

Gas exchange and mass distribution of the cowpea (*Vigna unguiculata* [L.] Walp.) under water deficit

Intercambio gaseoso y distribución de biomasa de caupí (*Vigna unguiculata* [L.] Walp.) bajo déficit hídrico

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ABSTRACT

Drought tolerance is important for the survival and productivity of plants in environments where drought periods are increasing as a result of climate variability attributable to natural causes and climate change caused by human activities. The objective of this study was to evaluate the dynamics of photosynthesis (A), stomatal conductance (gs) and intrinsic water-use efficiency (WUE=A/g_s) as a function of soil moisture content over a period of drought and the post-stress recovery of 14 cowpea genotypes. The studied genotypes tolerated soil moisture tensions close to -2 Mpa with no permanent wilting. Starting at a soil hydric potential of -0.7 MPa, decreases in photosynthesis (A), stomatal conductance (gs) and transpiration (E) were evident, as well as an increase in A/g_s, which varied by genotype. Estimating with regression models allowed for the discrimination of the degrees of drought tolerance between the cultivars. At 4 days after resuming hydration, no significant differences were found between the means of A, gs, A/g_s and E, suggesting drought tolerance in all genotypes. The genotypes: L-047 and L-034 conserved between 4 and 6 leaves, displaying the highest delayed leaf senescence during drought. Furthermore, they presented the highest biomass at 16 days post-stress recovery.

Key words: stomatal conductance, photosynthesis, drought, delayed leaf senescence.

RESUMEN

La tolerancia a sequía es importante para la supervivencia y productividad de las plantas en ambientes con aumentos de sequía, como resultado de la variabilidad climática por causas naturales y, el cambio climático por actividades humanas. El objetivo de este estudio fue evaluar las dinámicas de fotosíntesis (A), conductancia estomática (gs) y la eficiencia en el uso del agua (EUA=A/g_s) en función del contenido de humedad del suelo durante un periodo de sequía, y la recuperación pos-estrés en 14 genotipos de caupí. Los genotipos estudiados toleraron tensiones de humedad del suelo cercanas a -2 Mpa, sin presentar marchitamiento permanente. A partir de potencial hídrico del suelo de -0,7 Mpa, fueron evidentes las disminuciones en A y gs, así como el aumento en A/g_s, las cuales variaron con el genotipo; sus estimaciones con modelos de regresión permitieron discriminar grados de tolerancia a sequía entre cultivares. A los 4 días después de reasumir la hidratación, no se encontraron diferencias significativas entre las medias de tales parámetros. Los genotipos L-047 y L-034 conservaron entre 4 y 6 hojas, se mostraron como los de mayor retraso en senescencia foliar durante la sequía y presentaron mayor biomasa a los 16 días de recuperación post-stress.

Palabras clave: conductancia estomática, fotosíntesis, sequía, senescencia foliar tardía.

Introduction

The Cowpea (*Vigna unguiculata* [L.] Walp.) is one of the most important food legumes in the tropic and sub-tropic regions (Cardona-Ayala *et al.*, 2013), where drought is a major production constraint and is always a potential problem due to low and erratic rainfall (Agbicodo *et al.*, 2009; Ahmed and Suliman, 2010).

The physiological strategies of plants against microclimatic changes are directed toward reducing water loss; one strat-

egy is stomatal closure, which reduces transpiration but also restricts the entry of CO₂ into plants, decreasing the rate of photosynthesis and the translocation of assimilates to different organs of the plant as well as reducing the pressure gradient necessary for the entry of nutrients through the root, a situation that is aggravated with increasing temperatures and radiation (DaMatta, 2004).

In general, drought stress induces an array of morphological, physiological, biochemical, and molecular responses, in which photosynthesis is affected by a reduction in stomatal

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conductance (gs), which decreases the diffusion of CO₂ through the stomata into the intercellular spaces and then, through the mesophyll toward carboxylation sites (Singh and Reddy, 2011). Another limitation is of a biochemical nature, known as non-stomatal limitation, and is related to the limitation of photophosphorylation, regeneration of RuBp (ribulose 1,5-bisphosphate) and Rubisco activity (Singh and Reddy, 2011).

In the cowpea, a water deficit decreases stomatal conductance, leaf water potential and productivity of grains, which appear in a wide variability in drought tolerance, according to the degree of water deficit (Nascimento, 2009).

Studies conducted by Oliveira *et al.* (2005) show that stomatal conductance is an indicator of water stress in the cowpea and report values between 0.03 and 0.18 mol H₂O m⁻² s⁻¹. The highest recorded values, when comparing genotypes under the same water stress conditions, indicate more tolerance to drought (Nascimento, 2009).

The root/aerial part ratio is also an important trait in studies of drought adaptation because water absorption can be enhanced through an extensive and deep root system to allow water absorption in a greater volume of soil (Reis and Hall, 1987). In the cowpea, long root density, rooting depth and root dry matter per unit area are parameters that characterize the root system and can be used as drought tolerance criteria along with specific leaf area (Matsui and Singh, 2003).

Furthermore, it was found that the detrimental effects of drought in the photosynthetic apparatus can last for weeks or even months, so that the ability to recover a stressed plant's photosynthetic capacity can be associated with the ability of the stomata to reopen, partially or completely, and of protein synthesis to overcome the damage to the photosynthetic apparatus (Kozłowski and Pallardy, 1996).

The objective of this study was to determine the drought tolerance of 14 genotypes of cowpea obtained from a heterogeneous-homozygous population, assessing physiological responses related to gas exchange and biomass in early stages of the species cycle, for use as selection criteria of improved genotypes.

Materials and methods

Location. An experiment was conducted in a greenhouse covered with plastic between November of 2012 and February of 2013 at the Universidad de Córdoba, Colombia (8°48' N, 78°53' W). The climatic classification of the region

is tropical dry forest, with a dry period from December to April, an annual average temperature of 27.4°C, precipitation of 1,346 mm/year, 85% relative humidity (RH) and 2,108 h sunshine per year (Palencia-Severiche *et al.*, 2006).

Experimental material. Thirteen cowpea lines obtained from a heterogeneous- homozygous population called Criollo-Córdoba (included as a control), pre-selected by yield and nutritional quality, under suitable hydration conditions, were subjected to water stress starting at 16 d after sowing. The lines were planted in 8.5 L-plastic pots, filled with disturbed alluvial soil from the Sinú Valley of a silty loam texture.

Experimental design. We used a randomized complete block design with 14 treatments and three replications. 252 pots were used, 18 per genotype; three seeds were sowed per pot and thinning was carried out at 6 d after sowing, leaving one seed per pot.

Water dynamics of the substrate. To characterize the water dynamic of the substrate and its relationship with the gas exchange parameters, a moisture retention curve was obtained at water potentials of 0, 0.01, 0.03, 0.05, 0.1, 0.5, 1.0 and 1.5 MPa along with their corresponding gravimetric moisture content, using the hydric tension container method, combined with the membrane under pressure. A water retention curve relates soil water content with suction (matric potential=water potential) and reflects the ability of a soil or substrate to retain water depending on the exerted suction (tension); its characterization is necessary to know the hydraulic properties of soils (López-Canteñs *et al.*, 2010).

With the moisture content at 0.03 MPa (field capacity) and bulk density of the soil, the daily amount of water needed to replenish the evapotranspired plants was determined by difference in weight, in order to ensure a field capacity threshold until day 16. At that time, the water supply was suspended, when the plants had two fully expanded trifoliolate leaves.

In each pot, a plastic bag was used to prevent loss of substrate by the lower holes. After 32 d of drought, the plants were rehydrated for 16 d. The total study duration was 64 d.

The average air temperature was 31.67±7.92°C, the maximum mean 45.41±2.81°C, recorded between 09 and 15 h, and the minimum average 24.24±0.79°C, and recorded between 04 and 06 h. The maximum recorded temperature was 50.9°C and the minimum was 22.7°C. The average relative humidity was 69.59±23.42%, with values fluctuating

between 22 and 70% during the day and between 80 and 97% at night. These measurements were taken automatically with a Humidity & Temperature Datalogger Model RH520 (Extech Instruments Corporation, Nashua, NH).

Measurement of soil water content. The water content of the soil (SWC) was determined every 4 d, at depths of 6, 12 and 18 cm, using two pots with plants of the same age. Drying of the samples was done at 105°C for 24 h; simultaneously, the volumetric soil moisture was measured with a TDR 300 at a depth of 12 cm in all the pots.

Measurements of gas exchange. Measurements of gas exchange parameters: photosynthesis (A), stomatal conductance (gs), transpiration (E), as well as the intrinsic water-use efficiency ($WUE=A/g_s$), temperature and vapor pressure deficit (VPD) were recorded every a d during 32 d of drought and 16 d of rehydration, using the infrared gas analyzer (IRGA) model CIRAS-2 portable photosynthesis (PP Systems International, Amesbury, MA). These measurements were recorded from a leaflet of the second, third or fourth fully expanded leaf from the apex of each selected plant, between 10 and 13 h.

Direct and indirect measurements of growth and development of plants. From the 20th d after withholding irrigation, the number of trifoliolate leaves without signs of wilting and the presence of reproductive organs was recorded: flowers and developing pods. After 16 d of rehydration, the dry mass of the stem and leaves (vegetative biomass), reproductive structures (reproductive biomass: flowers and pods), and total mass were measured.

Statistical analysis. The relationship between soil volumetric moisture content and soil water potential was tested for polynomial, logarithmic and exponential functions and the best fit regression was selected.

The relationships between the soil water content and the different parameters of gas exchange (A, g_s , E and WUE) were tested for polynomial, linear, logarithmic and exponential functions and the best fit regressions were selected.

To select models, the following diagnostic criteria suggested by Rincón (2009) were taken into account: coefficient of determination (R^2), mean square error, studentized residuals (s_i), externally studentized residuals (t_i), Cook's distance (D_i), influence of each observation on its prediction (DF-FITS_i), and the impact of each observation on the residual sum of squares (Q(i)), for which the procedures: GLM, REG and IML of SAS® v. 9.2 (SAS, 2008) were used.

In order to estimate the effects of the variation of soil moisture content on the direct measurements of growth and development of the genotypes, analyzes of variance were conducted for each variable. For dry biomass, the transformation square root of $x+1$ was used.

Results and discussion

Soil moisture content and water potential

According to the laboratory determinations and the estimated values using the shown equation (Fig. 1) for 0.03 MPa equivalent to 0.3 atmospheres (field capacity), the substrate used in the experiment showed a moisture content of 27.88%, while at 1.5 Mpa equivalent to 15 atmospheres, corresponding to the permanent suction wilting point, where much of the plants wither, the moisture content was 12.03%.

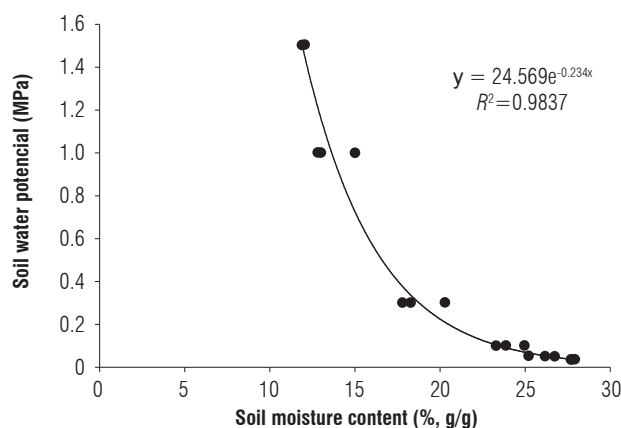


FIGURE 1. Functional relationship between gravimetric moisture content and soil water potential of Sinú Valley, silty loam texture, used to evaluate drought tolerance in 14 cowpea genotypes.

At 32 d of drought, the soil moisture content was 10.86% and the soil water potential was estimated at -1.9 Mpa. Considering the fact that the leaf water potential and soil water potential are in balance at pre-dawn (Tardieu and Simonneau, 1998; Sellin, 1999), the cowpea is classified as moderately drought tolerant (Boyer, 1978). The latter author reported nonlethal values of -1, 4 to -2.5 MPa for this species, assuming that a lethal water potential in many plants is in the range of -1.4 to -6.0 MPa.

Gaseous exchange under water stress

The combined analysis of the 14 cowpea genotypes showed that after 12 d of suspension of the water supply, with a soil gravimetric moisture of 15.24% (45.34% of the moisture at field capacity) and a soil water potential of -0.7 MPa, A, g_s and E decreased (Tab. 1). The plants showed a deviation

of physiology before water stress, since the average net photosynthesis decreased 55.08%, stomatal conductance 41.2% and transpiration, 39.02%, suggesting that stomatal regulation was the biggest limitation of photosynthesis (Singh and Reddy, 2011). At the same time, the intrinsic water-use efficiency (A/g_s) increased nearly 100%, from a soil water potential of -0.75 Mpa and soil gravimetric moisture content of 14.90%. Simultaneously, the leaf temperature and vapor pressure deficit (VPD) increased 6.8% and 8.18%, respectively, due to stomatal closure (Salah and Tardieu, 1997; Tardieu and Simonneau, 1998).

The progressive decrease of A , g_s and E suggests the morphological and metabolic mechanisms' activation of adaptation, to counteract the soil moisture deficit (Taiz and Zeiger, 2010; Singh and Reddy, 2011). In particular, at 16 d of drought, stomatal conductance showed an average reduction of 84.52% and continued decreasing progressively until day 32.

The reduction of g_s , due to the closure of the stomata, occurs when the mesophyll begins to dehydrate and is regulated by abscisic acid (ABA) (Moreno, 2009). Although, in this study, the ABA content was not measured, the values of g_s suggest an increase of the phytohormone in the leaves, due to its redistribution from the chloroplast of mesophyll cells and the synthesis and transport from the roots of this hormone (Taiz and Zeiger, 2010). ABA is released to the apoplast and reaches the guard cells through the transpiration stream (Zhang and Outlaw, 2001; Moreno 2009). ABA causes the loss of potassium, chlorine or malate in guard cells, which causes decreases in cell volume due to cytoplasm water exit, leading to stomatal closure (Moreno, 2009; Taiz and Zeiger, 2010).

The combined analysis of 14 genotypes showed quadratic relationships between gas exchange parameters and SWC

(Fig. 2). Decreases in photosynthesis, stomatal conductance and transpiration varied progressively, with soil drying as a result of evapotranspiration. After 24 d of water supply suspension, with a gravimetric moisture content of 11.21% and a soil water potential of -1.8 MPa, the average of these parameters were significantly reduced and, after 32 d of drought, net assimilation and stomatal conductance were in the proximity of zero, a situation that presaged the collapse of the plants (Agbicodo *et al.*, 2009). However, the 14 genotypes maintained green stems and only some leaves showed signs of senescence and basal regrowth in some plants; also, there was a reduction in the size of the stems and leaves as a result of decreased turgor pressure (Taiz and Zeiger, 2010). Similar results were reported by Hall (2004) for the cowpea, with a water potential of -1.8 MPa.

The magnitudes of A and g_s decreased at different rates among the genotypes, with effects that were linear, logarithmic or quadratic during the drought period (Tab. 2), suggesting genetic differences in drought tolerance among the genotypes. Likewise, the increase of A/g_s was expressed at different rates between the studied genotypes.

Gradual decreases of A and g_s indicate partial or complete closure of stomata to reduce water loss through transpiration. It has been reported that stomatal closure as a result of decreasing soil moisture content, mediated by changes in the water status of the root (Bates and Hall, 1981), is a response to a water deficit in the cowpea (Cruz de Carvalho *et al.*, 1998; Anyia and Herzog, 2004; Hamidou *et al.*, 2007; Singh and Reddy, 2011) and, according to Nascimento (2009), higher stomatal conductance values indicate a high drought tolerance.

In this sense, intrinsic water-use efficiency (A/g_s) has been recognized as a measure of carbon gain per unit of water loss and is inversely proportional to the ratio of intercellular

TABLE 1. Mean values and standard errors of A , g_s , E , T and VPD for specific soil gravimetric moisture contents, soil water potentials and drought times, evaluated in 14 cowpea genotypes.

Time (d)	θ_g	ψ_m	A	g_s	EUA	E	T	DPV
0	27.88	-0.033	10.53±0.04	100.86±0.58	0.10±0.0005	4.10±0.018	35.3±0.02	42.65±0.06
4	22.97	-0.114	8.53±0.15	73.27±1.17	0.12±0.0257	3.39±0.042	36.6±0.03	47.46±0.17
8	16.93	-0.577	13.05±0.16	99.02±1.09	0.13±0.0008	4.25±0.034	37.2±0.03	46.91±0.14
12	15.24	-0.694	3.20±0.08	59.31±0.73	0.08±0.0009	2.50±0.024	37.7±0.03	46.14±0.12
16	14.90	-0.752	4.87±0.09	15.48±0.32	0.31±0.0040	0.89±0.015	38.0±0.02	58.34±0.07
20	13.00	-1.173	3.15±0.09	10.62±0.25	0.30±0.0070	0.60±0.012	38.1±0.03	59.78±0.11
24	11.21	-1.783	1.51±0.07	5.76±0.24	0.26±0.0055	0.34±0.011	37.7±0.02	60.16±0.10
28	10.90	-1.917	1.02±0.06	3.90±0.13	0.26±0.0057	0.24±0.007	37.1±0.01	58.36±0.06
32	10.86	-1.935	0.09±0.02	1.29±0.04	0.07±0.0037	0.12±0.002	40.4±0.02	71.58±0.11

θ_g = soil gravimetric moisture; ψ_m = soil water potential; A = photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); g_s = stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); EUA = A/g_s = water-use intrinsic efficiency ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$); E = transpiration ($\text{mmol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$); T = temperature ($^{\circ}\text{C}$); DPV = vapor pressure deficit.

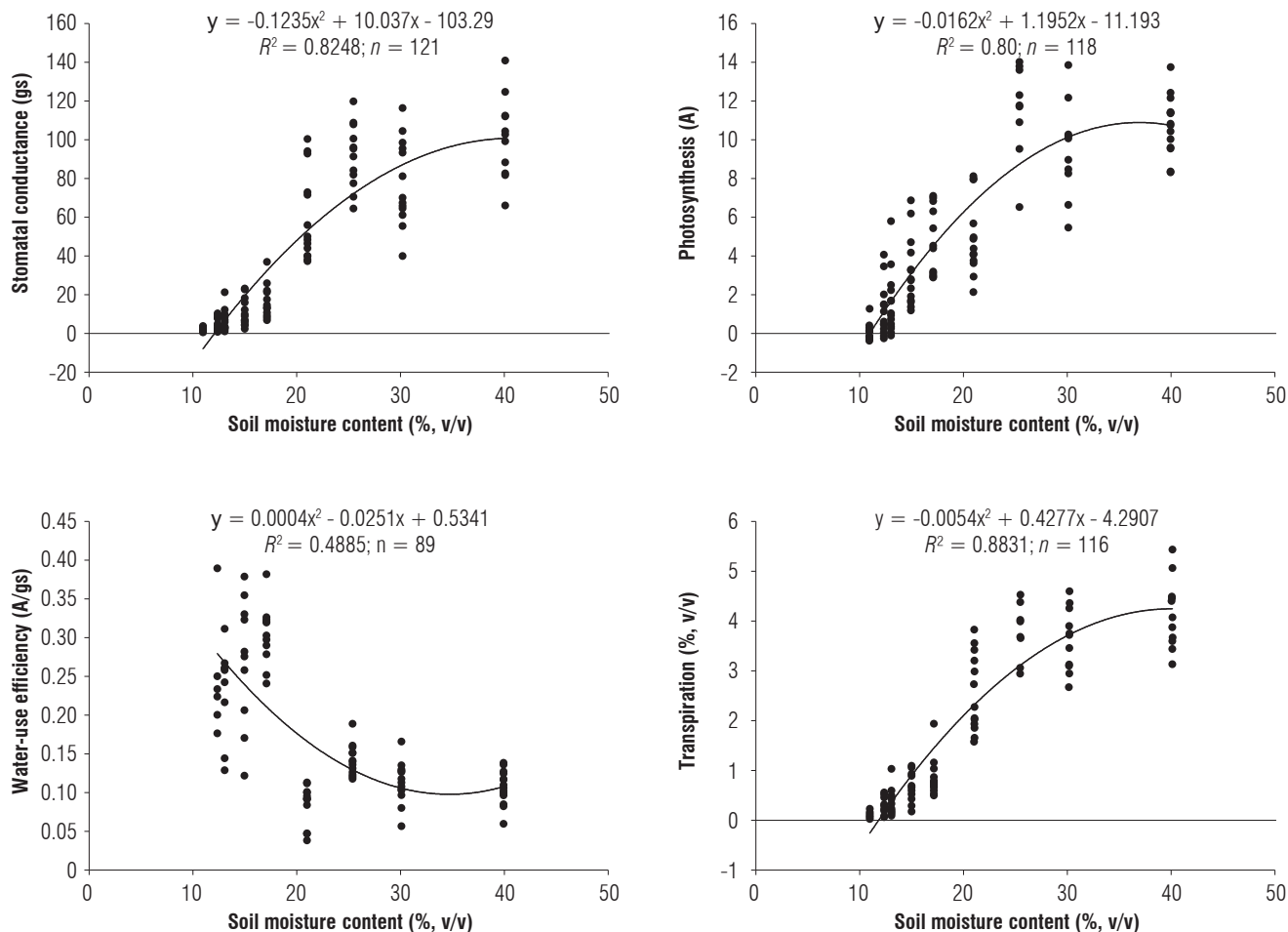


FIGURE 2. Functional relationships between soil moisture content (SWC) and stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), photosynthesis ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), intrinsic water-use efficiency ($\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$) and transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) evaluated in 14 cowpea genotypes.

TABLE 2. Characteristics of regression equations describing functional relationships of photosynthesis (A), stomatal conductance (gs), and intrinsic water-use efficiency (A/g) with soil moisture content for 14 cowpea genotypes. $P \leq 0.01$ and n varied from 16 to 26.

Genotype	A (coefficients)				gs (coefficients)				A/gs (coefficients)			
	y_0	a	b	R^2	y_0	a	b	R^2	y_0	a	b	R^2
L-001	-5.333	0.497	NA	0.82	-30.98	2.95	NA	0.79	0.675	-0.037	0.0006	0.92
L-005	-4.017	0.378	NA	0.76	-116.85	10.97	-0.140	0.79	1.753	-0.102	0.0016	0.86
L-006	-15.234	6.996	NA	0.54	-182.56	76.04	NA	0.70	0.749	-0.186	NA	0.54
L-009	-19.630	8.027	NA	0.79	-164.22	66.53	NA	0.67	1.071	-0.067	0.0010	0.70
L-014	-3.763	0.330	NA	0.69	-145.99	59.19	NA	0.70	1.010	-0.059	0.0009	0.71
L-019	-2.358	0.283	NA	0.75	-34.76	3.25	NA	0.86	0.902	-0.052	0.0008	0.75
L-020	-4.407	0.389	NA	0.88	-31.17	2.67	NA	0.81	1.374	-0.080	0.0012	0.92
L-024	-19.270	7.989	NA	0.72	-135.47	12.60	-0.150	0.82	1.397	-0.087	0.0014	0.72
L-030	-18.344	8.067	NA	0.70	-47.04	4.46	NA	0.87	0.659	-0.035	0.0005	0.71
L-034	-16.798	1.762	0.028	0.75	-180.14	17.96	-0.270	0.76	0.659	-0.031	0.0010	0.52
L-036	-4.407	0.389	NA	0.88	-41.44	3.392	NA	0.84	1.044	-0.262	NA	0.39
L-037	-18.293	7.621	NA	0.73	-38.04	3.168	NA	0.75	0.781	-0.040	0.0006	0.57
L-047	-18.400	1.886	-0.029	0.91	-291.26	115.18	NA	0.86	0.969	-0.055	0.0008	0.62
Cr-Co	-14.414	6.032	NA	0.66	-25.382	2.13	NA	0.83	1.087	-0.269	NA	0.62

Adjusted regression models were: for A: [$y_0 + ax$] in the genotypes L-001, L-005, L-014, L-019, L-020 and L-036; [$y_0 + a \ln x$] in L-006, L-009, L-024, L-030, L-037 and Cr-Co; [$y_0 + ax + bx^2$] in L-034 and L-047. For gs: [$y_0 + ax$] in L-001, L-019, L-020, L-030, L-036, L-037 and Cr-Co; [$y_0 + a \ln x$] in L-006, L-009, L-014 y L-047; [$y_0 + ax + bx^2$] in L-005, L-024 and L-034. For A/gs: [$y_0 + a \ln x$] in L-036, L-006 and Cr-Co; [$y_0 + ax + bx^2$] in L-001, L-005, L-009, L-014, L-019, L-020, L-024, L-030, L-034, L-037 and L-047. NA = not applicable.

and environmental CO₂ (Ci/Ca) concentrations (Singh and Reddy, 2011). It is evident that a higher intrinsic water-use efficiency and a higher photosynthetic rate can improve crop yield under water stress (Condon *et al.*, 2002; Parry *et al.*, 2005; Singh and Reddy, 2011).

An efficient mechanism to transfer information from the roots to the leaves may partially explain the drought avoidance shown by this plant (Bates and Hall, 1981; Moreno, 2009). Furthermore, the osmotic adjustment due to accumulation of inorganic and organic solutes and other strategies associated with adaptation to constraining environmental factors could be occurring (Rodríguez, 2006; Moreno, 2009; González-Rodríguez *et al.*, 2011).

The accumulation of free proline in *Helianthus annuus* under water stress has been reported, but its synthesis takes place earlier or later, depending on the degree of drought tolerance of the cultivar (Unyayar *et al.*, 2004). In cowpea plants under water stress, significant increases of proline favoring osmotic adjustment have been reported (Lobato *et al.*, 2008). Also, increases of total carbohydrates and amino acids and decreased amounts of total protein have been reported (Costa *et al.*, 2008; Lobato *et al.*, 2009).

The best fit functional relationships were linear, quadratic and logarithmic for A and gs, while, for A/gs, they were quadratic and logarithmic, in relation to the decrease in soil volumetric water content (Tab. 2). Singh and Reddy (2011) fitted linear equations for A and exponential ones for gs and A/gs in 15 genotypes originating from the USA, Senegal, Nigeria and India. The trend is the same: photosynthesis and the stomatal conductance decrease, whereas intrinsic water-use efficiency increases when the soil moisture content decreases.

The variation in rates of A, gs and A/gs in each genotype allows for the estimation of these parameters at a time interval as a function of soil moisture content (Singh and Reddy, 2011) and, in this study, allowed for the discrimination of the degrees of potential drought tolerance among the cultivars.

Gaseous exchanges after rehydration

At 4 d after resuming hydration, the analysis of variance showed no significant difference among the means of A, gs, A/gs and E. The mean values observed were 15.5±3.6 μmol (CO₂) m⁻² s⁻¹, 104.6±19.9 mmol (H₂O) m⁻² s⁻¹ and 0.15±0.02 μmol (CO₂) mmol⁻¹ (H₂O), respectively. These values are similar to and, in some cases, higher than those obtained when starting the water supply suspension, suggesting drought tolerance in all genotypes. Similar

results were found at 8, 12 and 16 d after resuming hydration (data not shown).

When plants that are subjected to a water deficit period are irrigated, the rate of photosynthesis may or may not return to previous levels, depending on the genetic material, severity and duration of the drought and relative humidity (Kozłowski and Pallardy, 1996). Plants with the ability to photosynthesize as they did before a water stress are more drought-tolerant. In this regard, Cruz de Carvalho *et al.* (1998) showed that stomatal conductance and the net assimilation rate measured during and after a water stress treatment are reliable parameters to identify drought tolerance in *Phaseolus vulgaris* and *Vigna unguiculata*.

Moreover, it was found that the detrimental effects of drought on the photosynthetic apparatus can last for weeks or even months, so the ability to recover a stressed plant's photosynthetic capacity can be associated with the ability of stomata to reopen, partially or completely, and of protein synthesis to overcome the damage to the photosynthetic apparatus (Kozłowski and Pallardy, 1996).

Electron transport seems to be very resistant to inhibition under a water deficit, while the photosynthetic metabolism and phosphorylation appear to be more susceptible to dehydration. Phosphorylation might be sensitive to the toxic effects of high concentrations of magnesium accompanying the chloroplast water removal of dehydrated leaves (Kozłowski and Pallardy, 1996; Taiz and Zeiger, 2010).

Number of leaves and dry mass of organs

At 32 d after sowing (16 d of drought), some genotypes developed flowers and initiated the formation of 1 or 2 underdeveloped pods. At 48 d (32 d of drought), the values of A, gs and E were in the vicinity of zero and, from this state, the water supply was resumed to observe post-stress recovery for 16 d. Reproductive biomass, only present in some genotypes, was represented by flowers and underdeveloped pods (Tab. 3).

No significant differences were found in the number of trifoliolate leaves between 20 and 32 d (Tab. 3), the time in which the water was retained in the soil matrix with suctions between -1 and -2 MPa. At 32 d of progressive drought, 8 genotypes showed 2 to 3 leaves, 4 between 3 and 4, and only 2, L-047 and L-034, conserved between 4 and 6 leaves, displaying the highest delayed leaf senescence.

Delayed leaf senescence has been reported as a characteristic that contributes to drought adaptation (Muchero,

TABLE 3. Observed average values of the variables: number of trifoliolate leaves and dry biomass during drought and rehydration evaluated in 14 genotypes of cowpea.

Genotype	Times of drought (20 and 32 d) and rehydration (4, 8 and 16 d)						
	Trifoliolate leaves (number)				Dry biomass (g)		
	20 d	32 d	4 d	8 d	Vegetative (16 d)	Reproductive (16 d)	Total (16 d)
L-001	3.58 a	2.83 a	2.50 a	2.58 ab	2.92 ab	0.37 a	3.29 ab
L-005	3.17 a	2.92 a	2.58 a	1.92 b	2.28 ab	0.14 a	2.41 ab
L-006	3.25 a	2.83 a	3.08 a	4.17 ab	4.09 ab	0.18 a	4.28 ab
L-009	3.25 a	2.75 a	2.67 a	4.17 ab	1.16 b	0.10 a	1.27 b
L-014	2.58 a	2.25 a	2.42 a	4.58 ab	3.64 ab	0.00 a	3.64 ab
L-019	3.33 a	3.17 a	3.83 a	5.17 a	3.27 ab	0.00 a	3.27 ab
L-020	3.42 a	2.83 a	2.75 a	3.42 ab	4.33 ab	0.56 a	4.90 ab
L-024	3.92 a	3.33 a	3.42 a	5.33 ab	4.67 ab	0.00 a	4.67 ab
L-030	3.17 a	2.75 a	2.67 a	3.75 ab	2.46 ab	0.00 a	2.46 ab
L-034	4.42 a	4.42 a	5.67 a	8.50 a	8.97 ab	0.04 a	9.00 a
L-036	3.17 a	3.00 a	3.08 a	4.50 ab	2.91 ab	0.00 a	2.91 ab
L-037	3.83 a	3.42 a	3.67 a	6.50 ab	7.08 ab	0.46 a	7.55 ab
L-047	4.00 a	3.83 a	4.92 a	7.92 ab	8.64 ab	0.11 a	8.75 ab
Cr-Co	3.75 a	2.75 a	3.67 a	4.58 ab	6.54 ab	0.23 a	6.77 ab
Means	3.48±0.48	3.08±0.52	3.30±0.68	4.79±1.25	4.50±1.75	0.16±0.14	4.65±1.77

Means with different letters indicate significant differences according to Tukey test ($P \leq 0.05$).

2009) because it increases the survival of the plants after a dry period and it permits the emission of a second stream of pods once the water supply is restored (Ehlers and Hall, 1997; Hall, 2004; Agbicodo *et al.*, 2009).

On the other hand, 8 d after resuming hydration, the analysis of variance showed significant differences between the genotypes and Turkey's test allowed for the separation of L-034 from L-005. However, the former did not differ significantly from the rest of the materials tested.

Moreover, significant differences were found in total biomass (represented in the stems, leaves and reproductive structures), indicating that the genotype L-034 accumulated more biomass than L-009. However, L-034 was not statistically different from the 12 remaining genotypes. The differences in the partition of assimilates were reflected in the total dry biomass, explained in part, by the differences in the parameters of gas exchanges.

It is noteworthy that 50% of cultivars did not develop flowers or that those that did, did so at a very slow rate; while for the remaining cultivars, the reproductive biomass represented 3 to 18%. This suggests that, in the genotypes studied, the mechanisms of adaptation to drought: escape, avoidance and tolerance (Mitra, 2001), although they could operate jointly or separately, are expressed differently. However, the ways in which these responses are associated with these mechanisms are not yet known (Agbicodo *et al.*, 2009).

Additionally, the early flowering genotypes possess delayed leaf senescence and undetermined growth habits and should exhibit drought adaptation and stability in yield in various environments (Ehlers and Hall, 1997). It is possible that these characteristics are associated with a higher density of deeply distributed roots, which allow for a higher water absorption (Matsui and Sing, 2003; Polanía *et al.*, 2012) and might suggest drought avoidance.

With estimates of stomatal conductance, photosynthesis, intrinsic water-use efficiency and total dry biomass, the genotypes: L-034, L-047, L-006 and L-001 were identified as the most potentially drought tolerant because they presented higher values of A, gs and A/gs between 12 and 28 d of drought, which corresponded to a soil water content between 15.24 and 10.90%, equivalent to soil water potentials of between -0.7 and -1.9 MPa and higher values of total dry biomass.

Conclusions

Rates of photosynthesis and stomatal conductance decreased starting at a soil water potential of -0.70 MPa, while intrinsic water-use efficiency increased progressively with soil drying. Photosynthesis and stomatal conductance reached zero values when the soil moisture content was 10.86% and the soil water potential was close to -2 Mpa without producing permanent wilting of plants in any genotype. Decreases in photosynthesis and stomatal conductance and increased intrinsic water-use efficiency under water stress conditions varied with the genotypes and


the estimates, with regression equations, allowed for discrimination of the degrees of drought tolerance among the cultivars. Fourteen genotypes showed full recovery of the gas exchange parameters after resuming the water supply.

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