PHYSIOLOGICAL AND BIOCHEMICAL EXPRESSIONS OF A DETERMINATED GROWTH COMMON BEAN GENOTYPE (*Phaseolus vulgaris* L.) TO WATER DEFICIT STRESS PERIODS

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ABSTRACT

Climate change has had a major impact on agriculture, affecting rainfall patterns. This study was undertaken to understand the effect of different water deficit periods on the physiological and biochemical responses of 'ICA-Cerinza' plants subjected to four water deficit periods (0, 5, 10 and 20 days). Sixty four1.5-L pots with one plant in each pot were arranged in a completely randomized design. Thirty-two pots watered with daily irrigation dose of 100% evapotranspitation (ET_o) needs through the experiment (Control). The other 32 pots were irrigated with 50% ET (water deficit). The lowest relative water content (RWC) values were observed at the end of the 10- and 20-day deficit periods (~73 and ~30%, respectively). Photosynthesis rate (P_n), stomatal conductance (g_s), chlorophyll and fluorescence ratios decreased in all deficit treatments. However, the effect was more severe in plants subjected to 20 days under water deficit, where P_n and the F_v/F_m ratio decreased about 40% compared to control plants (P_n = 9.6 µmol·m^{-2}·s^{-1} and F_v/F_m = 0.52 in deficit vs. P_n = 16 µmol·m^{-2}·s^{-1} and F_v/F_m = 0.79 in control plants). The electrolyte leakage, leaf carotenoids, malondialdehyde (MDA) and proline were higher in plants under prolonged (20 days). Results obtained suggested that 'ICA-Cerinza' plants could not adapt well and to landscaping situations where periods of extreme drought can be expected, since leaf gas exchange properties and membrane stability are seriously affected, which can have a negative effect on yield.

Key words: lipid peroxidation, leaf gas exchange, proline, chlorophyll fluorescence, drought

INTRODUCTION

Phaseolus vulgaris L. is widely consumed as dry grain for its content of protein, carbohydrates, vitamins and minerals (Beebe *et al.* 2014). Latin America is the world region with the highest bean production, covering 50% of the world production (Beebe *et al.* 2013). Also, it is estimated that 60% of the bean crops are cultivated under the risk of either intermittent or terminal drought (Polania *et al.* 2016). However, common bean production is subject to frequent droughts in highland Mexico, in the Pacific coast of Central America, in northeast Brazil, and in eastern and southern Africa from Ethiopia to South Africa (Beebe *et al.* 2013). In South America, common bean crops are mainly cultivated in the Andeans where this region can be affected by severe drought periods (Omae *et al.*, 2012).

Crops have been subjected to inclement weather conditions exacerbated by climate change in recent years (Dai, 2011). Monasterio *et al.* (2011) stated that climate change has increased areas affected by low water availability, encouraging more episodes of water deficit. Climate variation phenomena such as El Niño/Southern Oscillation (ENSO) cause reductions in rainfall and increased temperatures in Colombia (Ruiz and Pabon, 2013).

Water stress is known to be the major limiting factor of crop production worldwide (Dorostkar *et al.* 2016). Plants exhibit different anatomical, physiological and biochemical changes to temporary, or long periods, of low water availability, causing negative effects on growth, development and yield (Segura-Monroy *et al.* 2015). Türkan *et al.* (2005) reported that plants of *P. vulgaris* L. and *P. acutifolius* Gray exposed to varying lengths days of water deficit were adversely affected in accumulation of fresh and dry matter, and lengths of roots and aerial parts of the plant. These effects were more significant when the deficit period lasted for 14 days.

Photosynthetic and transpiration rates tend to decrease as a result of stomatal closure under water deficit (Rosales *et al.* 2012). Miyashita *et al.* (2005) reported that when common bean plants were subjected to water deficit, water loss was reduced by reduced transpiration. Periods of water deficit can cause disturbances of components of the light phase such as a low leaf chlorophyll content (Farooq *et al.* 2009). The maximum photochemical efficiency of PSII (F_v/F_m) and photochemical quenching decrease, while non-photochemical quenching increases as a strategy of heat energy dissipation under water deficit conditions (Aranjuelo *et al.* 2011).

A series of biochemical responses are generated under drought (Mohamed and Ibrahim, 2011). Physiological and biochemical responses of plants to drought are conditioned by duration of the water deficit period (Ashraf and Harris, 2013). Short or extended periods of water deficit can affect stability of membranes due to lipid peroxidation, causing production of peroxide ions and malondialdehyde (MDA) (Sanchez-Rodriguez *et al.* 2010). Changes in MDA concentration are an indicator of structural integrity of membranes as a result of water deficit (Cao *et al.* 2008). Sun *et al.* (2013) found that water deficit stimulates production of the secondary metabolite proline, which fulfill functions in plants in processes such as regulating osmosis, maintaining membrane stability, and being a carbon source. Ahmed and Hassan (2011) reported that a high production of proline is associated with genotypes with a better acclimation to environmental stress.

In Latin American, the studies available on the physiological behavior of bush bean genotypes in relation to duration of periods of water deficit are important because the available information has mainly focused on characterization of genotypes in a specific drought period (Beebe *et al.* 2013). The characterization of physiological and biochemical responses in bean seedlings due to different durations of water deficit are important because these ones allow knowing the possible acclimation and survival responses to adverse environmental conditions. This study was undertaken to determine effects of water deficit periods on physiological responses and biochemical responses in an indeterminate bush bean genotype.

MATERIALS AND METHODS

Plant Material and Growing conditions: 'ICA-Cerinza' seeds were planted in 1.5 L pots containing quarzitic sand, in order to have a low moisture retention substrate, and ensure a water deficit condition. The trial was conducted in the greenhouse and laboratories of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, located in the city of Bogota at 2556 M.A.S.L. (4°35'56" and 74°4'51") from September 4th to November 10th of 2014. Greenhouse growing conditions during the experiment were the following: average temperature 22°C, relative humidity between 60 and 90% and a natural photoperiod of 12 h (photosynthetically active radiation at noon was 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

After seed germination, plants were fertirrigated with a nutrient solution containing: 2.08 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1.99 mM $MgSO_4 \cdot 7\ H_2O$, 2.00 mM $NH_4H_2PO_4$, 10.09 mM KNO_3, 46.26 nM H_3BO_3, 0.45 nM $Na_2MoO_4 \cdot 2H_2O$, 0.32 nM $CuSO_4 \cdot 5H_2O$, 9.19 nM $MnCl_2 \cdot 4H_2O$, 0.76 nM $ZnSO_4 \cdot 7H_2O$, and 19.75 nM $FeSO_4 \cdot H_2O$. The fertigation volume was adjusted according to pot capacity and plant water requirements, obtaining a quantity of 100 mL every other day between 1-32 days after emergence (DAE) and 200 mL between 33-65 DAE. Finally, the experiment lasted 65 days after seeds germination.

Water deficit treatments: Treatments were started when plants reached phenological stage 13-14 according to the BBCH scale (formation of 3 or 4 fully expanded trifoliate leaves) at approximately 45 DAE. Treatments were the periods of exposure to water deficit: 0 (control), 5, 10 and 20 days. Additionally, plants from 5- and 10-day treatments had a recovery period of 15 and 10 days, respectively. Water deficit was established by the method described by Segura-Monroy *et al.* (2015) where 50% of the daily evapotranspiration (ET$_o$) needs were covered, which was determined by the weight loss of pots. Plants were watered with 50% of the daily ET$_o$ because plants exhibited a reduction between 40 and 60% in their leaf water relation.

Leaf relative water content: Leaf relative water content (RWC) was determined on fully expanded leaves from the middle portion of the canopy. The leaves were collected at 5, 10 and 20 days after water deficit treatments started (DAT) in each treatment. The RWC was calculated by the following equation:

$$RWC = \left[\frac{FW - DW}{TW - DW}\right] \times 100 \qquad \text{(Equation 1)}$$

FW is fresh weight, TW is turgid weight measured after 24 hours in distilled water at 4°C in a dark room, and DW is dry weight determined after 48 hours in an oven at 70°C.

Leaf photosynthesis and stomatal conductance: Photosynthesis was estimated using a portable photosynthesis meter (LI-COR 6200, Lincoln, NE, USA) at 45, 50, 55 and 65 DAE (0, 5, 10 and 20 DAT). Internal CO_2 concentration (C_i) was estimated at between 1000 and 1500 h. Intercellular to atmospheric carbon ratio (C_i/C_a) was also calculated. Stomatal conductance and transpiration were measured in the second fully expanded trifoliate leaf with a portable porometer (LI-COR 1600, Lincoln, NE, USA) and water use efficiency (WUE) was calculated as the ratio between photosynthesis to transpiration. Data collections were performed between 1000 and 1500 hours on fully sunny days. During measurements, the licor's conditions were: photosynthetically active radiation ≥600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, leaf temperature 27±5°C, and leaf-air vapor pressure difference 1.8±0.5 kPa.

Chlorophyll fluorescence and leaf chlorophyll content readings: A modulated fluorometer was used to determine photosystem II (PSII) fluorescence parameters (MINI-PAM, Walz, Effeltrich, Germany). Measurements were taken at the same time, and on the same leaf, used for gas exchange measurements at 45, 50, 55 and 65 DAE

(0, 5, 10 and 20 DAT). Leaves were previously adapted to darkness for 10 min. Then, fluorescence ratios were determined with a maximum light intensity up to 2600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For measurement of chlorophylls and carotenoids, 0.3 g tissue samples from the second fully expanded trifoliate leaf were homogenized in 2 mL of 80% acetone, and then samples were centrifuged (Model 420101, Becton Dickinson Primary Care Diagnostics, MD, USA) at 5000 rpm for 10 min to remove particles. The supernatant was diluted to a final volume of 6 mL by adding acetone (Sims and Gamon, 2002). Chlorophyll content was determined at 663 and 646 nm and carotenoids were determined at 470 nm using a spectrophotometer (Spectronic BioMate 3 UV-vis Thermo, Madison, WI). The equations for acetone described by Wellburn (1994) were used to estimate leaf photosynthetic pigments.

Malondialdehyde, electrolyte leakage and proline: The thiobarbituric acid method (TBA) described by Hodges *et al.* (1999) was used to estimate lipid oxidation (Malondialdehyde-MDA). Approximately 0.3 g of homogenized plant material was stored in liquid nitrogen. Samples were centrifuged at 5000 rpm, and then their absorbances were estimated at 440, 532 and 600 nm with a spectrophotometer (Spectronic BioMate 3 UV-Vis, Thermo, Madison, WI). Finally, an extinction coefficient

was used (157 $M \cdot mL^{-1}$) to obtain the concentration of MDA.

The electrolyte leakage was calculated by the method described by Jiang and Zhang (2001). Five discs (0.5 cm) of the second fully expanded trifoliate leaf were collected and 25 mL of deionized water used as a medium in 50 mL falcon tubes. The tube initial electrical conductivity (EC_1) was recorded after 2 h in a water bath (Model B-480, Postfach, Flawil, Switzerland) at 30°C with a conductivity meter (Model P700, Oakton Instruments Vernon Hills, IL, USA). The final electrical conductivity (EC_2) was obtained after 20 min in a water bath at 90°C. The percentage of electrolytes was calculated using the following equation:

$$Electrolyte\ Leakage\ (\%) = \left(\frac{CE_1}{CE_2}\right) \times 100 \qquad (Equation\ 2)$$

The method described by Bates *et al.* (1973) was used for determination of proline. Also, 0.3 g of homogenized plant material were extracted from the second fully expanded trifoliate leaf and stored in liquid nitrogen. Absorbance was measured at 520 nm using a spectrophotometer (Spectronic BioMate 3 UV - Vis, Thermo, Madison, WI, USA). Proline content was determined by a standard curve and calculated in fresh weight based on Equation 3:

$$\frac{\mu mol\ Proline}{Fresh\ vegetal\ material} = \frac{\left(\dfrac{\dfrac{\mu g\ Proline}{mL} \times mL\ Toluene}{\dfrac{115.5\ \mu g}{\mu mol}}\right)}{\left[\dfrac{g\ sample}{5}\right]} \qquad (Equation\ 3)$$

Experimental design and data analysis: The experiment was arranged in a completely randomized design. Each treatment had 4 replicates per sample. All percent values were transformed using arcsine transformation before analysis. When significant differences were obtained, the comparative Tukey test was used to separate means. Data were analyzed using the program Statistix v 9.0 (Analytical Software, Tallahassee, FL, USA).

RESULTS AND DISCUSSION

Leaf RWC varied through the experiment (Figure 1). In general, the RWC kept constant (90%) in control and plants exposed to water deficit for 5 days during the experiment. Differences were observed between treatments at 10 (DAT). At this point, common bean plants exposed for 10 and 20 days to water deficit showed a lower RWC (~75%) in comparison to control and 5-day water deficit plants (~ 90%). At 20 DAT, plants exposed for 20 days to water deficit showed a lower RWC (~30%). Leaf RWC is important because it allows quantifying the plant water status under normal and drought conditions (Keyvan, 2010). It can infer that

water relations may be affected by an adverse abiotic condition when RWC decreases, causing a lower photosynthesis rate (Costa-França *et al.*, 2000).

Photosynthesis (P_n), stomatal conductance (g_s) and transpiration (E) varied during the experiment (Figures 2-A, -B and -C). Differences were found on the leaf photosynthesis between treatments at 5 DAT (Figure 2 -A). Pn was higher (~15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in control plants compared to plants exposed to water deficit for 5, 10 and 20 days (~9 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Similar trends were observed at 10 DAT. At 20 DAT, P_n was still higher (16 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in plants without water deficit. Plants exposed for 5 days to water deficit showed an increase in the photosynthesis rate, reaching values similar to control plants. Plants subjected to water deficit for 10 and 20 days had low photosynthetic rates (~9.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Differences were only found on stomatal conductance (g_s) at 10 DAT (Figure 2-B). g_s decreased approximately 45% in all water deficit treatments compared to control plants. At 20 DAT, g_s exhibited a greater reduction (about 95%) in plants exposed to 20 days of water deficit. Whereas, g_s showed a recovery in plants exposed for 5 and 10 days to water deficit with respect to control plants. Likewise, leaf transpiration rate (E) exhibited a drop at 5

DAT, obtaining a lower E under water deficit treatments in comparison to controls. At 10 DAT, E still remained lower in plants under different water deficit periods. At 20 DAT, plants exposed for 5 and 10 days showed a recovery, presenting values similar to control plants. The plants exposed to water deficit for 20 days had the lowest E at the end of the trial (Figure 2-C).

De Laat (2014) stated that a lower RWC is associated with a low photosynthesis rate mainly due to limitation by stomatal closure, which is reflected in low stomatal conductance and transpiration values. Huang *et al.* (2011) indicated that stomatal adjustment is a common strategy in plants to mediate drought, causing a lower P_n.

Significant differences were found on WUE at 5 DAT (Table 1). Control plants had the lowest ratio compared to plants exposed to water deficit treatments. At 10 DAT, WUE continued to be slightly higher in water deficit treatments than in control plants. At 20 DAT, plants under water deficit for 20 days had a WUE 10-times greater compared to other treatments. Huang *et al.* (2011) reported that a high WUE provides evidence of the role of stomata in maintaining a high-water content in leaves during water deficit conditions. A high WUE can lead to high photosynthetic activity. However, they indicated that morphogenetic and metabolic studies are required to better understand the relation between stomatal closure and WUE. On the other hand, differences were observed in the intercellular carbon (C_i) among treatments at 10 DAT. C_i showed an increase of approximately 15% in plants subjected to the water deficit treatments compared to control plants. At the end of the experiment, bean plants that were under water deficit for 20 days had a greater C_i while plants exposed to 5 and 10 days of water deficit had internal CO_2 levels similar to control plants. Finally, Table 1 also summarized the effect of water deficit treatments on Ci/Ca ratio in common bean leaves. Higher ratios were observed in plants subject to 10 and 20 days of water deficit at 10 DAT. At 20 DAT, plants under water deficit for 20 days exhibited differences compared to the other treatments, showing a higher Ci/C_a ratio (~20%) compared to control treatment.

Differences were found on maximum photochemical quantum yield of photosystem II (F_v/F_m), effective photochemical quantum yield of photosystem II (Y (II)), non-photochemical quenching (NPQ) and photochemical quenching (qP) at 20 DAT (Figures 3 -A, -B, -C and -D). A lower Fv/F_m, Y(II) and qP were observed in plants subjected to 20 days of water deficit. Whereas, NPQ exhibited an increase, indicating that energy dissipation in photosystem II. The effects generated by water deficit on F_v/F_m ratio, Y(II), and photochemical and non-photochemical quenching (Pq and NPQ) are conditioned by the duration and severity of the stressful condition (Aranjuelo *et al.*, 2011). A long

water deficit period (20 days) reduced the photosystem II parameters (F_v/F_m, Y(II) and qP). Similar observations were reported by Dias and Brüggemann (2010), who found that *P. vulgaris* plants had a considerable reduction of qP under water deficit compared to control plants. Similarly, Gorbe and Catalayud (2012) stated that a higher NPQ occurs as the primary measure of energy dissipation in PSII under abiotic stress. In our results, a higher was especially found in plants exposed to 20 days of water deficit, indicating a protective mechanism against photoinhibition and photodamage due to water deficit (Cousins *et al.* 2002).

Leaf photosynthetic pigments (chlorophyll a, b, and total, and total carotenoid content) changed during the experiment (Figure 4). Differences were not obtained in leaf chlorophyll readings at 0, 5 and 10 DAT. At 20 DAT, there were reductions of ~25, ~7 and ~17% in the leaf chlorophyll content (a, b and total chlorophyll, respectively) in plants subjected to the longest water deficit period (20 days). At the end of the experiment, leaf carotenoids content was low in control plant (Figure 4-C). Plants exposed to 20 days of water deficit had an increase of approximately twice the concentration of total carotenoids compared to the control treatment.

Total chlorophyll content was lower in common bean plants exposed to 20 days of water deficit since this group of plants exhibited a decrease of total chlorophyll content compared to the control. In that sense, Herbinger *et al.*, (2002) stated that a drop of leaf chlorophyll content under water deficit is a mechanism to avoid damage by reactive oxygen. Total carotenoids content in plants was higher under prolonged water deficit period as a strategy of acclimation (Efeoğlu *et al.*, 2009).

Differences were observed on electrolyte leakage, MDA, and proline due to water deficit periods at 10 DAT (Figure 5). At 20 DAT, a higher electrolytes leakage was observed in plants subjected to 20 days of water deficit (Figure 5-A). This can be related to an increase in MDA content where the highest values were obtained for plants exposed to 20 days of water deficit (Figure 5-B). Similar trends were obtained on proline production where plants exposed to 20 days of water deficit had the highest values at the end of this trial compared to the others treatments (Figure 5-C).

The biochemical markers MDA and proline are used to assess the degree of acclimatization of plants to conditions of water deficit (Keyvan, 2010). The relation between the accumulation of proline and environmental stress indicates that this amino acid may function as a type of protection during the time plants are in the process of acclimatization to the stress condition (Farooq *et al.*, 2009). Migdadi *et al.* (2016) also found that the high values of proline content were recorded under the highest drought treatment in faba leaves. On the other hand, Cao *et al.* (2008) reported that production and accumulation of MDA in plants exposed to stress are

another marker of the structural integrity of membranes under water deficit. A greater accumulation of MDA was found (50% higher in stressed plants), indicating that plants suffered severe damage caused by the stressful condition (Türkan *et al.*, 2005; Keyvan, 2010).

Water deficit periods of 5- and 10-days did not have severe effects on the plant physiology of 'ICA-Cerinza'plants, obtaining a recovery of their physiological functions after stressful conditions. Water deficit periods exceeding 15 days caused negative effects on leaf gas exchange properties and membrane stability. In that sense, results obtained suggested that ICA-Cerinza genotype could not be adapted well and to landscaping situations where periods of extreme drought can be expected.
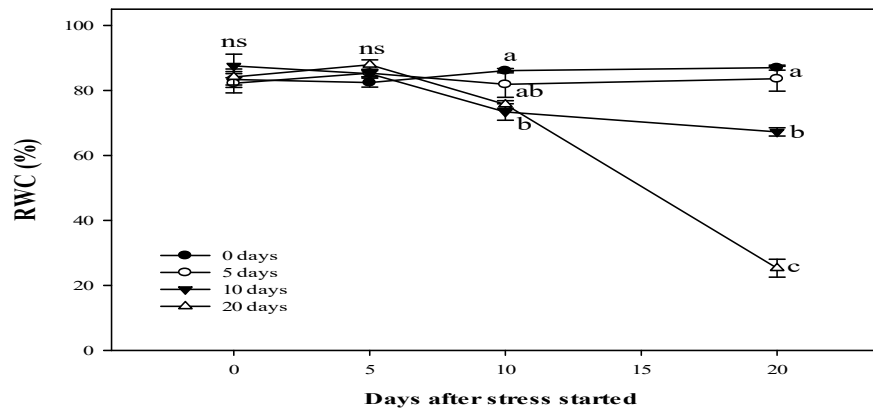

Figure 1. Effect of four periods of water deficit on leaf relative water content (RWC) in 'ICA-Cerinza' plants. Points are the average of four data. Bars represent ± standard error. Column averages followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).

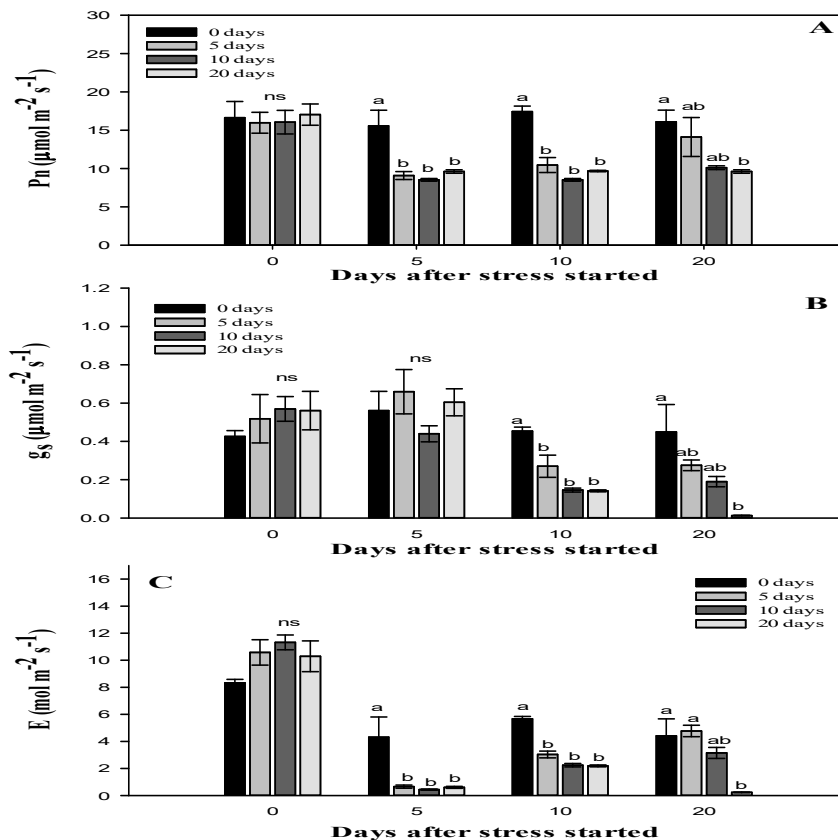**Figure 2. Effect of four period of water deficit on A). Net photosynthesis (P_n),B). Stomatal conductance (g_s) and C). Transpiration (E) of 'ICA-Cerinza' leaves. Bars represent the average of four data ± standard error. Column averages followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).**
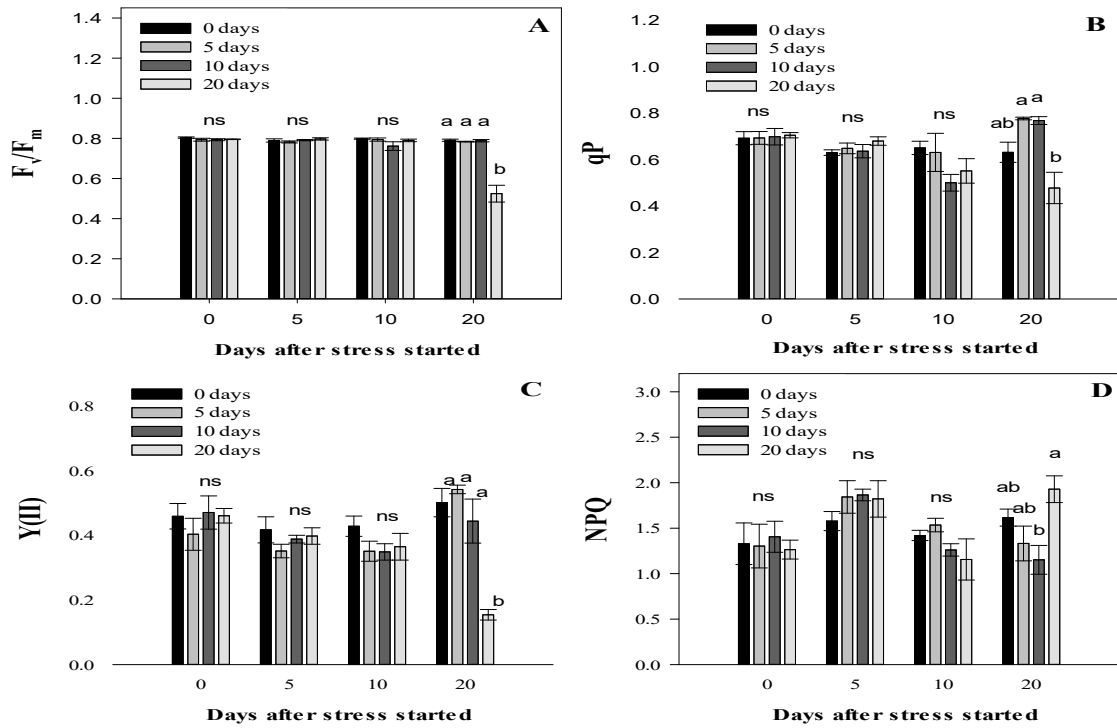
123

Figure 3. Effect of four periods of water deficit on A). Maximum quantum efficiency of photosystem II (F_v/F_m); B). Photochemical quenching (Pq); C). Actual efficiency of photosystem II (Y(II)) and D). Non-photochemical quenching (NPQ) of 'Ica-Cerinza' leaves. Bars represent the average of four data ± standard error. Averages followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).
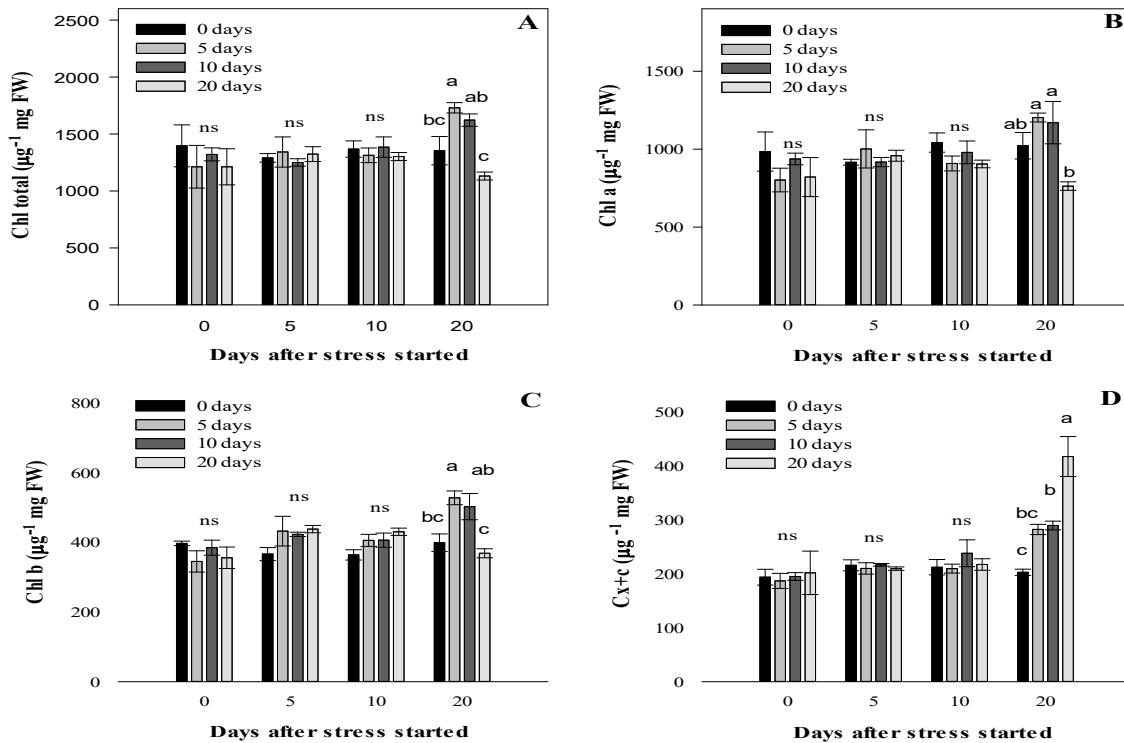

Figure 4. Effect of four periods of water deficit on A). Total chlorophyll content; B). Chlorophyll a content; C). Chlorophyll b content and D). Total carotenoid content of 'ICA-Cerinza' leaves. Bars represent the average of four data ± standard error. Averages followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).
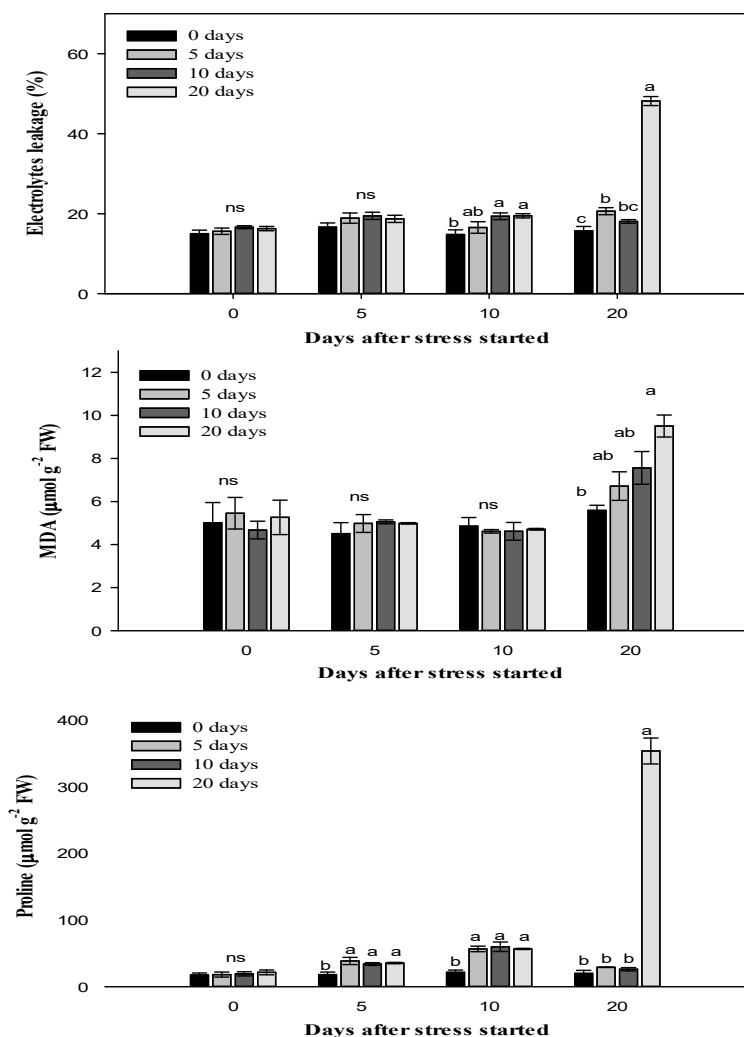
Figure 5. Effect of four periods of water deficit A). Electrolyte leakage; B). Malondialdehyde production (MDA) and C). Proline production of 'ICA-Cerinza'leaves. Bars represent the average of four data ± standard error. Averages followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$).

Table 1. Effect of four periods of water deficit on Water Use Efficiency (WUE), intercellular carbon (C_i) and intercellular carbon to atmospheric Carbon ratio (C_i/C_a) in'ICA-Cerinza' leaves.

	WUE(μmol mol^{-1} H$_2$O)			
	Days after stress started			
Treatment(days)	0	5	10	20
0	1.87 ± 0.33	5.52 ± 2.00 b^z	3.08 ± 0.12 b	4.66 ± 1.22 b
5	1.44 ± 0.20	14.94 ± 2.30 a	2.99 ± 0.05 b	2.98 ± 0.51 b
10	1.41 ± 0.19	17.74 ± 0.62 a	4.00 ± 0.06 a	3.37 ± 0.40 b
20	1.73 ± 0.29	16.55 ± 1.85 a	4.30 ± 0.06 a	43.78 ± 5.67 a
Significance	NS^y	**	***	***
C.V. (%)^x	29.96	28.02	4.71	37.88
	C_i (ppm)			
	Days after stress started			
Treatment(days)	0	5	10	20
0	325.70 ± 7.98	332.36 ± 6.61	336.33 ± 5.30 b	332.36 ± 6.61 b
5	311.31 ± 2.78	318.05 ± 3.61	355.70 ± 7.54 b	318.05 ± 3.61 b

Treatment(days)	0	5	10	20
10	313.76 ± 9.06	328.21 ± 2.09	399.53 ± 8.41 a	328.21 ± 2.09 b
20	308.63 ± 2.66	317.65 ± 2.35	396.69 ± 6.40 a	385.15 ± 9.87 a
Significance	NS	NS	***	***
C.V. (%)	4.08	2.52	3.66	3.69

C_i/C_a

Days after stress started

Treatment(days)	0	5	10	20
0	0.81 ± 0.04	0.81 ± 0.02	0.82 ± 0.01 b	0.81 ± 0.02 b
5	0.78 ± 0.01	0.77 ± 0.01	0.80 ± 0.03 b	0.77 ± 0.01 b
10	0.79 ± 0.02	0.80 ± 0.01	0.94 ± 0.00 a	0.80 ± 0.01 b
20	0.78 ± 0.01	0.79 ± 0.00	0.93 ± 0.00 a	0.95 ± 0.02 a
Significance	NS	NS	***	***
C.V. (%)	4.43	2.27	2.97	3.45

[z]Values within one column followed by different letters are significantly different to P≤ 0.05 according to Tukey Test.
[y]N.S.= Non-significant (*P≤ 0.05*). **, *** Significant to P≤ 0.01 o P≤ 0.001
[x]C.V.: Coefficient of variation.

REFERENCES

Ahmed, J., and M. Hassan (2011). Evaluation of seedling proline content of wheat genotypes in relation to heat tolerance. Bangladesh J. Bot. 40:17-22.

Aranjuelo, I., G. Molero, G. Erice, J.C. Avice, and S. Nogués (2011). Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). J. Exp. Bot. 62(1): 111-123.

Ashraf, M., and P.J.C. Harris (2013). Photosynthesis under stressful environments: An overview. Photosynthetica 51:163-190.

Bates, L., R. Waldren, and I. Teare (1973). Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207.

Beebe, S.E., I.M. Rao, M.W. Blair, and J.A. Acosta-Gallegos (2013). Phenotyping common beans for adaptation to drought. Front. Physiol.: 4 (35): 1-20.

Beebe S.E., I.M. Rao, D.M. Jyostna, and J. Polania (2014). Common beans, biodiversity, and multiple stresses: challenges of drought resistance in tropical soils. Crop Pasture Sci. 65: 667-675.

Cao, Y.Y., H. Duan, L.N. Yang, Z.Q Wang, S.C. Zhou, and J.C. Yang (2008). Effect of heat stress during meiosis on grain yield of rice cultivars differing in heat tolerance and its physiological mechanism. Acta Agron. Sinica 34: 2134-2142.

Costa-França, M. G., A.T. Pham Thi, C. Pimentel, R.O. Pereyra Rossiello, Y. Zuily-Fodil, and D. Laffray (2000). Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. Environ. Exp Bot. 43 (3): 227-237.

Cousins, A. B., N.R Adam, G.W. Wall, B.A. Kimball, P.J. Pinter, M.J. Ottman, S.W. Leavitt, and A.N. Webber (2002). Photosystem II energy use, non-photochemical quenching and the xanthophyll cycle in *Sorghum bicolor* grown under drought and free-air CO_2 enrichment (FACE) conditions. Plant Cell Environ. 25 (11): 1551-1559.

Dai, A. (2011). Drought under global warming: A review. Wiley Interdiscip. Rev. Clim. Chang. 2:45–65.

De Laat, D., C. Colombo, A. Chiorato, and S. Carbonell (2014). Induction of ferritin synthesis by water deficit and iron excess in common bean (*Phaseolus vulgaris* L.). Mol. Biol. Rep.41 (3): 1427-1435.

Dias, M. C., and W. Brüggemann (2010). Limitations of photosynthesis in *Phaseolus vulgaris* under drought stress: gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. Photosynthetica 48(1): 96-102.

Dorostkar, S., A. Dadkhodaie, and B. Heidari (2016). Changes in biochemical characteristics of thirty-six iranian wheat landraces in response to drought stress and their classification using multivariate analysis. J. Anim. Plant. Sci. 26(6):1740-1749.

Efeoğlu, B., Y. Ekmekci, and N. Cicek (2009). Physiological responses of three maize cultivars to drought stress and recovery. S. Afr. J. Bot.75 (1):34-42.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S.M.A. Basra (2009). Plant drought stress: Effects, mechanisms and management. Agron. Sustain. 29: 185-212.

Gorbe, E., and A. Calatayud (2012). Applications of chlorophyll fluorescence imaging technique in horticultural research: A review. Sci. Hort., 138: 24-35.

Herbinger K., M. Tausz, A. Wonisch, G. Soja, A. Sorger, and D. Grill (2002). Complex interactive effects of drought and ozone stress on the antioxidant defense systems of two wheat cultivars. Plant Physiol. Biochem. 40: 691-696.

Hodges, D.M., J.M. DeLong, C.F. Forney, and R.K. Prange (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207: 604-611.

Huang, Z., Z. Zou, C. He, Z. He, Z. Zhang, and J. Li (2011). Physiological and photosynthetic responses of melon (*Cucumis melo* L.) seedlings to three *Glomus* species under water deficit. Plant Soil 339:391-399.

Jiang, M., and J. Zhang (2001). Effect of abscisic acid on active oxygen species, antioxidative defense system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol. 42:1265-1273.

Keyvan, S. (2010). The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. J. Anim. Plant Sci. 8(3): 1051-1060.

Migdadi, H.M., E. H. El-Harty, A. Salamh, and M. A. Khan (2016). Yield and proline content of faba bean genotypes under water stress treatments. J. Anim. Plant Sci. 26(6): 1772-1779.

Miyashita, K., S. Tanakamaru, T. Maitani, and K. Kimura (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ. Exp. Bot. 53(2): 205-214.

Mohamed, S.A., and H. Ibrahim (2011). Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. Bangladesh J. Bot. 40(1):75-83.

Monasterio, P.P., F. Pierre, T. Barreto, C. Marin, O. Mora, J. Tablante, and C. Mendoza (2011). Influencia del fenómeno el niño/oscilación del sur sobre la precipitación y rendimiento del cultivo de maíz en el municipio peña, estado Yaracuy, Venezuela el niño/southern. Agronomía Trop. 61: 59-72.

Omae, H., A. Kumarand, and M. Shono (2012). Adaptation to High Temperature and Water Deficit in the Common Bean (*Phaseolus vulgaris* L.) during the Reproductive Period. J. Bot. 2012:1-6.

Polania, J., I.M. Rao, C. Cajiao, M. Rivera, B. Raatz and S. Beebe (2016). Physiological traits associated with drought resistance in Andean and Mesoamerican genotypes of common bean (*Phaseolus vulgaris* L.). Euphytica 210: 17-29.

Rosales, M.A., E. Ocampo, R. Rodríguez-Valentín, Y. Olvera-Carrillo, J. Acosta-Gallegos, and A.A. Covarrubias (2012). Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. Plant Physiol. Bioch. 56:24-34.

Ruiz, A.D.C., and J.D. Pabón (2013). Efecto de los fenómenos de El Niño y La Niña en la precipitación y su impacto en la producción agrícola del departamento del Atlántico (Colombia). Rev. Col. de Geog. 22:35-54.

Sánchez-Rodríguez, E., M. Rubio-Wilhelmi, L.M. Cervilla, J.J. Blasco, J.J. Rios, M.A. Rosales, L. Romero, and J.M. Ruiz (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci. 178:30-40.

Segura-Monroy, S.D., A. Uribe-Vallejo, A. Ramírez-Godoy, and H. Restrepo-Diaz (2015). Effect of kaolin application on growth, water use efficiency, and leaf epidermis characteristics of *Physalis peruviana* L. seedlings under two irrigation regimes. J. Agri. Sci. Tec. 17:1585-1596.

Sims, D.A., and J.A. Gamon (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81: 337-354.

Sun, J., J. Gu, J. Zeng, S. Han, A. Song, F. Chen, and S. Chen (2013). Changes in leaf morphology, antioxidant activity and photosynthesis capacity in two different drought-tolerant cultivars of chrysanthemum during and after water stress. Sci. Hort.161: 249-258.

Türkan, İ., M. Bor, F. Özdemir, and H. Koca (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci.168: 223-231.

Wellburn, A.R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol.144: 307-313.