessed during the period of water deficit are shown in Table 2. It was observed that the length and volume of the roots, in both genotypes, was greater in the first layer (5 to 25 cm depth) than in the second (25 to 45 cm depth), when grown under optimal water conditions. In contrast, when the genotypes were grown under water restriction, only the BAT 477 used deeper soil layers. The superiority of the root system of BAT 477 plants in relation to those of Perola, under conditions of low water availability, was on average around 50% in length, surface area, and volume in the second soil layer.

Water deficit and its effects on the physiology of common bean plants

Gas exchange measurements were used to assess the photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) in BAT 477 and Perola plants, under conditions without and with stress (Table 3). Under irrigation, both genotypes exhibited similarity in their gas exchanges; however, in the water-stress treatment, BAT 477 plants exhibited a significant reduction in the A and E, while Perola maintained their rates similar to those of the control plants. With regard to the g_s (number and activity of stomata), it was observed that, even in the control, BAT 477 exhibited a mean value ($0.54 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), which was significantly less than that exhibited by the plants of the sensitive genotype, Perola ($1.27 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). As for conditions of water deficit, the reduction observed in g_s was 50% and 44% in the BAT477 and Perola plants, respectively. In relation to water use efficiency (WUE), BAT 477 plants exhibited a value greater than that found in Perola, regardless of the water regime applied. In addition, it was possible to

observe that in both genotypes, WUE exhibited an increase under water deficit conditions.

To evaluate the ability of genotypes in retaining water under water deficit conditions, the following determinations were made in stressed and non-stressed plants at 30 days after suspending irrigation: relative water content (RWC), osmotic potential (Ψ_s) and osmotic adjustment (OA) (Table 4). The RWC values were approximately 80% in BAT 477 and Perola kept under adequate water conditions. However, under conditions of low soil water availability, BAT 477 maintained its RWC, whereas there was a significant reduction for Perola when compared to the respective control treatments. As well as the maintenance of RWC, BAT 477 plants had a significant reduction in Ψ_s (intracellular increase of solute concentration) and, consequently, they were osmotically adjusted (0.30 MPa). In contrast, Perola plants, in addition to having exhibited a reduction in RWC, showed no reduction in their Ψ_s , indicating that they were not capable of being osmotically adjusted (Table 4).

Water deficit and its effects on the agronomic characteristics of common bean plants

Under water deficit conditions, grain yield and most of its components, regardless of the genotype, had reduced values when compared to the control treatment (Table 5). In the irrigated treatment, a yield difference among the genotypes was not observed. However, in the treatment without irrigation, BAT 477 and Perola had a 33% and 53% reduction in yield, respectively, compared to that of the control plants (Table 5). It was also observed that when a water deficit was imposed, most of the yield components for BAT 477 genotype were greater than the values found for Perola plants.

Discussion

Understanding the factors that regulate the antagonism between carbon assimilation and water loss and those that direct the partitioning of assimilates between photosynthetic and non-photosynthetic, reproductive and non-reproductive structures, in relation to water availability, it is essential to identify plant mechanisms able to promote perception, signaling, prevention and/or preparation of the cellular environment, in advance to the lack of water in the production system. Thus, this study using the common bean genotypes BAT 477 and Perola with contrasting responses towards drought showed that the shoot structures of both genotypes were reduced (significantly in the sensitive genotype) as a result of the low water availability from the soil. This may be interpreted as a form of plant survival since reduction in the growth rate of the shoots means a reduction of the structures with high transpiration capacity and, therefore, a reduction in the water demand by the plant (Chaves et al., 2009). In addition, it was observed that Perola, a genotype sensitive to water deficit, had a more accentuated reduction in these structures, which resulted in a greater rate of growth reduction. Studies undertaken by Asfaw et al. (2012) and Ghanbari et al. (2013) corroborate these results since they showed that common bean genotypes sensitive to water-stress reduce their shoot growth in a more significant manner. In this study, a smaller reduction in shoot growth of the tolerant genotypes (BAT 477) under water deficit, compared to the sensitive genotype, may also have been due to the remobilization of photoassimilates for the growth of deep roots since an increase of length and volume in the root system in deeper soil layers was observed, probably to

Table 1. Shoot dry matter biomass (SDMB) and leaf area (LA) of two genotypes of common bean (*Phaseolus vulgaris* L.), BAT 477 and Perola, grown under conditions without stress (WOS) and with stress (WS), at 30 days after the suspension of irrigation.

Genotype	SDMB (g)		LA (cm ²)	
	WOS	WS	WOS	WS
BAT 477	9.76Aa	4.90Ab	2605.8Aa	1532.1Ab
Perola	5.89Ba	1.72Bb	1511.2Ba	449.7Bb

Capital letters compare the genotypes within each water regime and small letters the water regimes within each genotype. Different letters indicate significant differences with ≤ 0.05 probability of error by the Tukey test. $CV_{SDMB} = 13.13$; $CV_{LA} = 11.76$.

Table 2. Root length, area of contact of the roots with the acrylic surface, and root volume of common bean (*Phaseolus vulgaris* L.) plants at two soil depths (5 – 25 cm and 25 – 45 cm) of common bean genotypes, BAT 477 and Perola, grown under conditions without stress (WOS) and with stress (WS), at 30 days after the suspension of irrigation.

Genotypes	1st depth*		2nd depth**	
	WOS	WS	WOS	WS
	Length (cm)			
BAT 477	270.69Aaa'	102.75Bbb'	195.49Aba'	155.46Aaa'
Perola	145.80Baa'	173.66Aaa'	67.95Bba'	67.72Bba'
	Surface (cm ²)			
BAT 477	52.33Aaa'	23.11Aab'	35.11Aaa'	29.62Aaa'
Perola	23.59Baa'	27.13Aaa'	12.93Baa'	16.00Aaa'
	Volume (cm ³)			
BAT 477	0.80Aaa'	0.42Bab'	0.45Aba'	0.45Aaa'
Perola	0.49Baa'	0.67Aaa'	0.20Bba'	0.24Aba'

Capital letters compare the genotypes within each water regime in the different layers; small letters, the different layers within each water regime in each genotype; and small letters followed by an apostrophe, the water regimes within each genotype in the different layers. Different letters indicate significant differences with ≤ 0.05 probability of error by the Tukey test. $CV_{Length} = 20.24$; $CV_{Surface\ area} = 38.59$; $CV_{Volume} = 27.63$. * 5 – 25 cm; ** 25 – 45 cm.

Table 3. Photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and water use efficiency (WUE) of common bean (*Phaseolus vulgaris* L.) genotypes, BAT 477 and Perola, grown under conditions without stress (WOS) and with stress (WS), at 30 days after the suspension of irrigation.

Genotype	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		gs ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		WUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	
	WOS	WS	WOS	WS	WOS	WS	WOS	WS
	BAT 477	14.32Aa	10.43Bb	3.71Aa	2.88Bb	0.54Ba	0.27Ba	26.71Ab
Perola	13.42Ab	14.91Aa	3.96Aa	3.69Aa	1.27Aa	0.71Ab	11.33Bb	21.52Ba

Capital letters compare the genotypes within each water regime and small letters the water regimes within each genotype. Different letters indicate significant differences with ≤ 0.05 probability of error by the Tukey test. $CV_A = 5.39$; $CV_E = 8.89$; $CV_{gs} = 32.65$; $CV_{WUE} = 21.21$.

Table 4. Relative water content (RWC), osmotic potential (Ψ_s), and osmotic adjustment (OA) of common bean (*Phaseolus vulgaris* L.) genotypes, BAT 477 and Perola, grown under conditions without stress (WOS) and with stress (WS) from water deficit, at 30 days after the suspension of irrigation.

Genotype	RWC (%)		Ψ_s (MPa)		OA (MPa)	
	WOS	WS	WOS	WS	WOS	WS
	BAT 477	81.03Ab	90.04Aa	- 0.89Ab	- 1.17Aa	-*
Perola	77.95Aa	68.74Bb	- 0.64Ba	- 0.70Ba	-*	0.06B

Capital letters compare the genotypes within each water regime and small letters the water regimes within each genotype. Different letters indicate significant differences with ≤ 0.05 probability of error by the Tukey test. $CV_{RWC} = 3.61$; $CV_{\Psi_s} = 5.17$; $CV_{OA} = 14.34$. * - Without osmotic adjustment.

Table 5. Grain yield (grams of grain soil-column⁻¹) and its components: number of grains soil-column⁻¹ (NG), number of grains pod⁻¹ (NGP), number of pods soil-column⁻¹ (NP), and 100 grain weight (W100), of two common bean (*Phaseolus vulgaris* L.) genotypes, BAT 477 and Perola, grown under conditions without stress (WOS) and with stress (WS) from water deficit, at 30 days after the suspension of irrigation.

Geno-type	Yield		NG		NGP		NP		W100	
	WOS	WS	WOS	WS	WOS	WS	WOS	WS	WOS	WS
BAT 477	45.97Aa	30.76Ab	182.67Aa	114.67Ab	3.42Aa	3.11Aa	47.67Aa	37.33Ab	24.33Bb	27.75Aa
Perola	39.47Aa	18.42Bb	109.00Ba	59.00Bb	2.50Ba	2.66Ba	41.33Aa	25.67Bb	35.12Aa	29.49Ab

Capital letters compare the genotypes within each water regime and small letters the water regimes within each genotype. Different letters indicate significant differences with ≤ 0.05 probability of error by the Tukey test. $CV_{Yield} = 14.40$; $CV_{NG} = 11.30$; $CV_{NGP} = 6.37$; $CV_{NP} = 12.15$; $CV_{W100} = 4.72$.

explore regions with greater moisture content. Similar results, in which genotypes more tolerant to water deficit expanded their roots in the direction of deeper layers of the soil profile, were described by Sponchiado et al. (1989) and Polanía et al. (2012). It should also be noted that the genotype BAT 477, in the two water regimes assessed, showed a more robust root system compared to the Perola genotype, showing that it may be an important constitutive character that probably confers an adaptive advantage for drought tolerance.

In regard to physiological properties, an important aspect of this report was to observe that BAT 477 had a perceived low water availability in the soil prior to Perola since a reduction in their photosynthetic and transpiration rates was observed at 30 days after the suspension of irrigation. This suggests that a longer period of soil desiccation is necessary for Perola to reduce gas exchanges, indicating that perception and signaling events under water-stress probably occur late in this genotype. Moreover, the fact that BAT 477 had shown a sharper reduction in g_s in comparison to the Perola genotype under water deficit conditions backs up the hypothesis that this genotype must have mechanisms for the optimization of carbon dioxide (CO_2) usage for seed production (Cuellar-Ortiz et al., 2008). On a molecular level, this decrease in the rate of net uptake of photosynthetic carbon is due to limitation through CO_2 , in preference to a reduction in photosynthetic capacity. According to Acosta-Díaz et al. (2009), stomatal closure may occur prior to alterations of the leaves' water status, which suggests the existence of early communication between the shoots and the root system, under soil dehydration, by mediators such as the plant hormone abscisic acid (ABA), known as the main factor that controls stomatal opening and closing (Lizana et al., 2006). Based on this information, it is probable that BAT 477 have more effective control of their stomatal opening and closing since they exhibited a reduction in their photosynthetic and transpiration rates before the Perola genotype.

The superior performance of common bean cultivars with a tolerance to water deficit is also a result of a better WUE, expressed as the ratio between the A and g_s (Rosales et al., 2012). It was possible to observe that the BAT 477 genotype, in both water regimes, has a greater ability to overcome the limitation in the diffusion of CO_2 through the stomata, probably through a greater efficiency of mesophyll diffusion of CO_2 and effective fixing of CO_2 . This physiological behavior in the tolerant genotype seems to be a constitutive trait since the leaf stomatal conductance was lower, compared to Perola, and even so, the genotype maintained a yield potential equal to or greater than what was shown by the sensitive genotype.

In a similar manner, it was observed that the WUE of BAT 477, regardless of the water regime applied, was significantly greater than that shown by Perola. Therefore, differences among the genotypes assessed, related to the perception of water availability in the soil, may result in the activation of many adaptation mechanisms located in different organs. It thus becomes evident that the greater values of g_s ($0.71 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and E ($3.69 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) observed in Perola at the end of 30 days of dehydration caused by water restriction, compared to those obtained in BAT 477 ($0.27 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and $2.88 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, respectively), are important characteristics that explain the origin of the difference in sensitivity to water scarcity.

The results also indicated that the superior performance of BAT 477, in relation to Perola, grown under water restriction resulted from the OA since its occurrence was verified after 30 days of dehydration caused by water restriction. OA in response to water stress is important due to the fact that

cellular elongation (cell expansion) and stomatic movement are processes dependent on turgescence, and maintaining this turgescence is crucial in adaptation of the plant to the water deficit condition. OA in BAT 477 resulted from a greater concentration of solutes within their cells (lower Ψ_s) and maintenance of cell turgor, shown by the greater value of RWC. In contrast, the sensitive genotype did not significantly increase intracellular concentration of solutes and, at the same time, did not maintain its RWC; therefore, it did not make OA. That means that Perola was not efficient in maintaining the water balance under stress conditions since they showed a greater E, greater g_s , and lower RWC than the tolerant genotype BAT 477. Considering that, under water restriction, the tolerant genotype, BAT 477, promotes stomatal closure and maintains a high RWC, the findings of this study suggest that the osmotic adjustment is the more effective mechanism for water deficit tolerance. That does not mean that the turgor maintenance is the only strategy, but it could, to some degree, maintain a proper physiological condition to allow coping with periods without water and to sustain yield (Rosales et al., 2013).

In crops such as the common bean, in which the product of interest is the grain, the main criterion for the selection of cultivars tolerant to low water availability is related to the characteristics that result in a high grain production (Rosales et al., 2012). In this study, BAT 477 presented a significantly increased grain production when compared to Perola, grown under water restriction. This is in accordance with what had been previously reported in the literature (Porch et al., 2009; Terán and Singh, 2002).

Thus, the lower reduction in BAT 477 yield may also be explained, in part, by the denser root system, less reduction in SDMB and LA, greater quantity of water in the cell, OA, and, very probably, a greater efficiency in its mechanism of carboxylation and greater mobilization of reserves from the stem to the grain.

Different phenotyping methods to recognize the relevant traits related to the sources of tolerance are needed, since several mechanisms of drought tolerance are available (Beebe et al., 2013). In this study, throughout the analysis of two genotypes under a particular type of drought, allowed us to build a realistic idea of the mechanisms involved in providing tolerance to water shortages during specific plant developmental stages as well as in particular environments. This knowledge will help to define selection criteria for drought tolerance in the common bean, cultivated in tropical regions, which could be used after validation in common bean breeding programs.

Materials and Methods

Plant material

We used two genotypes of common bean (*P. vulgaris* L.) which are known for their drought tolerance behaviors. The BAT 477 genotype line was released by Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia) and has tolerance characteristics to multiple stresses, including water deficit and low availability of phosphorus in the soil (Terán and Singh, 2002; Porch et al., 2009; Rao et al., 2013; Recchia et al., 2013). The Perola cultivar was released by the common bean breeding program of Embrapa Arroz e Feijão with the attributes of high yield, broad environmental adaptation and consumer acceptance (Yokoyama et al., 1999), but it is sensitive to water deficit (Beebe et al., 2008). The identity and genetic purity of the genotypes BAT 477 and Perola were

certified through molecular analyses using microsatellite markers, as described in Cardoso et al. (2013).

Experimental conditions

The experiment was conducted in a greenhouse at Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil) from September to December 2012. The plants were submitted to the minimum and maximum mean values of temperature of 23.8 °C/40.3 °C and relative air humidity of 25.0%/70.4% to impose a significant evapotranspiration demand. Nevertheless, the dimension of the column and the quantity of the water contained in the soil up to the 10th DAE of the common bean plants, provided conditions for plants to gradually realize the water deficit.

The soil type was a Latossolo Vermelho (Oxisol) with clay-like texture, with a field capacity (FC) of 6.55 KPa (292.2 g kg⁻¹) and wilting point of 1505.66 KPa (172.6 g kg⁻¹). It was sieved (125 mm mesh) to remove the larger aggregates and enriched with fertilizer for the purpose of the common bean plants nutritional suitability (Ribeiro et al., 1999).

The treatments consisted of the contrasting genotypes being subjected to two irrigation conditions, with and without water-stress. For that purpose, BAT 477 and Perola seeds were germinated in plastic containers with the commercial substrate Plantmax® (vermiculite, pine/eucalyptus bark, charcoal, limestone, simple superphosphate, ammonium nitrate, cattle manure, and meals and bagasse of plant origin). Three seedlings per genotype were transplanted on the 5th DAE in PVC tubing columns of 30 cm diameter and 120 cm height. For the control treatment (without water-stress), the amount of water in the surface layers of the soil was equivalent to 80% FC established and maintained throughout the crop cycle. As for the treatment with water deficit, the quantity of water was maintained at 80% FC up to 10 DAE (V2 vegetative phase, primary leaves) (Fernández et al., 1982). After this period, the irrigation was suspended until 40 DAE, when the plants were in the R6-R7 reproductive phase, flowering and the beginning of the pod formation (Fernández et al., 1982). For the water-stressed treatment the amount of water lost from the soil was monitored by weighing two columns kept on electronic balances (Libratec, model WT 3000-I), located at strategic points inside the greenhouse. That way, it was possible to observe that after the period without irrigation the soil reached on average 53.5% of its FC and, as a result, moderate stress was considered to have been imposed on the common bean plants.

Traits measurements

On the last day of water-stress treatment, assessments were made in regard to growth of structural parts of the plant shoots: LA in cm², by the LI-COR leaf area meter; and SDMB in g, through drying the samples at 65°C in a laboratory oven until a constant weight was achieved.

The growth of the root system was assessed by measuring the length (cm), surface area (cm²), and volume (cm³) of the roots through images generated on a root scanner CI – 600 Cano Scan (CID Bio-Science, Version 3.1.19), with quantification by the WinRhizo software (Regent Instruments Canada Inc., version 2008a 32 bits). Root images corresponding to depth 1 (5 to 25 cm) and to depth 2 (25 to 45 cm) were taken weekly. The graphs of root development were generated over a period of four weeks. In addition information was recorded regarding root length (cm), area (cm²) of contact of the roots with the acrylic surface, and root

volume (cm³) according to the methodology described by Terra (2014).

On the last day of water-stress, the assessments of gas exchanges were made on the upper leaves (completely expanded and with good exposure to the sun), as following: A (μmol CO₂ m⁻² s⁻¹), E (mmol H₂O m⁻² s⁻¹), and gs (mol H₂O m⁻² s⁻¹), determined by the portable infrared gas analyzer (LCpro+, ADC BioScientific) in the period from 8:00 to 10:00 in the morning. WUE (μmol CO₂ mol⁻¹ H₂O) was expressed as the ratio between A and gs (Rosales et al., 2012).

On the last day of water deficit, leaves on the upper part of the plant were used to assess the RWC as a %, Ψ_s in MPa, and OA in MPa, according to the methodology described by Bajji et al. (2001).

After the period of water restriction, irrigation (80% FC of the soil in the first layers) was reestablished in the columns of the stressed plants, up to the physiological maturity of the grains, for the assessment of agronomic traits such as grain yield (grams of grain column⁻¹ of soil) and its components - number of grains column⁻¹ of soil, number of grains pod⁻¹, number of pods column⁻¹ of soil, and 100 grain weight (Pinheiro et al., 2009).

Statistical analysis

The experimental design was completely randomized, in a double factorial arrangement, having the genotype and water regime as factors, for the physiological and agronomic properties of the shoots. For the triple factorial arrangement the root system depth was used as factor. Three replications were used per treatment, and a column containing three plants was considered as a plot. The analysis of variance (ANOVA) was performed using the Sisvar 5.1 software (Ferreira, 2011) and the mean values were compared by the Tukey test at 5% probability of error (p ≤ 0.05).

Conclusion

In this study, deepening of roots and osmotic adjustment were the main physiological characteristics which indicated tolerance to stress from water deficit in common bean plants grown under tropical conditions. This study provides important advances for the physiological mechanisms involved in tolerance to water-stress that should be characterized for the particular types of drought applied during specific stages of plant development, as well as for particular environments. This knowledge will be useful for the broad set of experiments conducted by the breeding programs aiming to identify and select plants with a superior performance to drought.

Acknowledgments

To the National Council for Scientific and Technological Development (CNPq) for the grants to RPV; and to the Brazilian Agricultural Research Corporation (EMBRAPA/MP2) for the research's financial support.

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