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# Tolerance to Drought in Leguminous Plants Mediated by *Rhizobium and Bradyrhizobium*

Allan Klynger da Silva Lobato,
Joaquim Albenísio Gomes da Silveira,
Roberto Cezar Lobo da Costa and
Cândido Ferreira de Oliveira Neto

Additional information is available at the end of the chapter

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## 1. Introduction

The water availability is considered the climatic factor with large effect on agricultural productivity, being responsible to determine species distribution in different climate zones around the globe [1]. Effects of drought depend of plant development stage, inten‐ sity, and duration of the water restriction. In other hand, plant adaptive strategies will determine the tolerance level, and consequently your survival on these conditions of in‐ adequate water supply [2].

Water deficit is an abiotic factor that affects the agricultural production with high frequency and intensity, influencing aspects related to plant development, such as decrease in photo‐ synthesis rate, reduction in leaf area [3], and stomata closing [4]. Crops normally present performance affected by water deficiency, which can cause lower growth and development (Figure 1), with progressive reduction in leaf dry matter [5] and consequent repercussion on production parameters, such as number of grains and pods per plant.

Root system presents complex strategy aiming to maintain water supply in conditions of water deficit, by increasing the root elongation rate and completely inhibiting the shoot [6]. On the other hand, plants growing in low water potentials normally present root thinner [7], and this morphological modification is an adaptation to increase water absorption efficien‐ cy. Therefore, a combination of changes in morphological, physiological and biochemical levels are necessary to plant survival in environments affected by drought.
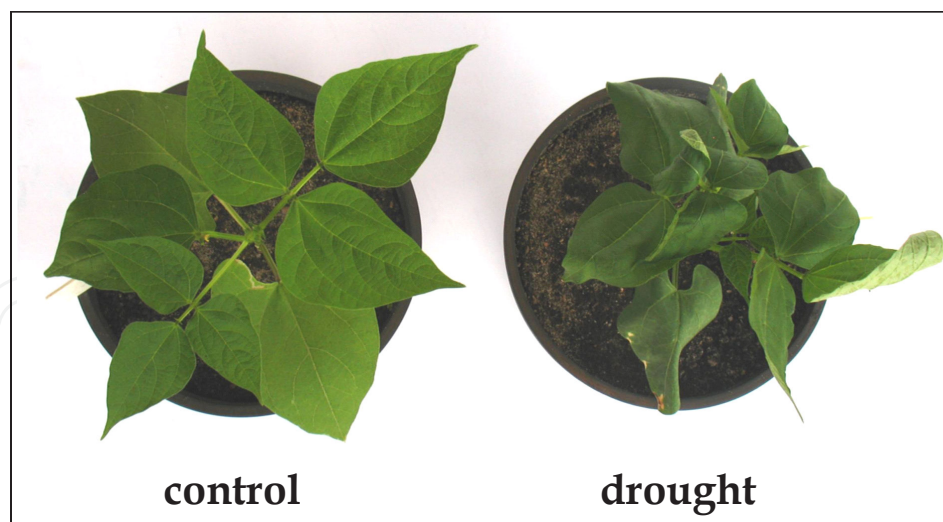
**Figure 1.** Visual aspect of shoot in *Phaseolus vulgaris* plants exposed to drought by four days.

The biological fixation of nitrogen is the capacity of an organism to divide the molecule of nitrogen ($N_2$) and to combine hydrogen atoms ($H^+$), forming ammonium ($NH_4^+$) [8], being carried out by a distinct group of microorganisms, singly or under symbiosis. The *Bradyrhizobium* and *Rhizobium* genders are described as soil bacteria that have ability to infect root hair of leguminous plants, and it to induce nodule formation (Figure 2), with subsequent fixation of nitrogen [9].



**Figure 2.** Visual aspect of root system of *Vigna unguiculata* plants inoculated with *Bradyrhizobium*. The red arrows indicate nodules formed after infection process.

The persistence of rhizobial strains, and their symbiotic performance in current and subsequent seasons are affected by numerous biotic and abiotic factors [10], with drought stress and nitrogen deprivation, being among the most significant in many parts of the world [11]. Other important factor is the root exudation ability, which it will determine plant microbe associations so that the survival and tolerance of rhizobia during water restriction.

Molybdenum is an essential element for soil microorganisms, since it serves as a cofactor for different enzymes involved in the metabolism of nitrogen, carbon and sulfur. Before the synthesis of molybdoenzymes, uptake of molybdate, which is the more stable form of molybdenum), its activation to an appropriate form, and its incorporation into the organic fraction of the molybdenum-cofactors, are required [12].

The presence of molybdenum is necessary during formation of several proteins, including the nitrogenase, the molybdoenzyme that reduces atmospheric dinitrogen ($N_2$) into ammonia ($NH_4^+$) [13]. This bacterium is also capable of denitrification, via nitric oxide (NO) and nitrous oxide ($N_2O$) to $N_2$, when the cells are cultured under oxygen-limiting conditions [14]. The first reaction of denitrification, is carried out by the periplasmic Mo-containing nitrate reductase [15]. In addition, the reaction under normal conditions is described as $N_2 + 8\,e^- + 8\,H^+ + 16\,MgATP \rightarrow 2\,NH_3 + H_2 + 16\,MgADP + 16Pi$.

The enzyme mechanism requires reduction of the Fe protein by electron donors such as ferredoxin and flavodoxin, transfer of single electrons from the Fe protein to the MoFe protein (which is dependent on MgATP hydrolysis) and, finally, internal electron transfer in the MoFe protein by the P cluster to the FeMo cofactor substrate-binding site. Each electron-transfer step requires an obligatory cycle of association of the Fe and MoFe proteins to form a complex (Figure 3), after which the two components dissociate [16].
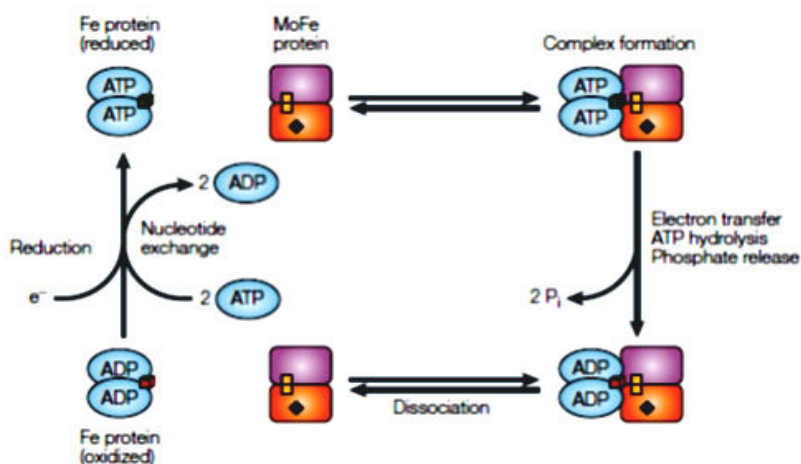


**Figure 3.** Schematic representation of the nitrogenase Fe protein cycle. The Fe protein dimer is shown in light blue with the cube representing the [4Fe–4S] cluster coloured black to indicate the reduced form and red to represent the oxidized form. The α and β subunits of the MoFe protein are depicted as orange and pink, respectively, the yellow squares represent the P cluster and the black diamond represents the FeMo cofactor. Changes in the oxidation state of the MoFe protein are not shown [16].

Several leguminous such as *Vigna unguiculata* and *Cicer arietinum* are considered tolerant to water deficit, and important mechanisms were developed by this species to tolerate inadequate water supply. For example, biochemical modifications in carbon metabolism, such as increase in sucrose [17], as well as significant interference in nitrogen metabolism, like reduction of soluble proteins [5] and increase in total amino acids [18] contribute to osmotic adjustment of these plants (Figure 4).
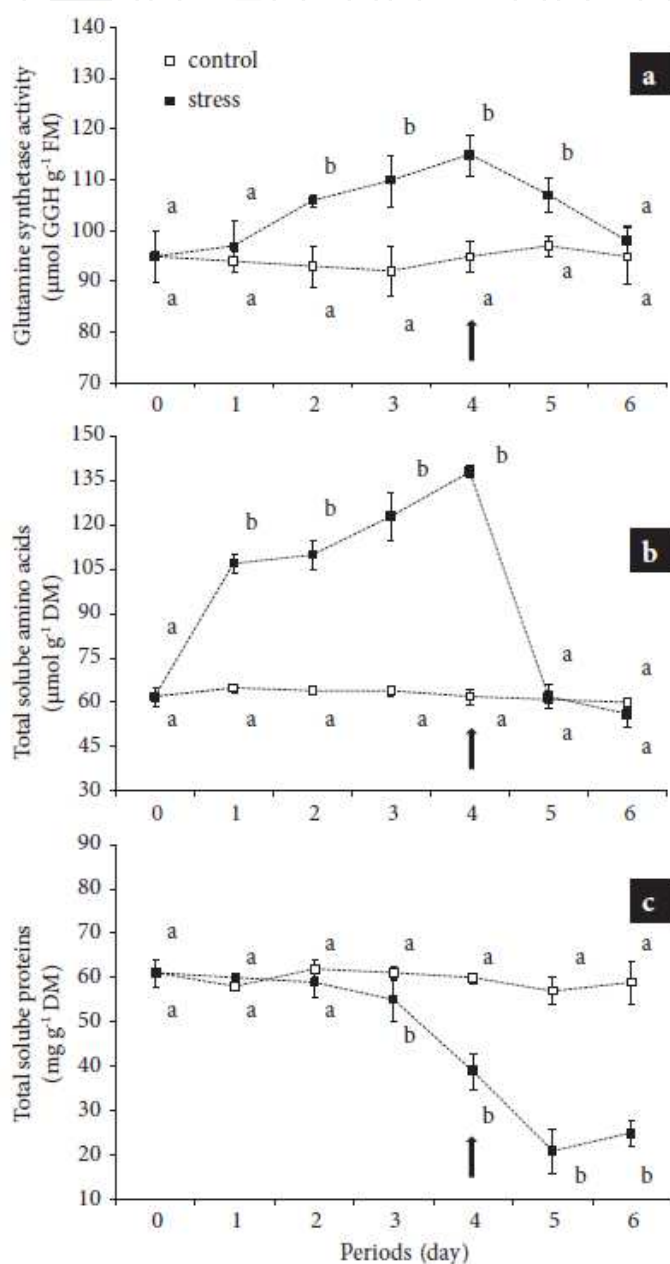


**Figure 4.** Glutamine synthetase activity (a), total soluble amino acids (b) and total soluble proteins (c) in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. Means followed by the same letter are not significantly different by the Tukey test at 5% of probability. The bars represent the mean standard error and the arrow the arrow indicates the rehydration point [5].

## 2. Objective

Aims of this chapter is to define (i) water deficiency and biological fixation of nitrogen, to explain (ii) as this symbiotic process can promote beneficial repercussions to plant and microorganism, and to present (iii) the attenuation of negative impacts on nodule and plant, besides nitrogen compounds and morphological parameters of plants exposed to water restriction.

## 3. Water maintenance in leaf and nodule produced by inoculation

Drought is environmental component that affect crop yields worldwide. In nature, this stress is multifaceted problems that are usually associated with other adverse circumstances, which limit plant performance such as water shortage and nutrient deficits. In order to as-sess the osmotic stress, Sassi et al. [19] monitored two *Phaseolus vulgaris* cultivars inoculated with *Rhizobium*, being cvs. 'Flamingo' (tolerant) and Cv. 'Coco Blanc' (sensitive).

Leaf osmotic potential (Ψo) decreased in stressed plants in both cultivars. A minimum value of −2.3 MPa was reached in Cv. 'Flamingo' plants under mannitol-induced osmotic stress (Figure 5 A). Ψo decreased in stressed nodules, reaching −1.3 MPa in Cv. 'Coco Blanc' and −1.7 MPa in Cv. 'Flamingo' (Figure 5 B). Therefore, Cv. 'Flamingo' showed a better osmotic adjustment response to osmotic stress both in leaves and nodules [19].
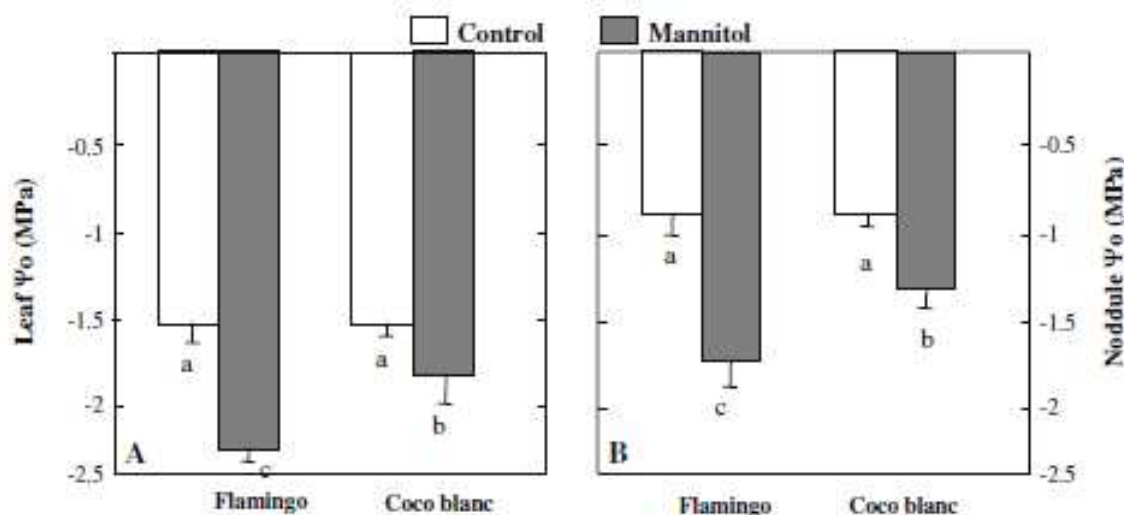


**Figure 5.** Variation of osmotic potential (Ψo) in response to osmotic stress in leaves (A) and nodules (B) mediated by 50 mM mannitol. Values represents mean ± SE (n=6) [19].

In control leaves of both cultivars, RWC remained close to 80% (Figure 6 A). After 15 days of osmotic treatment, RWC was 65%in mannitol-treated plants of Cv. 'Flamingo', and only

45% in Cv. 'Coco Blanc'. These results indicate that osmotic stress caused an important reduction in shoot water supply. The same trend was observed in nodules (Figure 6 B). Indeed, data showed decreased nodule RWC in both stressed cultivars. This decrease was higher in Cv. 'Coco Blanc' treated nodules [19].
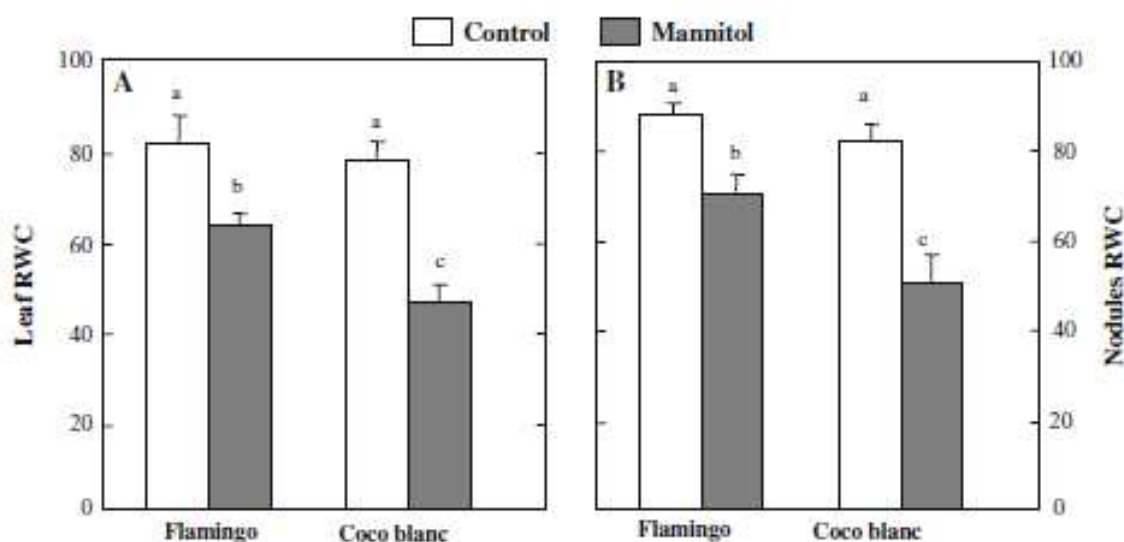


**Figure 6.** Effect of mannitol-induced osmotic stress on relative water content (RWC) in Flamingo and Coco blanc bean cultivar leaves (A) and nodules (B). Values represents mean ± SE (n=6) [19].

Mannitol-induced water deficit produced substantial dehydration that led to decreasing $\Psi_o$ (Figure 6). The decrease in $\Psi_o$ is considered a potential mechanism of cellular drought resistance as it enables turgor maintenance and growth continuation [20]. Cv. 'Flamingo' exhibited lower $\Psi_o$ under osmotic treatment. It was able to uptake more water and then grow more when exposed to decreased $\Psi_o$, thus it turned out to be a better drought tolerant cultivar than Cv. 'Coco Blanc' [21]. This may be attributed to maintenance of the leaf and nodule water status under stressed conditions (Figure 5). Several mechanisms could be involved in contributing to water retention.

## 4. *Bradyrhizobium* ameliorates negative effects in plants exposed to drought

The relationship between the water status in the plant and N2 fixation, mainly under water stress, and the changes in nodule morphology have been studied in some temperate legumes [22]. However, tropical legumes growing in arid regions, have not received adequate attention.

Even where information is available, the degree of water stress in the plants was not clearly defined, which makes it difficult to make comparisons. The structural basis for the differ-

ence in sensivity of N2 fixation in tropical legumes, under water stress, is not clearly understood [23]. Based in these problems reported, Figueiredo et al. [24] investigated Vigna unguiculata plants exposed to 3 inoculation forms (BR-2001, EI-6, and control) combined with 6 different degrees of water stress (-1.5, -2.0, -4.0, -6.0, -8.0, and -10.0 kPa).

Water deficit response in cowpea appears to be directly related to a reduction in nodule mass (Table 1), which may (after a severe stress, S6) have affected nodule structural constituents. However, in moderate stress (S3) the impact on nodule water content was higher than on the changes in nodule mass [24].

| Matric potentials[1] (kPa) | LHb (mg g$^{-1}$ nod.DM) | | | UN (nmol ml$^{-1}$) | | | N$_2$ase (nmol C$_2$ H$_4$ pl$^{-1}$ h$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Strains | | | | | | | | |
| | BR-2001 | EI-6 | C | BR-2001 | EI-6 | C | BR-2001 | EI-6 | C |
| S1 | 26.16ab$^A$ | 28.06ab$^A$ | 23.00ab$^B$ | 5179a$^A$ | 5351ab$^A$ | 4000a$^B$ | 10697a$^A$ | 11274b$^A$ | 9288a$^B$ |
| S2 | 28.56a$^A$ | 29.70a$^A$ | 24.23a$^B$ | 5840a$^A$ | 6099a$^A$ | 4402a$^B$ | 11670a$^A$ | 12410a$^A$ | 9571a$^B$ |
| S3 | 25.40b$^A$ | 27.23ab$^A$ | 20.40bc$^B$ | 3740b$^B$ | 4488bc$^A$ | 2906b$^C$ | 2563b$^B$ | 3400c$^A$ | 2093b$^B$ |
| S4 | 25.40b$^A$ | 26.50b$^A$ | 20.40bc$^B$ | 3366bc$^B$ | 4114cd$^A$ | 2733b$^B$ | 2403b$^{AB}$ | 2633cd$^A$ | 1630bc$^B$ |
| S5 | 24.03b$^A$ | 25.90b$^A$ | 20.30bc$^B$ | 2819bc$^B$ | 3567cd$^A$ | 2388b$^B$ | 1130c$^B$ | 2277d$^A$ | 1000c$^B$ |
| S6 | 23.36b$^B$ | 25.90b$^A$ | 19.26c$^C$ | 2589c$^B$ | 3337d$^A$ | 2071ab$^B$ | 1121c$^{AB}$ | 1756d$^A$ | 837c$^B$ |
| F (plot) | 13.90** | | | 132.87** | | | 142.15** | | |
| F (subplot) | 132.36** | | | 67.67** | | | 67.48** | | |
| % CV (plot) | 5.54 | | | 7.44 | | | 23.94 | | |
| % CV (subplot) | 4.57 | | | 9.54 | | | 20.25 | | |

[1]For S1 to S6 see Table 1. *, **Significant at the 0.05 and 0.01 probability level. In each column (lower letters) and in each line (capital letters), the means followed by the same letter do not differ statistically ($p < 0.05$) from each other, according to Tukey's test

**Table 1.** Nodule dry matter (NDM) and nodule water content (NWC) in cowpea with (BR-2001 and EI-6) and without (C) *Bradyrhizobium* spp. inoculation at different degrees of water stress [24].

# 5. Interference positive on nitrogen compounds of plants inoculated and exposed to water deficit

Cowpea (*Vigna unguiculata* [L.] Walp.) is a leguminous with high protein content, large capacity of fixation of the atmospheric nitrogen (N$_2$) and low requirements to soil fertility [25], being frequently cultivated by farmers in Northern and Northeastern regions of the Brazil. This species constitutes the main subsistence culture, being the grain used as protein source in feeding [26]. Cowpea presents important agronomical characteristics, such as rusticity and precocity, besides being considered a plant adapted to conditions of limited or insufficient water availability [27].

Beneficial effects proportioned by the inoculation on growth parameters as leaf, stem and root are largely explored and well known in leguminous plants [28-30], but informations more specific of this symbiotic process on essential compounds such as amino acids and proteins are limited. Figueiredo et al. [24] report that inoculation using *Bradyrhizobium* can alleviate the negative consequences in *Vigna unguiculata* plants induced to water deficiency, but study conducted by Serraj and Sinclair [31] revealed that water supply presents repercussion on symbiotic efficiency.

Based on this overview, Barbosa et al. [32] carried out a study aiming to investigate if nitrogen compounds exercise influence on accumulation of dry matter in *Vigna unguiculata* plants exposed to combined action of inoculation and water deficit.

The concentration of total soluble amino acids in plants subjected to inoculation was higher only in tolerant plants, if compared with same treatments of plants non-inoculated (Figure 7 A). Water deficit promoted a significant increase in this variable to all treatments. The tolerant cultivar presented lower changes, in comparison with same treatments in sensitive cultivar.

Total soluble proteins of inoculated plants presented higher values (Figure 7 B), when compared to same treatments in non-inoculated plants. Water deficit caused a significant decrease in both cultivars, presenting higher variation in sensitive plants.

For proline the inoculated plants presented higher values, comparing with same treatments in non-inoculated plants (Figure 7 C). The two cultivars demonstrated higher values in water deficit, when compared with respective controls. These results present a greater variation in tolerant plants, if compared with same treatments in sensitive plants.

Tolerant plants submitted to inoculation presented significant increase in amino acids, and these results are attributed to biological fixation of nitrogen. The nitrogenase enzyme promotes the nitrogen absorption in form of nitrogen gas ($N_2$) and conversion to ammonium ($NH_4^+$). In addition, the higher formation of amino acids probably is linked to increase in activity of enzymes glutamine synthetase (GS), being your activity depending of ATP (adenosine-5'-triphosphate), and glutamate synthase (GOGAT). In addition, the increase in amino acids of plants exposed to inoculation is due to greater flux and better assimilation of nitrogen in form of ammonium, concomitantly with higher activity of GS and GOGAT enzymes. Ramos et al. [33] evaluating the responses in Glycine max plants under water deficit and inoculation of Bradyrhizobium japonicum observed also an increase in concentration of total soluble amino acids.

The concentration of total soluble amino acids in plants under water deficit increased in all treatments. This increment occurred probably due to increase in activity of protease enzymes, responsible by breakdown of proteins aiming to adjust osmotically the plant [34]. Similar results on increase in amino acids were obtained to Costa et al. [35] investigating *Vigna unguiculata* plants. Delfini et al. [36] evaluating the responses of two *Arachis hypogaea* cultivars submitted to inoculation of *Bradyrhizobium sp.* showed significant increase in amino acids.
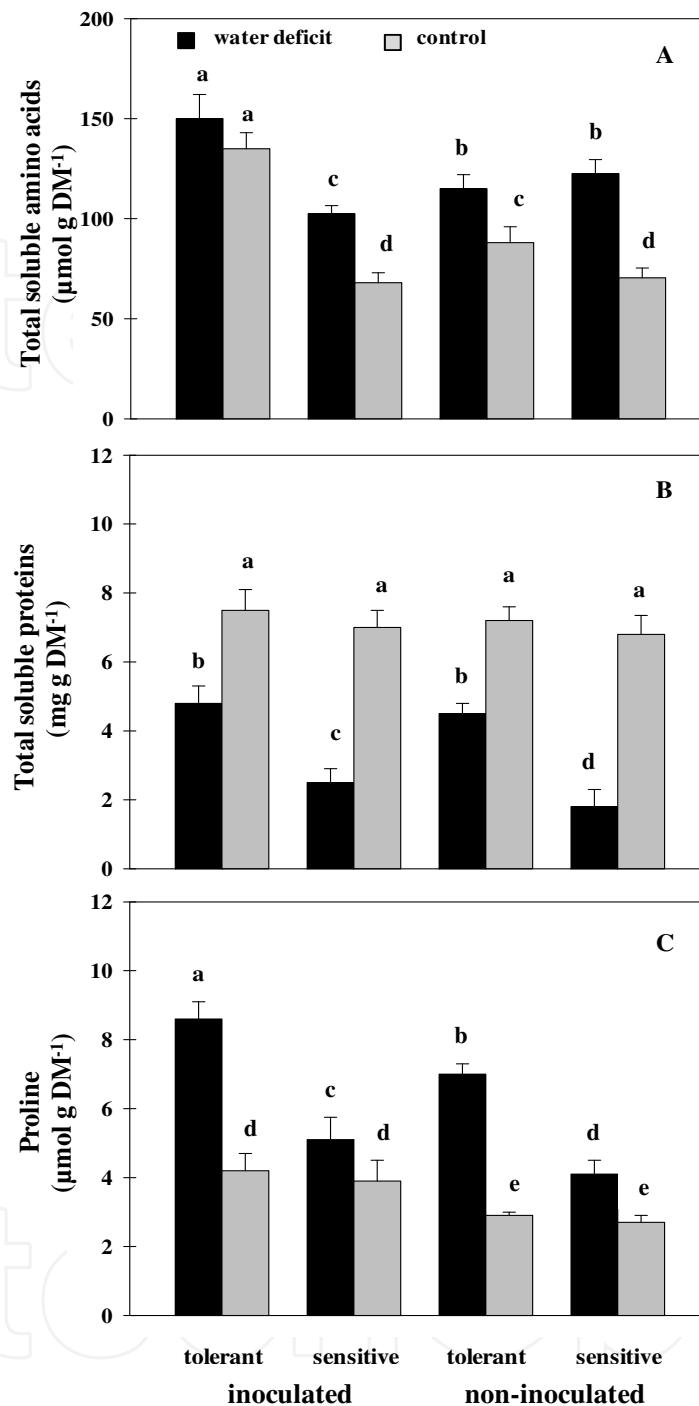
**Figure 7.** Total soluble amino acids (A), total soluble proteins (B), and proline (C) in two contrasting *Vigna unguiculata* plants under water deficit and subjected to inoculation. Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error [32].

The increase showed in total soluble proteins induced by inoculation suggests that bacteria action resulted in increase in nitrogen supply through secondary route, that is regulated by the nitrogenase [37], because in this study was not verified increase in activity of the nitrate reductase after inoculation. Hristozkova et al. [38] evaluating the responses

in *Pisum sativum* plants under inoculation and molybdenum application also obtained increase in protein levels.

The decrease in protein levels promoted by the water deficit is associated to decrease of the protein synthesis combined with increase of proteolytic enzymes, responsible by breakdown of soluble proteins in plants [39]. Costa [40] obtained similar results studying *Vigna unguiculata* subjected to water deficit, corroborating these results.

The increase of proline levels provoked by the inoculation is probably linked to better amino acids utilization such as glutamic acid and arginine, being the glutamic acid the precursor of the proline, while arginine can suffer reaction mediated by enzyme called of pyrrolline-5-carboxylate reductase (P5CR) and consequently to liberate proline [33].

Kohl et al. [41] also observed higher amounts of proline in *Glycine max* plants inoculated with *Bradyrhizobium japonicum*, contributing with results of this study. The increase of proline in plants under water deficit is a response to loss of cell turgescence [42]. Nogueira et al. [43] describe that the proline accumulation has been related with drought tolerance in higher plants, actuating as osmoregulator agent with the objective to keep water in plant tissue [44]. Similar behavior was described by González et al. [45] working with *Pisum sativum* plants under water restriction.

## 6. *Bradyrhizobium* producing better performance on morphological parameters

Beneficial effects proportioned by the inoculation on growth parameters are largely explored in crops as *Phaseolus vulgaris* and *Glycine max*, but informations on dry matter accumulation of *Vigna unguiculata* under water deficit is limited. Barbosa et al. [32] conduced an experiment with 2 cultivars (tolerant and sensitive) combined with 2 water regimes (water deficit and control), and 2 inoculation forms (inoculated and non-inoculated), totalizing 8 treatments.

In shoot dry matter the inoculation provoked increase, considering same treatments in plants non-inoculated (Figure 8 A). However, this increase only was significant in tolerant cultivar under inoculation and irrigation. Water deficit occasioned a significant decrease in shoot dry matter, with exception in tolerant cultivar non-inoculated. The tolerant plants presented better results, if compared with sensitive plants independently of treatment (Figure 8 A).

The inoculation provoked increase in plant dry matter, with exception in sensitive cultivar under irrigation (Figure 8 B), comparing to same treatments in plants non-inoculated. Water deficiency induced decrease in plant dry matter for all treatments, being these significant results when compared with control plants. Independently of treatments was showed that sensitive cultivars presented lower values, if compared to tolerant cultivars.
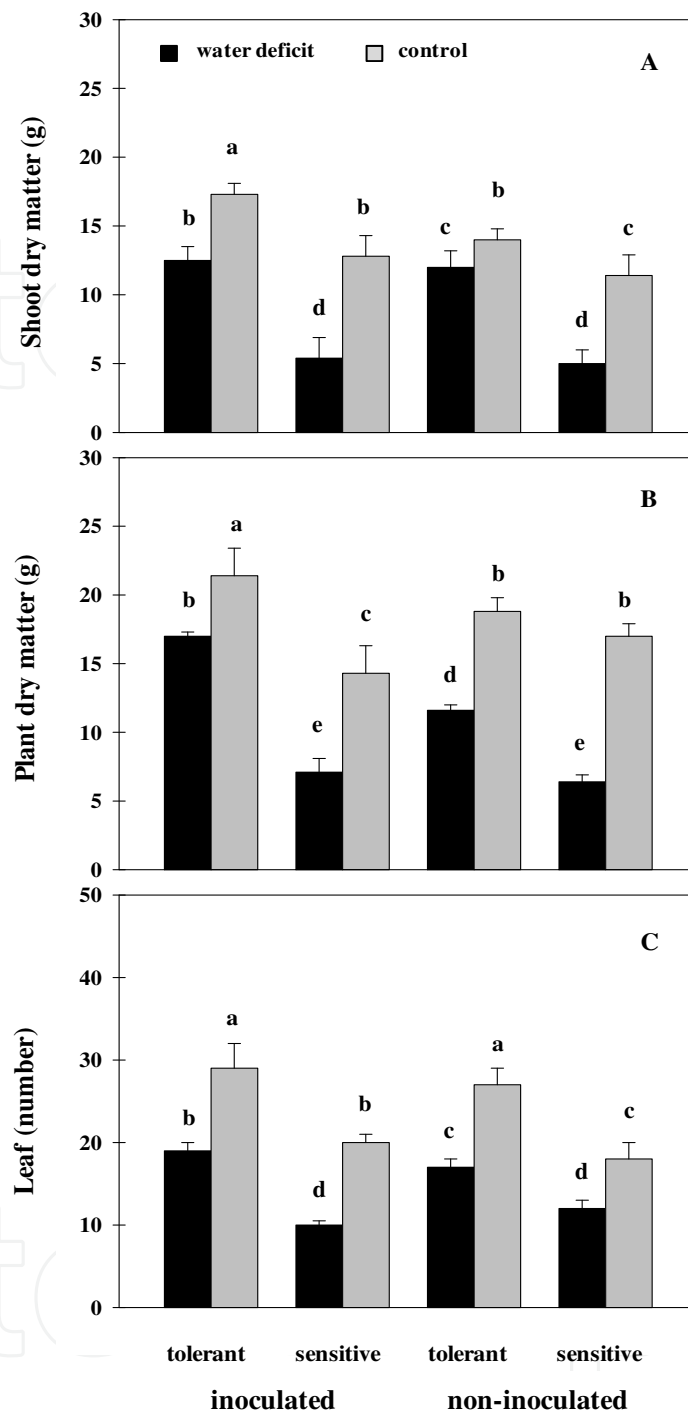
**Figure 8.** Shoot dry matter (A), plant dry matter (B), number of leaves (C) in two contrasting Vigna unguiculata plants under water deficit and subjected to inoculation. Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error [32].

For leaf number occurred increase after inoculation, with exception in sensitive plants under water deficiency (Figure 8 C). Water deficit proportioned decrease in values of leaf number, being significant in comparison with control plants. In tolerant cultivar were obtained higher values of leaf number, if compared with sensitive cultivar.

Shoot dry matter was maximized after the inoculation procedure, being this fact linked to probably increase in nodule number in root (data not shown), as well as it proportioned higher absorption and availability of nitrogen to plant [46-47]. Similar results linked to shoot dry matter were found by Figueiredo et al. [24] in research with *Vigna unguiculata* plants exposed to inoculation of *Bradyrhizobium*.

The water deficit reduced the production of shoot dry matter, with these effects associated to negative interference of water deficiency on biochemical processes as nitrate assimilation and biological fixation of nitrogen [35], modifying indirectly the partitioning of photo-assimilates in root and shoot, and consequent decrease in accumulation of shoot biomass [48]. Similar results were found by Mendes et al. [49] working with two *Vigna unguiculata* cultivars submitted to drought during two stages.

The inoculation proportioned increase of total dry matter, and this result must be linked to better development and efficiency of root system, in which presents higher nitrogen absorption using the nodulation process. I addition, normally the higher nitrogen fixation will produce increase in amino acids and also proteins [36], and it exercises influence on photoassimilates availability.

Similar responses were described by Sassi et al. [50] investigating two *Phaseolus vulgaris* cultivars subjected to inoculation with bacteria of *Rhizobium* gender. Plants under water deficiency frequently have the production of dry matter reduced, being this decrease related to fact that water deficit affects several metabolic processes such as absorption of water and nutrients, which are fundamental to keep adequate growth and development rates.

Nascimento [51] also reported that water deprivation affects the osmotic mechanism, and by consequence reduces the $CO_2$ supply, that is essential in photosynthetic process. Similar results were found by Leite and Virgens Filho [52] studying *Vigna unguiculada* plants exposed to water deficit.

The increase in leaf number promoted by inoculation is occasioned by the higher number of nodules in root, and consequently due to the better biological fixation of nitrogen [53]. Araújo et al. [54] studying *Vigna unguiculata* and *Leucaena leucocephala* plants also reported an increase of this variable, confirming the results obtained in this work. The lower leaf number after water deficiency is caused by the process of leaf abscission, and this fact occurs due to substrate not to present water and nutrient sufficient to supply the plant exigencies [4]. Correia and Nogueira [55] obtained similar results with *Arachis hypogaea* plants under water deficit.

## 7. Final considerations

This chapter was structured with recent informations on capacity of *Bradyrhizobium* and *Rhizobium* to mediate tolerance in leguminous plants submitted to water deficit, which it can be used by students, teachers, researchers, scientists and farmers. It revealed concepts, effects, and results on water deficiency and your consequences on plants, as well as explored sever-

al possibilities linked to symbiosis between plant-microorganisms. Additionally, it presented essential compounds such as molybdenum and reactions during process of biological fixation of nitrogen. Also was demonstrated the water maintenance in leaf and nodule produced after inoculation. Based in novel results, were related interference positives on nitrogen compounds such as total soluble amino acids, proline, and total soluble proteins. Other results prove the beneficial repercussion produced by inoculation with *Bradyrhizobium* on morphological parameters.

## Acknowledgements

## Author details

Allan Klynger da Silva Lobato[1], Joaquim Albenísio Gomes da Silveira[2],
Roberto Cezar Lobo da Costa[1] and Cândido Ferreira de Oliveira Neto[1]

1 Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia, Paragominas, Brazil

2 Laboratório de Metabolismo e Estresse de Plantas, Universidade Federal do Ceará, Fortaleza, Brazil

## References

[1] Rockström J, Falkenmark M. Semiarid crop production from a hydrological perspective: Gap between potential and actual yield. Crit. Rev. Plant Sci 2000;19 319-326.

[2] Kramer PJ, Boyer JS. Water relations of plant and soils. Academic Press, New York. 1995.

[3] Fontana DC, Berlato MA, Bergamaschi H. Micrometeorological alterations in soybeans grown under different water regimes. Pesquisa Agropecuária Brasileira 1992;27 661-669.

[4] Santos RF, Carlesso R. Water deficit and morphologic and physiologic behavior of plants. Revista Brasileira de Engenharia Agrícola e Ambiental 1998;2 287-294.

[5]   Costa RCL, Lobato AKS, Silveira JAG, Laughinghouse HD. ABA-mediated proline synthesis in cowpea leaves exposed to water deficiency and rehydration. Turkish Journal of Agriculture and Forestry 2011;35 309-317.

[6]   Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT. Root growth maintenance during water deficits: Physiology to functional genomics. Journal of Experimental Botany 2004;55 2343-2351.

[7]   Sharp RE, Silk WK, Hsiao TC. Growth of the maize primary root at low water potentials. I. Spatial distribution of expansive growth. Plant Physiology 1988;87 50-57.

[8]   Albino UB, Campo RJ. Effect of sources and levels of molybdenum on *Bradyrhizobium* survival and on biological nitrogen fixation in soybean. Pesquisa Agropecuária Brasileira 2001;36 527-534.

[9]   Mercante MM, Gol SR, Franco AA. Importance of phenolics compounds in the interactions between leguminous species and rhizobia. Ciências da Vida 2002; 22 65-81.

[10]  Bordeleau LM, Prevost D. Nodulation and nitrogen fixation in extreme environments. Plant Soil 1994;116 115–125.

[11]  Squartini A, Dazzo FB, Casella S, Nuti MP. The root-nodule symbiosis between "*Rhizobium hedysari*" and its drought-tolerant host *Hedysarum coronarium*. Symbiosis 1993;15 227–238

[12]  Pau RN, Lawson DM. Transport, homeostasis, regulation, and binding of molybdate and tungstate to proteins. Metal Ions in Biological Systems 2002;39 31–74.

[13]  Lawson DM, Smith BE. Molybdenum nitrogenases: a crystallographic and mechanistic view. Metal Ions in Biological Systems 2002;39 75–119.

[14]  Bedmar EJ, Robles EF, Delgado MJ. The complete dentrification pathway of symbiotic N-fixing bacteria *Bradyrhizobium japonicum*. Biochemical Society Transactions 2005;1 11–16.

[15]  Delgado MJ, Bonnard N, Tresierra-Ayala A, Bedmar EJ, Müller P. The *Bradyrhizobium japonicum napEDABC* genes encoding the periplasmic nitrate reductase are essential for nitrate respiration. Microbiology 2003;149 3395–3403.

[16]  Dixon R, Kahn D. Genetic regulation of biological nitrogen fixation. Nature Reviews Microbiology 2004;2 621-631.

[17]  Lobato AKS, Costa RCL, Oliveira Neto CF, Santos Filho BG, Alves GAR, Freitas JMN, Cruz FJR, Marochio CA, Coimbra GK. Responses of the pigments and carbon metabolism in *Vigna unguiculata* cultivars submitted to water deficit. Research Journal of Biological Sciences 2009a;4 593-598.

[18]  Lobato AKS, Oliveira Neto CF, Costa RCL, Santos Filho BG, Cruz FJR, Laughinghouse IV HD. Biochemical and physiological behaviour of *Vigna unguiculata* (L.) Walp. under water stress during the vegetative phase. Asian Journal of Plant Science 2008a;7 44-49.

[19] Sassi S, Aydi S, Gonzalez EM, Arrese-Tgor C, Abdelly C. Understanding osmotic stress tolerance in leaves and nodules of two *Phaseolus vulgaris* cultivars with contrasting drought tolerance. Symbiosis 2010;52 1-10.

[20] Bajji M, Lutts S, Kinet JM. Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus culture issued from durum wheat (*Triticum durum*) cultivars differing in drought resistance. Journal of Plant Physiology 2000;156 75–83.

[21] Sassi AS, Aydi S, Gonzalez EM, Abdelly C. Osmotic stress affects water relations, growth, and nitrogen fixation in *Phaseolus vulgaris* plants. Acta Physiologiae Plantarum 2008a;30 441–449.

[22] Sprent JJ. Nitrogen fixation. In Physiology and Biochemistry of drought resistance in plants. Eds. L C Paleg and D Aspinall. pp 131–143. Academic Press. 1981.

[23] Venkateswarlu B, Neelam S, Maheswari M. 1990 Nodulation and $N_2$ ($C_2H_2$) fixation in cowpea and groundnut during water stress and recovery. Field Crops Research 1990;25 223–232.

[24] Figueiredo MVB, Vilar JJ, Burity HA, França FP. Alleviation of water stress effects in cowpea by *Bradyrhizobium ssp*. Inoculation. Plant and Soil 1999;207 67-75.

[25] Alves JMA, Araújo NP, Uchôa SCP, Albuquerque JAA, Silva AJ, Rodrigues GS, Silva DCO. The agroeconomic evaluation of the production of cowpea varieties intercropped with varieties of cassava in The State of Roraima. Revista Agroambiente 2009;3 15-30.

[26] Frota KMG, Soares RAM, Arêas JAG. Chemical composition of cowpea [*Vigna unguiculata* (*L.*) *Walp*], BRS-Milênio cultivar. Ciência e Tecnologia de Alimentos 2008;28 470-476.

[27] Oliveira AP, Sobrinho JT, Nascimento JT, Alves AU, Albuquerque IC, Bruno GB. Evaluation of breeding lines and cultivars of cowpea-beans in Areia, Paraiba, Brazil. Horticultura Brasileira 2002;20 180-182.

[28] Ramos MLG, Gordon AJ, Minchin FR, Sprent JI, Parsons R. Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris L.*). Annals of Botany 1999;83 57-63.

[29] Silveira JAG, Matos JCS, Cecatto VM, Viegas RA, Oliveira JTA. Nitrate reductase activity, distribution, and response to nitrate contrasting *Phaseolus* species inoculated with *Rhizobium* spp. Environment and Experimental Botany 2001;46 37- 46.

[30] Moraes WB, Martins Filho S, Garcia SGO, Caetano SP, Moraes WB, Cosmi FC. Evaluation of biological, fixation of nitrogen in *rhizobium* under water deficit. Idesia 2010;28 61-68.

[31] Serraj R, Sinclair TR. Processes contributing to $N_2$ fixation insensitivity to drought in the soybean cultivar Jackson. Crop Science 1996;36 961-968.

[32] Barbosa MAM, Lobato AKS, Viana GDM, Coelho KNN, Barbosa JRS, Moraes MCHS, Costa RCL, Santos Filho BG, Oliveira Neto CF. Relationship between total soluble proteins and dry matter in two contrasting cowpea cultivars induced to inoculation and water deficiency. The Scientific World Journal, 2012;paper is press.

[33] Ramos MLG, Parsons R, Sprent JI. Differences in ureide and amino acid content of water stressed soybean inoculated with *Bradyrhizobium japonicum* and *B. elkanii*. Pesquisa Agropecuária Brasileira 2005;40 453-458.

[34] Costa RCL, Lobato AKS, Oliveira Neto CF. Variation in content of total soluble amino acids in leaves of cowpea under water stress. In: Congresso Nacional de feijão caupi, pp. 1-19, Teresina, PI, maio, 2006a.

[35] Costa RCL, Cardoso BB, Silva JT, Gomes Filho JGF, J. A. G. Silveira JAG. Water stress strongly decreases the assimilation of nitrate and nodulation in cowpea (*Vigna unguiculata*, (L.) Walp.). *In* Reunião Nacional de Pesquisa de Caupi, pp. 78-79, Teresina, PI, Brazil, march, 1996.

[36] Delfini R, Belgoff C, Fernández E, Fabra A, Castro S. Symbiotic nitrogen fixation and nitrate reduction in two peanut cultivars with different growth habit and branching pattern structures. Plant Growth Regulation 2010;6 153-159.

[37] Marschner H, Mineral nutrition of higher plants, Academic Press, pp. 889, London, UK, 1995.

[38] Hristozkova M, Geneva M, Stancheva I, Response of inoculated pea plants (*Pisum sativum* L.) to root foliar fertilizer application with reduced molybdenum concentration. Plant Physiology 2006;45 73-79.

[39] Lechinoski A, Freitas JMN, Castro DS, Lobato AKS, Oliveira Neto CF, Cunha RLM. Influence of water stress on levels of soluble amino acids and proteins in leaves of teak (*Tectona grandis L. f.*). Revista Brasileira de Biociência 2007;5 927-929.

[40] Costa RCL. Nitrogen assimilation and osmotic adjustment in plants nodulated bean-to-string *Vigna unguiculata* (L.) Walp. Subjected to water stress. Ph.D Thesis. Universidade Federal do Ceará, Brasil, 1999.

[41] Kohl DH, Kennelly DJ, Zhy Y, Schubert KR, Sheader G. Proline accumulation, nitrogenase ($C_2H_2$ reducing) activity and activities of enzymes related to proline metabolism in drought-stressed soybean nodules. Journal of Experimental Botany 1991;42 831-837.

[42] Oliveira AAO, Barreto LP, Bezerra Neto E, Santos MVF, Costa JCA. Organic solutes in forage sorghum genotypes under salt stress. Pesquisa Agropecuária Brasileira 2006;14 31-35.

[43] Nogueira RJMC, Santos CR, Neto EB, Santos VF. Physiological behaviour of two peanut cultivars submitted to suppression watering. Pesquisa Agropecuária Brasileira 1998;33 1963-1969.

[44] Muchow RC, Carberry PS. Designing improved plant types for the semi-arids trop‐ ics: Agronomist` viewpoints. In: penning de vries, F.W.T. Teng, P. Metselaar, Eds., Dordrecht:Kluwer, pp. 37-61, Meryland, EUA, 1993.

[45] González EM, Aparicio-Tejo PM, Gordon AJ, Minchin FR, Igor CA. Water-deficit ef‐ fects on carbon and nitrogen metabolism of pea nodules. Journal of experimental Botany 1998;49 1705-1714.

[46] Epstein E, Bloom AJ. Nutrition and growth. In: *E.* Epstein and A. J. Bloom, Ed. pp. 251-286, Londrina, PR, Brazil, 2006.

[47] Souza RA, Hungria M, Franchini JC, Maciel CD, Campo RJ, Zaia DAM. Minimal set of parameters for evaluation soil microbiota and biological nitrogen fixation in soy‐ bean. Pesquisa Agropecuária Brasileira 2008;43 83-91.

[48] Correia GK, Nogueira CMJR. Evaluation of the growth of groundnut (*Arachis hypogae L.*) subjected to water deficit. Revista de Biologia e Ciências da Terra 2004a;4 56-60

[49] Mendes RMS, Távora FJAF, Pitombeira JB, Nogueira RJMC. Source-sink relation‐ ships in cowpea under drought stress. Revista Ciência Agronômica 2007;38 95-103.

[50] Sassi S, Aydi S, Gonzalez EM, Arrese-Tgor C, Abdelly C. Understanding osmotic stress tolerance in leaves and nodules of two *Phaseolus vulgaris* cultivars with con‐ trasting drought tolerance. Symbiosis 2010;52 1-10.

[51] Nascimento SP. Effect of water deficit in cowpea to identify genotypes with drought tolerance. Ph.D Thesis, Universidade Federal do Piauí, 2009.

[52] Leite ML, Virgens Filho JS. Dry matter production of cowpea [*Vigna unguiculata* (L.) Walp] plants submitted to water deficits. Exatas Terra 2004;10 43-51.

[53] Ferreira EPB, Martins LMV, Xavier GR, Rumjanek NG. Nodulation and grain yield by cowpea [*Vigna unguiculata* (L.) Walp.] Inoculated with rhizobia, isolates. Revista Caatinga 2011;24 27-35.

[54] Araújo ASF, Carneiro RFV, Bezerra AAC, Araújo FF. Co-inoculation *Rhizobia* and *Ba‐ cilus subtilis* in cowpea and Leucaena: effects on nodulation, $N_2$ fixation and plant growth. Ciência Rural 2009;40 43-48.

[55] Correia KG, Nogueira RJMO. Assessment of growth of peanut (*Arachis hypogaea L.*) subjected water deficit. Revista de Biologia e Ciência da Terra 2004b;4 240-248.