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Research Article

Physiological and Biochemical Responses to Drought Stress and Subsequent Rehydration in the Symbiotic Association Peanut-*Bradyrhizobium* sp.

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Drought stress is one of the most important environmental factors that regulate plant growth and development and limit its production. Peanut (*Arachis hypogaea* L.) is an agriculturally valuable plant with widespread distribution in the world serving as a subsistence food crop as well as a source of various food products. The aims of this work were to evaluate growth and nodulation as well as some physiological and biochemical stress indicators in response to drought stress and subsequent rehydration in the symbiotic association peanut-*Bradyrhizobium* sp. SEMIA6144. Drought stress affected peanut growth reducing shoot dry weight, nodule number, and dry weight as well as nitrogen content, but root dry weight increased reaching a major exploratory surface. Besides, this severe water stress induced hydrogen peroxide production associated with lipid and protein damage; however, the plant was able to increase soluble sugar and abscisic acid contents as avoidance strategies to cope with drought stress. These physiological and biochemical parameters were completely reversed upon rehydration, in a short period of time, in the symbiotic association peanut-*Bradyrhizobium* sp. Thus, the results provided in this work constitute the initial steps of physiological and biochemical responses to drought stress and rehydration in this nodulated legume.

1. Introduction

Peanut (*Arachis hypogaea* L.) is grown as an important crop in a wide range of environments between latitudes of 40°N and 40°S. Two-thirds of the global production occurs in rainfed areas of the semiarid tropics which are characterized by unpredictable periods of water deficit [1]. Along with the United States and China, Argentina is one of the major exporters of peanut for human consumption [2]. However, the crop production area suffers intermittently water deficit periods almost every year [3]. Peanut flowering and pod filling are quiet sensitive to drought stress [4], thus water deficit periods affecting these phenological stages may have a large negative impact on yield.

Drought stress causes cellular dehydration as a consequence of water release from cytosol and vacuoles to the apoplast. The plant responses to water stress include changes in stomatal conductance, growth, osmolyte accumulation, and expression of specific genes. In these processes, the abscisic acid (ABA) is defined as the major stress hormone due to its rapid accumulation in severe conditions and participation in physiological and biochemical processes that allow plants to survive to this challenge [5]. Sharp and LeNoble [6] suggested that ABA may be helpful to maintain shoot and root limited growth under water deficit. Understandably, maintenance of root growth under this condition would enhance drought tolerance due to an increased capacity of water uptake. On the other hand, both the maintenance of plant functions at a low water potential and their recovery after rehydration contribute to high yield achievement under cyclic drought periods [7]. Plant recovery after rehydration is an essential trait for plant survival and reflects the balance between damaged structures reconstruction and adequate metabolism restoration [8]. Therefore, comprehensive studies about plant responses to rehydration are essential.

It is well known that reactive oxygen species (ROS) production is linked with normal metabolic processes such as aerobic metabolism [9] and photosynthesis [10]. However, its production is increased under abiotic stress conditions through mechanisms such as the inhibition of $NADP^+$ regeneration, Mehler reaction, and photorespiration. Under these conditions, ABA accumulation triggers superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) production through inhibition of CO_2 uptake and alteration in transport electron chain in chloroplasts [11]. In addition, H_2O_2 has been detected in ABA response as a mediator of stomatal closure [12] or inhibition of stomatal opening [13]. Finally, the antioxidant system, which includes enzymatic and nonenzymatic compounds, is capable of detoxifying ROS under stress conditions. However, when ROS production exceeds the antioxidant activity, the cell can enter in an oxidative stress expressed by lipid peroxidation and protein and DNA oxidative damage [14]. Akcay et al. [15] demonstrated that drought impaired growth and induced oxidative damage in peanut seedlings: however, oxidative damage as well as ABA accumulation in nodulated peanut is essentially unexplored.

Most of legumes, including peanut, have particular features in response to drought such as reduced rates of nodulation and biological nitrogen fixation (BNF) [16]. In nodules, drought stress increases soluble sugars and decreases solute potential in cells maintaining the turgor at a low water potential. The effects of drought stress on biological nitrogen fixation have been focused on three factors: carbon metabolism, nodule permeability to oxygen, and nitrogen feedback. A common factor that links these three factors is the sensitivity of phloem flow to plant water status. Because of the high sensitivity of nodules to phloem volumetric flow, there are a number of possible consequences resulting in a high sensitivity of nitrogen fixation to drought stress [17]. Nodulated peanut showed differential tolerance responses to drought stress conditions depending upon the genotype [16]; however, the effects of water deficiency and subsequent rehydration on the symbiotic association peanut-*Bradyrhizobium* sp have not been studied yet. Taking into account the agronomic importance of this symbiotic pair, information on those responses would provide a valuable contribution to the topic. Therefore, the aims of this work were to evaluate growth and nodulation as well as some physiological and biochemical stress indicators in response to drought stress and subsequent rehydration in the symbiotic association peanut-*Bradyrhizobium* sp.

2. Materials and Methods

2.1. Plant Material and Treatments. Seeds of peanut (*Arachis hypogaea* L.) cv Granoleico (Criadero El Carmen, General Cabrera, Córdoba, Argentina) were surface sterilized [18] and pregerminated in Petri dishes for 96 h. Pregerminated seeds were transferred to pots which have a diameter of 8 cm and height of 12 cm. Each pot was filled with 200 g of sterile volcanic sand. Plants were grown in a controlled growth chamber (light intensity: 200 μmol m^{-2} sec^{-1}; 16-h day/8-h night cycle; 28°C; and a relative humidity of 50%). The strain *Bradyrhizobium* sp. SEMIA6144, able to infect peanut plants, was provided by MIRCEN, Porto Alegre, Brazil. Seven days after sowing, plants were inoculated with 4 mL of Yeast Extract-Manitol (YEM) culture containing 10^8 cells mL^{-1}. Plants were irrigated twice a week alternately with distilled water and Hoagland nutrient solution without nitrogen [19] in order to keep the field capacity (13%), which was determined through pressure-plate method [20]. Thirty days after sowing (DAS) plants in flowering phase (R1) [21] were separated at random into three experimental groups (a) control: plants were kept under normal irrigation conditions, (b) drought stress: the irrigation was suspended until plants exhibit wilting symptoms, (c) rehydrated: plants subjected to drought stress were reirrigated.

2.2. Plant Water Status. Along the experiment, the water condition of plants subjected to different treatments was measured in the second expanded leave from the top of the main stem of each plant collected between 10–12 a.m. Relative water content (RWC) was determined by weighting the leaves, afterwards imbibition (tissues floated in distilled water for 8 h) and finally oven dried at 60°C [22]. Osmotic potential (Ψo) was determined by measuring the freezing point of samples using an osmometer (Semi Micro K-700, Knauer) [23]. Also, osmotic potential was also measured in nodules (100 mg) which were pooled of different plants at the end of the stress and rehydration periods. Transpiration efficiency (TE) was determined following the method of Udayakumar et al. [24]. Briefly, biomass of plants was recorded at the beginning and at the end of treatments. The difference between these two values was related to the cumulative water transpired, which was daily measured by weighing the pot plants corresponding to the three treatments. This value was compared with pots containing only volcanic sand to calculate the difference between evapotranspiration and transpiration. Finally, TE was calculated as follows: $(DW_{final} - DW_{initial})/CWT$, where DW_{final} is the dry weight of plants recorded at the end of the experiment, while $DW_{initial}$ is the dry weight of plants recorded at beginning of it, and CWT is the cumulative water transpired along the experiment.

2.3. Growth and Biological Nitrogen Fixation Parameters. Treated plants were harvested at the end of the stress and rehydration periods (44 and 47 DAS, resp.) and control plants were collected at the end of the experiment (47 DAS). Then, they were used for the determination of shoot, root and nodule dry weight (Shoot DW, Root DW, and Nodule DW), normalized nodule weight (NNW), nodule number, and shoot nitrogen content (SNC) after drying the samples at 70°C during 72 h. The nitrogen content in shoots was determined according to the procedure proposed by Nelson and Sommers [25].

2.4. Drought Stress Indicators. Samples of roots, leaves, and nodules of treated plants (44 and 47 DAS) were immediately frozen in liquid nitrogen and stored at −80°C for further analysis. The amount of total chlorophyll was determined

by the method described by Arnon [26]. Briefly, about 0.1 g of peanut leaves was placed into a mortar, and the tissues were grinded to fine pulp after the addition of 80% acetone. The resulting extract was transferred to a Buchner funnel containing a pad of Whatman filter paper. While filtering the extract, the grinding of the leaves pulp was repeated to adjust the final volume of the filtrate to 10 mL. The optical density of the chlorophyll extract was read with a spectrophotometer set at 652 nm. The amount of total chlorophyll present in the extract was calculated on the basis of μg of chlorophyll per gram of leaf tissue, according to the following equation:

$$\text{Total chlorophyll} = [(OD_{652} \times 1000)/3.45] \times 10\,\text{mL}/1000 \times 0.1\,\text{g}. \quad (1)$$

Total soluble sugar concentration was determined in leaves and nodules. First, the sample (1 g for leaves and 0.3 g for nodules) was boiled in 5 volumes of 80% (v/v) ethanol for 5 minutes. Then, the fifth part of the alcoholic solution was evaporated at 80°C, and the residue was resuspended in 20 volumes of distilled water [27]. Finally, sugar content of the resulting solution was determined by Dische [28]. Abscisic acid content was measured according to technique of Zhou et al. [29]. Briefly, 150 mg of plant material was homogenized with liquid nitrogen in an acid extraction solvent (pH 2.8-3). As standard 5 ng of [2H6]-ABA (J.D. Chen, USDA-ARS of Beltsville, Maryland, USA) was added. The aqueous phase was purified by adding an equal volume of ethyl acetate and organic phase was evaporated at total drying at 35°C. Extract was resuspended in 100 μL of methanol (100%), placed in specific vials, and 10 μL of each sample was used to determine ABA content by liquid chromatography (LC) (Waters, New York, USA) tandem mass spectrometry (MS–MS) (Micromas, Manchester, UK) with a monitoring software (Masslink 4.1).

2.5. Hydrogen Peroxide Production and Oxidative Stress Indicators. Hydrogen peroxide was measured spectrophotometrically after reaction with KI [30]. Leaves were homogenized in liquid nitrogen with 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 20 min to yield a crude extract. The reaction mixture consisted of 0.16 mL 0.1% TCA leaf or nodule extract supernatant, 0.16 mL of 100 mM K-phosphate buffer, and 0.68 mL reagent (1 M KI w/v in fresh double-distilled water). The reaction was developed for 1 h in darkness and absorbance measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2. The level of lipid peroxides was determined as malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction, as described by Heath and Packer [31]. Samples of leaves or nodules (0.3 g) were homogenized in 3 mL of 0.1% (w/v) TCA solution. The homogenate was centrifuged at 10,000 g for 5 min, and 0.75 mL of 20% TCA containing 0.5% (w/v) TBA was added to a 0.75 mL aliquot of the supernatant. The mixture was heated at 95°C for 30 min, quickly cooled on ice, and then centrifuged at 10,000 g for 15 min. The sample was measured at 532 nm and corrected by the nonspecific absorption at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 $mM^{-1}\,cm^{-1}$. Protein carbonyl content was measured by derivatization with 2,4-dinitrophenylhydrazine according to Levine et al. [32] with some modifications. Proteins were extracted from 0.25 g of leaves or nodules with 2.5 mL of 100 mM potassium phosphate (pH 7.0), 0.1% (v/v) Triton X-100, 1 mM Na_2EDTA, and 2.5 mg of leupeptin to prevent proteolysis of oxidized proteins during sample preparation. After precipitation of possible contaminating nucleic acids in the samples with 1% (w/v) streptomycin sulfate, an aliquot of 0.4 mL of the extracts was reacted with 0.1 mL of 20 mM dinitrophenylhydrazine in 2 M HCl and another aliquot (control) with 0.1 mL of 2 M HCl for 1 h, with vigorous shaking every 10 to 15 min. Proteins were then precipitated with 10% (w/v) TCA, and the pellet was washed four times with 1 : 1 (v/v) ethanol : ethyl acetate. Precipitated proteins were solubilized in 6 M guanidine-HCl (pH 4.5) by incubation for 30 min with shaking. The insoluble material was removed by centrifugation, and the absorbance of the hydrazones (derivatized carbonyls) was measured at 370 nm. To obtain more accurate results, the amount of protein to be analyzed for carbonyl content was adjusted to 0.5 mg in all samples.

2.6. Statistical Analysis. The data were analysed using ANOVA and LSD Fisher's test at $P \leq 0.05$. Factorial design (days and treatments were considered as factors) was utilized for RWC and Ψo analysis. Prior to the test of significance, the normality and homogeneity of variance were verified using the modified Shapiro-Wilk and Levene tests, respectively. If homogeneity of variance was not given, data were transformed using an appropriate function.

3. Results

3.1. Establishment of Plant Water Status. The results obtained of the parameters related to plant water status such as relative water content (RWC) (Figure 1(a)) and osmotic potential (Ψo) (Figure 1(b)) kept quite constant in control plants along the experiment. These parameters significantly decreased reaching the lowest values when wilting symptoms were observed in stressed plants (approximately fourteen days from the beginning of the experiment). In rehydrated plants, RWC and Ψo values were similar to control plants after three days of rewatering. Nodule Ψo corresponding to control, stressed and rehydrated plants were -0.87 ± 0.02; -1.21 ± 0.01 and -0.74 ± 0.08, respectively, being the value of stressed plants significantly different to control and rehydrated ones. At harvest, the Ψo values were used as indicators of the plant water status for the physiological and biochemical determinations carried out in leaves and nodules. TE is used as a water used balance and biomass production indicator under stress conditions. TE and its related variables did not show significant differences in both treatments compared with control. Although differences were not significant, TE and its components were lower in stressed and rehydrated plants than in control ones (Table 1).

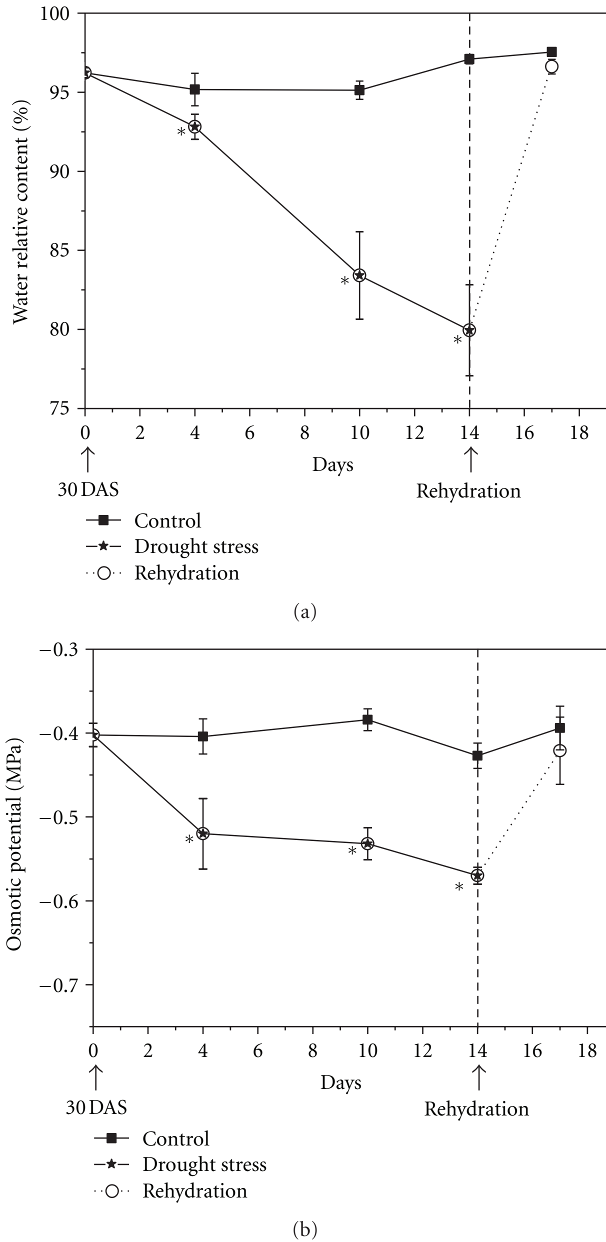

Figure 1: Water status of leaves of peanut plants exposed to drought stress and rehydration. Water relative content (a) and osmotic potential (b). Values are means ± SE ($n = 10$). * Indicates significant differences at $P < 0.05$ according to LSD Fisher's test.

3.2. Estimation of Growth and Biological Nitrogen Fixation in Peanut-Bradyrhizobium sp. Symbiosis under Drought Stress Condition. At the beginning of the experiment, peanut plants (30 DAS) had an average shoot and root DW of 471.48 ± 34.53 and 60.31 ± 11.88 mg, respectively. After exposing the plants to drought stress they showed wilting symptoms (flaccidity and modifications in leaf-angle arrangement), while rehydrated plants recovered leaf turgor. At harvest, shoot DW decreased and root DW was significantly higher in stressed and rehydrated plants than in control plants, causing an increase in root/shoot ratio in stressed and rehydrated plants (Table 2). Nodule number and DW significantly decreased in stressed and rehydrated plants. The values of NNW, obtained from the shoot DW and nodule DW ratio, did not show differences among treatments indicating that both values are positively correlated ($r = 0.8$) (data not shown). The SNC showed a significant reduction in stressed and rehydrated plants compared with control ones (Table 3).

Table 1: Effects of drought and rehydration on the transpiration efficiency parameters.

Treatments	TE ($g\,mL^{-1}$)	CWT (mL)	$DW_{final} - DW_{initial}$ (g)
Control	0.023 ± 0.005[a]	19.82 ± 3.23[a]	0.37 ± 0.04[a]
Drought stress	0.023 ± 0.007[a]	16.44 ± 5.88[a]	0.31 ± 0.05[a]
Rehydration	0.015 ± 0.004[a]	16.30 ± 5.76[a]	0.24 ± 0.08[a]

TE: transpiration efficiency, CWT: cumulative water transpired in the stress period, DW_{final}: dry weight of plants recorded at the end of the stress period; $DW_{initial}$: dry weight of plants recorded at beginning of the stress period. Values are means ± S.E. ($n = 6$). Different letters in each column indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

Table 2: Effects of drought stress and rehydration on peanut growth.

Treatments	Shoot DW (mg)	Root DW (mg)	Root/Shoot ratio
Control	806.93 ± 50.20[a]	74.64 ± 10.57[a]	0.090 ± 0.007[a]
Drought stress	530.13 ± 67.74[b]	121.53 ± 10.12[b]	0.183 ± 0.019[b]
Rehydration	639.36 ± 49.99[b]	113.75 ± 6.70[b]	0.146 ± 0.019[b]

Values are means ± S.E. ($n = 12$). Different letters in each column indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

3.3. Effect of Drought Stress on Chlorophyll, Sugar, and ABA Contents. Chlorophyll content remained unchanged in plants exposed to all treatments (Figure 2). Total soluble sugar increased in stressed plants leaves compared with rehydrated and control plants. In stressed plants nodules, soluble sugar content increased in 30%, while rehydrated plants showed an intermediate value related to other treatments (Figure 3). ABA endogenous content showed a significant increase in leaves and roots of stressed plants compared with control ones, reaching higher values in leaves. After rehydration, plant ABA levels were similar to control plants (Figure 4).

3.4. Influence of Drought Stress on H_2O_2 Production, Lipid Peroxidation, and Protein Oxidation. Hydrogen peroxide production increased in leaves and nodules of plants subjected to drought stress. After rehydration, H_2O_2 content was similar to well-irrigated plants (Figure 5(a)). Lipid peroxides (quantified as MDA content) increased in leaves and nodules of peanut plants exposed to drought stress (Figure 5(b)). Carbonyl groups content increased only in leaves, while in nodules, this value was similar to control plants (Figure 5(c)). In rehydrated plants, the indicators of

Table 3: Influence of drought stress and rehydration on peanut nodulation and nitrogen content.

Treatments	Nodule number	Nodule DW (mg)	NNW	SNC (mg plant^{-1})
Control	41.85 ± 2.92^a	22.24 ± 2.62^a	0.018 ± 0.003^a	23.52 ± 2.84^a
Drought stress	29.15 ± 1.50^b	15.83 ± 1.08^b	0.019 ± 0.001^a	10.14 ± 1.88^b
Rehydration	33.57 ± 2.14^b	16.65 ± 1.37^b	0.023 ± 0.003^a	10.42 ± 0.43^b

NNW: normalized nodule weight; SNC: shoot nitrogen content. Values are means ± S.E. ($n = 12$). Different letters in each column indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

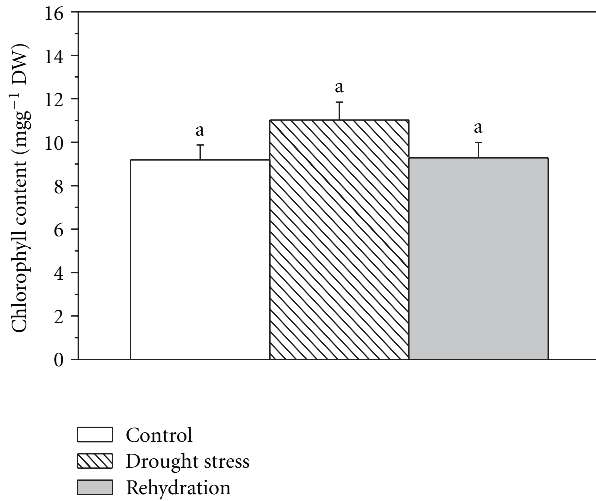

Figure 2: Chlorophyll content in peanut plants exposed to drought stress and rehydration. Values are means ± SE ($n = 10$). Different letters indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

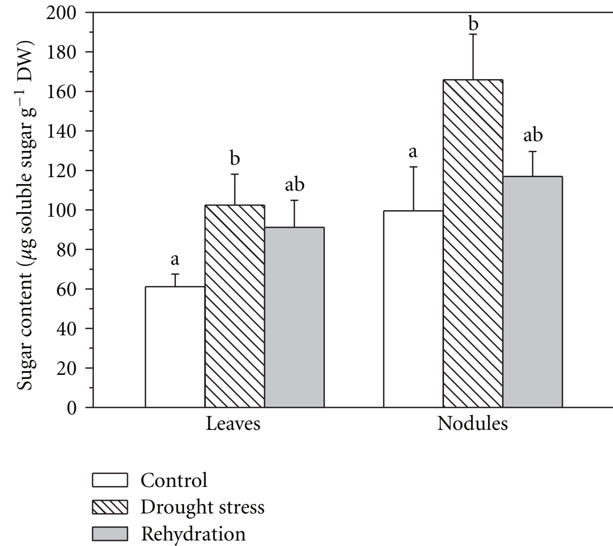

Figure 3: Soluble sugar content in leaves and nodules of peanut plants exposed to drought stress and rehydration. Values are means ± SE ($n = 10$). Different letters indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

oxidative stress reached the control values in leaves and nodules at the end of the rehydration period (three days).

4. Discussion

In general, drought stress reduces shoot growth through effects on plant water status, photosynthesis, and leaf expansion, whereas root growth can achieve different responses [33]. Therefore, an increased root/shoot ratio acts as an index that reflects changes induced by drought stress as differential growth rates between organs. In this work, peanut plants subjected to severe water stress (leaf $\Psi o = -0.56$ MPa) showed a decreased shoot DW and an increased root DW. These results are in agreement with those found by Puangbut et al. [34], who reported an increased root DW related to high root density and length. In addition, peanut ability to keep a viable root system during water stress is required for crop drought tolerance.

It is well-documented that abiotic stress affects nodule development as well as nitrogen fixing activity which reduces the N contribution to legume growth [31]. Drought stress is one of the major factors affecting nitrogen fixation by legume-*Rhizobium* symbiosis. Several mechanisms have been previously reported to be involved in the physiological response of symbiotic nitrogen fixation to drought stress,

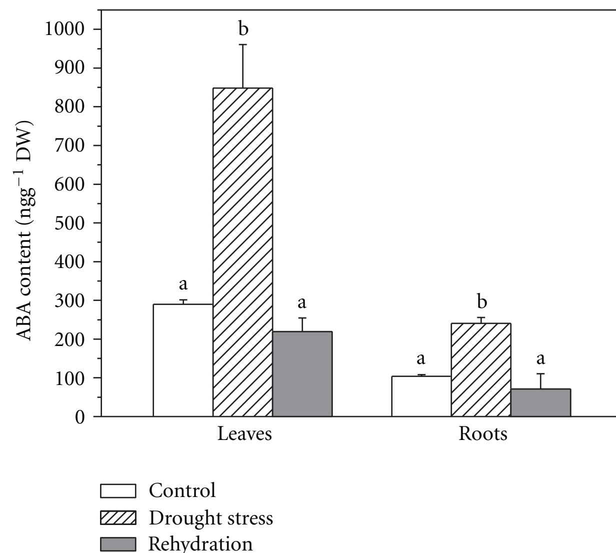

Figure 4: ABA content of peanut plants exposed to drought stress and rehydration. Values are means ± SE ($n = 5$). Different letters indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

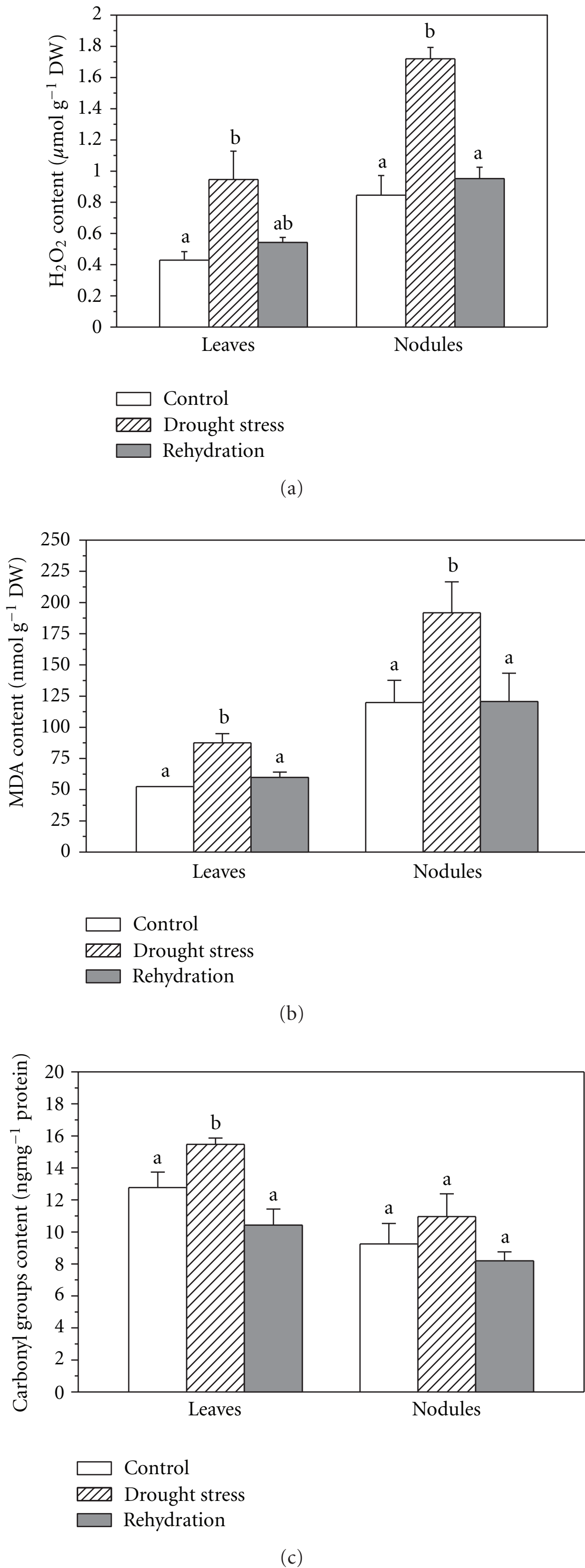

Figure 5: H_2O_2 content (a), MDA content (b), and carbonyl groups content (c) in peanut plants exposed to drought stress and rehydration. Values are means ± SE ($n = 8$). Different letters indicate significant differences at $P < 0.05$ according to LSD Fisher's test.

that is, carbon shortage and nodule carbon metabolism, oxygen limitation, and feedback regulation by the accumulation of N fixation products [35]. In this work, the symbiotic nitrogen fixation was estimated on the N content and nodule DW which frequently correlate well with shoot DW, the latter parameter provides an acceptable basis of N_2-fixing effectiveness [36]. The results obtained revealed that drought stress caused a decrease in nodulation as well as SNC, and these parameters remained unchanged during the short rehydration period indicating a negative impact of water deficit on symbiotic nitrogen fixation.

Arunyanark et al. [37] reported that stability in peanut chlorophyll content was related to drought tolerance due to the ability to keep constant biomass production, despite unfavourable conditions. Our findings revealed that chlorophyll content maintained unaltered, and this may be related to a higher root biomass production to increase its exploratory surface in order to improve water uptake. Besides, chlorophyll content may allow plants to deliver sufficient energy to deal with the energy-consuming adaptations to drought stress. Another possibility is that chlorophyll has a role in control of redox homoeostasis, that is, collaborates in heat dissipation of excess excitation energy within light-collecting chlorophyll and the carotenoid-binding protein complexes of photosystem (PS) II, which are considered major photoprotective mechanisms [38]. In this study, chlorophyll content led to an intriguing result, this parameter remained unchanged while nitrogen content decreased, although it is well known that both parameters are tightly related between them [39]. A possible explanation for decrease in foliar N concentration is attributable to drought-induced retranslocation of shoot N to roots or volatilization of foliar N [40].

Soluble sugar accumulation has been associated with drought tolerance in several plant species [41]. Coueé et al. [42] reported that soluble compounds accumulation may be a tolerance strategy associated with ROS-scavenging pathways for survival under stress conditions. Osmotic adjustment is recognized as an effective mechanism associated with drought tolerance, this involves the net accumulation of solutes mainly due to the increase in soluble sugar [43]. As a consequence of this net accumulation, the osmotic potential of the cell is lowered. In this work, independently of the effect of solute concentration due to the water loss from the tissue, both RWC and osmotic potential were used to determine if lower values in osmotic potential could be a consequence of tissue dehydration or osmotic adjustment. Our results showed a low osmotic adjustment (data not shown) suggesting that sugars had a minor role in it, and changes in osmotic potential could be, in part, an indirect result of water lost. Thus, osmotic potential is a good indicator of plant water status and soluble sugar accumulation could be involved, at least partially, in ROS scavenging and signaling response pathways in peanut leaves and nodules under stress conditions.

ABA accumulation has been widely associated with drought stress responses in plants, and interestingly, with plant capacity to keep shoot and root growth under these conditions, in which improved root growth leads to a marked

benefit in exploration of soil in terms of water search [6]. ABA accumulation has also been related to increased sugar content due to an enhanced expression of enzymes involved in starch hydrolysis [44]. Therefore, ABA accumulation could be responsible for root growth maintenance and sugar accumulation in stressed peanut plants.

Under stress conditions, hydrogen peroxide generation is essential in cellular signalling due to its role as a second messenger in plant defence [45]. ABA accumulation is known to trigger H_2O_2 production as a signal mediating stress tolerance responses [46]. Several studies demonstrated that ABA accumulation occurs prior to H_2O_2 appearance and both molecules are involved in stomatal closure as well as in stomatal opening inhibition [28]. Our results showed that lipid peroxidation and protein oxidation were enhanced in peanut leaves exposed to drought stress while after rehydration those values reached the control values. These indicators of oxidative damage are tightly related to ROS production, evidenced by H_2O_2 accumulation, suggesting an oxidative stress under this condition. In stressed peanut nodules, lipid but no protein damage was observed with a concomitant decrease upon rehydration. Naya et al. [47] reported similar results in alfalfa nodules exposed to drought. They concluded that oxidative damage of cellular components jointly with limitations in metabolic capacity of bacteroides would be contributing factors to the reduced N fixation. Thus, for the symbiotic pair peanut-*Bradyrhizobium* sp. SEMIA 6144 one of the most relevant findings was the rapid decrease of H_2O_2 content when peanut plants were rehydrated in a short period of time (three days) causing the reversion of oxidative stress which may be involved, at least partially, in N fixation reduction.

5. Conclusion

The severe water stress affected negatively peanut growth and nodulation. The enhance of H_2O_2 production caused lipid and protein damages; however, the plant was able to increase soluble sugar and ABA contents as avoidance strategies to cope with drought stress. These physiological and biochemical responses were completely reversed upon rehydration in the symbiotic association peanut-*Bradyrhizobium* sp in a short period of time. Further studies are required to elucidate the entire response pathway, specially the cause-effect relationship between ABA and ROS production in stressed nodulated plants as well as the antioxidant system activity and its implication in ROS removal in rehydrated legume plants.

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